

Effect of solvent polarity on total phenolic, antioxidant and antibacterial capacity of cherry leaves (*Muntingia calabura* L.)

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Abstract. The cherry (*Muntingia calabura* L.) is a neotropical tree widespread in Indonesia. This study will examine how solvent polarity affects total phenolic, total flavonoids, antioxidants, and antibacterial capacity. The maceration method extracts materials with ethanol, ethyl acetate, and hexane from cherry leaves. The maximum yield, phenolics, flavonoids, and antioxidant capacity are in ethanol solvent. Based on ANOVA at 95% confidence intervals, solvent polarity significantly affected metabolite and activity profiles. However, DPPH, ethyl acetate, and hexane solvents revealed similar antioxidant capacity. The antioxidant capacity of DPPH and FRAP was positively correlated. The DPPH method exhibited more antioxidant capacity, whereas the FRAP approach had superior precision. The Pearson correlation test demonstrated a favourable association between antioxidant capacity and total phenolic, with $r = 0.85$. The paper disc method for *Staphylococcus aureus* and *E. coli* antibacterial testing. As a positive control, Chloramphenicol had an apparent zone diameter of 13.3 mm (*Staphylococcus aureus*) and 15.8 mm (*Escherichia coli*). The samples had antibacterial capacity against *Staphylococcus aureus* and *Escherichia coli* at 7 mm and 6.5 mm (ethanol), 8.7 mm and 7.2 mm (ethyl acetate), and 9.5 cm and 7.5 cm (hexane). Chemically, hexane solvent was more antimicrobial than others.

1 Introduction

Plants produce massive natural products called secondary metabolites, biosynthetically derived from primary metabolites. Thousands of structurally unique secondary metabolites are formed within cultivation to protect plants from herbivores, bacteria, fungi, and viruses [1]. The cherry plant (*Muntingia calabura* L.) is a neotropical tree that originates in Brazil and is widespread in America, India, and South Asia [2]. Kersen has different names in each country, namely, Jamaican cherry, Panama berry, Singapore cherry, jam cherry, Japanese kerns [3], strawberry cherry, jam tree, Chinese cherry, madras pea pumpkin, cotton candy berry, Japanese cherry, gasagase hannina mara, and Siamese cherry

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[4]. Kersen plants in Indonesia are generally found along urban roads or in parking lots. Different parts of the cherry plant have been reported as traditional medicine. The leaves commonly used to treat ulcers, dysentery, diarrhoea, stomach ulcers, fever, and haemorrhoids [5]. Extracts from bark, flowers, and leaves are used as an antiseptic [6], relieve colds and headaches, and reduce prostate gland swelling and stomach ulcers. The root has been used as an abortifacient, and the fruit is solidly served in tarts or made into jam [7].

Pharmacologically, cherry leaves exhibit anti-obesity [8], anti-diabetic [9], antimicrobial [10], anti-inflammatory [11], antioxidant [12], antibacterial [13], and antifungal [14]. The content of secondary metabolites influences these pharmacological effects. The extraction method and the solvent type used determine the profile of extracted plant metabolites [15]. The polarity of the solvent affects the nature of the extracted metabolites because different bioactive metabolites with varying characteristics of chemicals and polarities may not dissolve in certain solvents [12]. Thus, this study aims to evaluate the activity effectiveness of cherry leaf extract using solvents with different polarities. The estimated activity was antioxidant and antibacterial capacity, which correlated with the total phenolic and flavonoid content.

2 Material and Method

2.1 Material

The materials used were ethanol, ethyl acetate, hexane, acetic acid, Folin-Ciocalteu reagent, sodium carbonate, gallic acid, aluminium chloride, potassium acetate, quercetin, 2,2'-diphenyl-1-picrylhydrazyl, phosphate buffer, trichloroacetic acid, potassium iron (III) cyanide, Muller Hinton agar (MHA), Triple Sugar agar (TSA). The tools used are glassware, visible light spectrophotometer, rotary vaporizer, hotplate, ovens, and autoclaves.

2.2 Method

2.2.1 Preparation of plant extracts

Cherry leaves were obtained from cherry plants around the Dramaga Campus of IPB in July 2022, and identified their toxicology at Herbarium Bogoriense. Kersen leaves were then dried using the oven for 48 h at 45 °C. The leaves were crushed to a size of 80 mesh using a disk mill.

2.2.2 Extraction method

The extraction process was based on Buhian et al. (2016) [16] with some modifications. Samples weighing 30 g were immersed in a 1:10 (w/v) mixture of ethanol, ethyl acetate, and hexane for 72 h before being filtered every 24 h. A rotary evaporator was used to concentrate the filtrate.

2.2.3 Determination of total phenolic content

Each extract's total phenolic content (TFC) was determined using a modified Folin-Ciocalteu method (Hossain et al., 2021). The 0.5 mL of the extract was added with 2.5 mL of Folin Ciocalteu reagent and then incubated for 20 min. The sample solution set is added with 2 mL of sodium carbonate solution 7.5%. The sample's absorption was measured using

a UV-Vis spectrophotometer at a wavelength of 725 nm. Gallic acid standard solutions with concentrations of 5, 10, 15, 20, 25, 30, and 50 ppm were treated the same as the samples. The TFC content of each extract was expressed as milligrams of gallic acid equivalent per gram dry (mg GAE/g dry sample).

2.2.4 Determination flavonoid content

The extract's flavonoid content (TPC) was determined using the aluminium chloride method. Total flavonoids were determined using the aluminium chloride by the method of Buhian et al. [16]. One milli liter of extract solution of 250 ppm is mixed with 3 mL of ethanol, 0.2 mL of aluminium chloride 10%, 0.2 mL of 1 M potassium acetate, and 5.6 mL of distilled water. The mixture was incubated at room temperature for 30 min and absorbed using a UV-Vis Spectrophotometer at a wavelength of 420 nm. Quercetin standard solutions of 5, 10, 25, 50, 75, and 100 ppm were treated the same as the samples [10]. Total flavonoids were calculated in milligrams of quercetin equivalent (QE) gram of dry powder (mg QE/g dry powder).

2.2.5 Determination of antioxidant capacity by DPPH method

The extract's antioxidant capacity was evaluated using a modified Blois 1956 technique [17]. 1 mL of 2,2'-diphenyl-1-picrylhydrazyl (DPPH) 0.075% was mixed with 2 mL of the extract. The mixture was incubated at room temperature in the dark for 30 min. The sample's absorbance was measured at 517 nm. The negative control was ethanol, while the positive control was ascorbic acid, which was generated in the following concentrations: 0.05, 0.1, 0.5, 1, 3, and 5 ppm. Antioxidant capacity is measured in millimoles of ascorbic acid on a gram of dry powder (mmol GAE/g dry powder).

2.2.6 Determination of antioxidant capacity by FRAP method

Antioxidant capacity was measured based on the ability of the sample to reduce $\text{Fe}^{3+}(\text{CN})_6$ to $\text{Fe}^{2+}(\text{CN})_6$ using a modified Oyaizu method (1986) [18]. 1 mL of the extract in methanol was mixed with 2 mL of sodium phosphate buffer (0.2 M, pH 6.6) and 2 mL of potassium ferricyanide [$\text{K}_3\text{Fe}(\text{CN})_6$] 1%. The mixture was incubated at 50 °C for 20 min. After incubation, the mixture was acidified with 2 mL of trichloroacetic acid 10%. Two mL of distilled water and 1 mL of FeCl_3 (0.1%) were added to the acidified mixture. Absorbance was measured at 700 nm. The calibration curve uses ascorbic acid with a concentration of quercetin at six concentrations of 0, 5, 10, 15, 20, 25, and 30 ppm. The antioxidant capacity is expressed in μmol ascorbic acid/g dry powder.

2.2.7 Determination of antibacterial activity

Antibacterial activity using the disc diffusion method refers to Buhian et al. research (16). Gram-positive bacteria (*Staphylococcus aureus*) and gram-negative bacteria (*Escherichia coli*) were cultured using TSA agar medium at 37 °C for 24 h and cultured into a test tube containing 5 mL of NaCl. The turbidity in the test colony suspension was standardized to McFarland standard (approx. 1.5×10^8 CFU/mL). MHA media was made by weighing the MHA as needed and dissolving it in an Erlenmeyer flask with distilled water until a volume of 500 mL was obtained, then heating until homogenous. The media is sterilized in an autoclave at 121°C for 15 min before being placed onto a 15 mL petri dish and allowed to harden. The test suspension was flattened and inoculated with a hockey stick

on 0.1 mL of MHA medium while being allowed to dry. Paper discs (0.5 in diameter) were soaked in the test solution (leaf extract) for 15 min at each concentration, then aseptically removed with sterilized tweezers and placed aseptically on the plate containing the test bacteria. Three repetitions were performed, with sterile distilled water as a negative control and chloramphenicol as a positive control. The press was incubated at 37 °C for 24 h. Data examination The data analysis displayed is the average standard deviation of three repetitions. Data were statistically analyzed using one-way analysis of variance (ANOVA) with a 95% confidence level (p0.05) and Duncan's test. A 95% confidence level (p0.05) indicated a significant disagreement. Graphpad Prisma 9 examined the relationship between total flavonoid concentration and antioxidant and antibacterial activities.

3 Results and discussion

3.1 Leaf Extraction

Pre-extraction treatment, including drying and milling, is essential for optimizing the extraction of bioactive components. The smoothing procedure requires increasing the sample's surface area, which interacts with the solvent [15]. The dry weight of the sample is used to determine its content throughout the drying process. Cherry leaves dried in an oven at 45 °C for 48 h had a water content of 5.93%. Earlier research found no significant changes in total phenolic content or the ability of extracts to trap radicals using air drying, freeze drying, or oven drying [12] but had a significant effect on antioxidant capacity and total polyphenol content [19].

The extraction method and solvent used in the extraction process affect the extracted primary and secondary metabolites. Their polarity influences the ability of a solvent to extract bioactive components contained in plants and is accountable for their bioactivity [20]. Kersen leaves were macerated in organic solvents of various polarities for 72 h, including ethanol, ethyl acetate, and n-hexane. Differences in solvent polarity resulted in substantially different yield percentages (Table 1). Compared to the other two solvents, ethanol produced the highest yield per cent. The ethanol solvent is a polar solvent that dissolves polar molecules such as polysaccharides, phenols, aldehydes, ketones, amines, and other oxygen-containing compounds that dissolve in water due to the creation of hydrogen bonds. As a result, ethanol solvent yields are higher with these compounds than ethyl acetate and n-hexane [20]. Samples were repeated three times, and a-c showed different standard deviations based on ANOVA analysis with Duncan's further significant test.

Table 1. The yield of cherry leaves on various polarities of the solution

Solvent	Yield percent*
Ethanol	32.97 ±2.33 ^a
Ethyl acetate	9.40±0.21 ^b
Hexane	6.09±0.07 ^c

3.2 Total Phenolics and Total Flavonoids

The total phenolic content was determined using Folin–Ciocâlțeu reagent (FCR) method with gallic acid as the standard. Figure 1 displays the standard curve for determining

total phenolic with line equation $y = 0.0079x + 0.0137$ and a coefficient of determination of 0.9964.

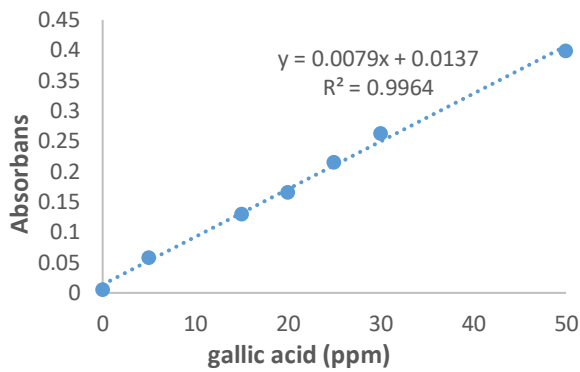


Fig. 1. Standard curve for gallic acid.

Total flavonoids were determined using the aluminium chloride method based on the reaction of the formation of the Al (III) complex with the carbonyl group at C4 and the hydroxyl groups at C3 and C5 (flavanols and flavones) [21]. The complex formed is measured using a visible spectrophotometer with a wavelength between 410 and 450 nm. Adding $AlCl_3$ to the quercetin standard shifts the maximal wavelength of quercetin from 367 nm to 427 nm (bathochromic effect). In this study, quercetin was reacted with aluminium chloride and potassium acetate before being measured at 420 nm [22]. Figure 2 displays the line equation $y = 0.0079x + 0.0137$ and the coefficient of determination produced by a standard quercetin solution.

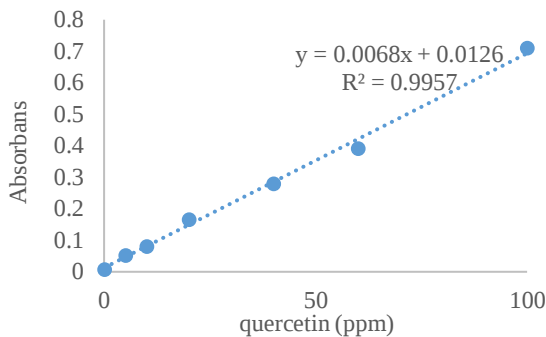


Fig. 2. Standard curve for quercetin

The results for TFC and TPC were substantially different depending on the polarity of the solvent (Table 2). Compared to ethyl acetate and hexane solvents, the ethanol extract showed highest TFC and TPC, 61.83 mg GAE/g dry sample (phenolic total) and 8.91 mg QE/g dry sample (total flavonoids). Flavonoids a part of the phenolic group, and based on experiments, the high phenolic content of cherry leaves is comparable with the flavonoids contain. The relationship between total phenolics and flavonoids is directly proportional in cherry leaves and stems [10]. Samples were repeated three times, and a-c showed different standard deviations based on ANOVA analysis with Duncan's further significant test.

Table 2. The yield of cherry leaves on various polarities of the solution

Solvent	TFC (mg GAE/g dry sample)	TPC (mg QE/g dry sample).
Ethanol	61.81 ± 3.13c	8.91 ±0.89a
Ethyl acetate	10.08 ± 0.23b	4.94 ± 0.22c
Hexane	3.85 ± 0.19a	2.37 ± 0.09b

3.3 Antioxidant capacity

The antioxidant capacity of cherry leaves was analyzed using the Ferric Reducing Antioxidant Power (FRAP) and the diphenyl picrylhydrazyl (DPPH) method. The antioxidant capacity of the FRAP method is proportional to the ability of the sample to reduce Fe³⁺ to Fe²⁺. At low pH, in the presence of TPTZ (Sigma Aldrich, India), the ferric-tripyridyl triazine (Fe³⁺-TPTZ) complex is reduced to iron (Fe²⁺-TPTZ), forming an intense blue solution with a maximum absorption of 593 nm [18]. Reducing power is based on the plant's ability to reduce the Fe (III)-TPTZ complex or 2,4,6-tripyridyl-s-triazine to produce Fe (II)-TPTZ [23]. The PRAF method used ascorbic acid as a standard curve with the equation 0.0041x + 0.5137 with a coefficient of determination of 0.9594 (Figure 3).

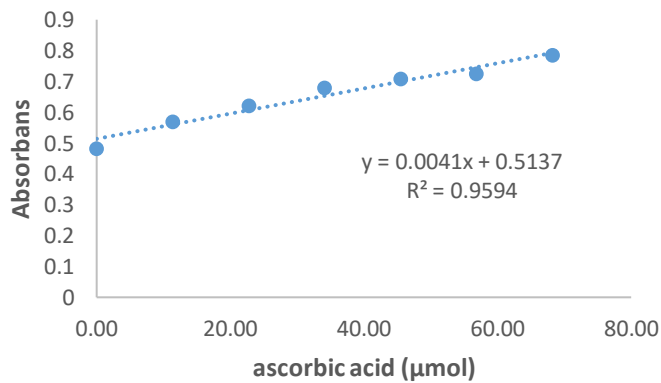


Fig. 3. The FRAP method's ascorbic acid standard curve.

The DPPH method for measuring antioxidant levels is based on the transfer of electrons and hydrogen from antioxidant molecules to DPPH. The radical DPPH has an intense purple hue. If the radical accepts electrons or hydrogen from an antioxidant molecule and produces a stable DPPH molecule, its maximal wavelength is approximately 515 nm, and its colour will change to yellow. Figure 4 depicts the ascorbic acid standard used in this method, with the line equation y=0.014x+0.4322 and a coefficient of determination of 0.986.

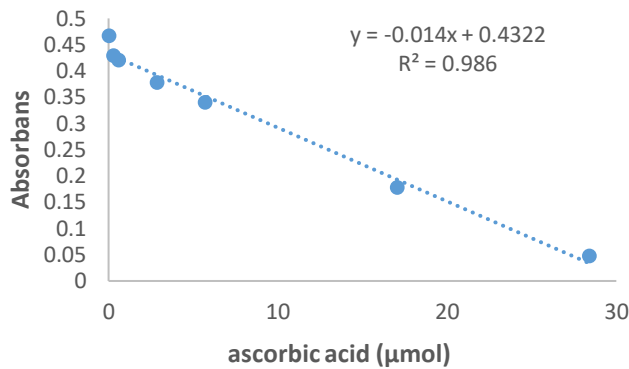


Fig. 4. The DPPH method's ascorbic acid standard curve.

The antioxidant capacity obtained by the two methods differs due to the two approaches varied underlying concepts. nevertheless, the two methods show that ethanol solvent has hight capacity than other solvents. Hexane solvent had the least amount of action (Table 3). This capacity is proportional to the phenolic and flavonoid concentration of the extract. The phenolic and flavonoid content of secondary metabolites is often favourably linked with activity [24].

Table 3. The Antioxidant capacity of cherry leaves on various polarities of the solution

Solvent	Antioxidant capacity (μmolAAE/g dry sample)l	
	FRAP	DPPH
Ethanol	61.81 ± 3.13c	8.91 ±0.89a
Ethyl acetate	10.08 ± 0.23b	4.94 ± 0.22c
Hexane	3.85 ± 0.19a	2.37 ± 0.09b

The potential correlation between the total phenolic content and cherry extract's antioxidant activity was examined using Pearson's coefficient (Figure 5). The findings demonstrated a statistically significant and positive association ($r = 0.85$) between the antioxidant activity measured by the FRAP technique and the total phenolic content of the ethanol extract. The impact of total phenolic content on antioxidant activity is consistent with findings reported in prior research. Polyphenols, commonly associated with antibacterial properties, generate extensive secondary metabolites through biosynthetic processes originating from primary metabolites. Flavonoids and other secondary metabolites formed from phenylpropanoids are known to collect within the vacuoles of epidermal cells in plants. This accumulation protects plants against various biotic and abiotic challenges while conferring immunity against diverse pathogens. Additionally, these compounds exhibit potent antimicrobial properties and function as immunological enhancers.

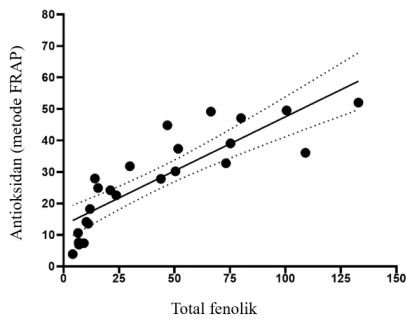


Fig. 5. The DPPH method's ascorbic acid standard curve.

The antibacterial efficacy of *Escherichia coli* and *Staphylococcus aureus* bacteria was assessed using antibacterial testing. The extent of antibacterial activity was determined by measuring the diameter of the clear zone inhibition. The measurement of the inhibition zone's diameter can be determined by observing the transparent region surrounding the paper disc. The extent of bacterial growth inhibition is directly proportional to the width of the clean zone. The figures for the diameter of the inhibitory zone can be observed in Table 4.

Table 4. Clear zone diameter of cherry leaves on various polarities of the solution

Solvent	Clear zone diameter (mm)	
	<i>Staphylococcus aureus</i>	<i>Escherichia coli</i>
Ethanol	7	6.5
Ethyl acetate	8.7	7.2
Hexane	9.5	7.5
Negative control	0	0
Positive control	13.3	15.8

As a positive control, chloramphenicol exhibited a clear zone diameter of 13.3 mm (*Staphylococcus aureus*) and 15.8 mm (*Escherichia coli*). The samples' antibacterial activity against *Staphylococcus aureus* and *Escherichia coli* was 7 mm and 6.5 mm (ethanol), 8.7 mm and 7.2 mm (ethyl acetate), and 9.5 cm and 7.5 cm (hexane). The antibacterial activity of hexane solvent was higher than that of other solvents, but the clear zone activity was still under the positive control of chloramphenicol. Activity variations in contrast to prior research, ethanol activity demonstrates bacterial activity against *Escherichia coli* cherry leaves [16]. According to the findings, the phenolic and flavonoid content of cherry leaves did not correlate with antibacterial activity.

4 CONCLUSION

The polarity of the solvent used in extracting cherry leaves resulted in considerably varied metabolite profiles and bioactivity in cherry leaf extract. When compared to ethyl acetate and hexane solvents, ethanol showed a greater percentage yield, total phenolics, total flavonoids, and antioxidant activity. The results of antioxidant testing utilizing the DPPH technique in ethyl acetate and hexane solvents were similar. When comparing the FRAP and DPPH methods for determining antioxidant activity, the DPPH approach has a value positively linked with higher antioxidant activity. However, it is inversely proportional to the precision value. Precision is higher when using the FRAP approach than the DPPH method.

The Pearson analysis, which returns a value of $r = 0.85$, demonstrates a positive connection between antioxidant activity and total phenolics. However, the association between antibacterial activity and total phenolic content could not be determined. As a result, the authors believe that further research into the association between cherry leaf secondary metabolites and antibacterial action is required. According to the findings, cherry leaves' phenolic and flavonoid content did not correlate with antibacterial activity.

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