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Original Article

Analysis of Solvent Concentration Effect and Extraction Method on The Total Phenolic of *Syzygium myrtifolium* Walp. Leaf Extract

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ABSTRACT

Background: *Syzygium myrtifolium* Walp., an Indonesian ornamental plant, contains phenolic compounds with antioxidant, anti-inflammatory, and antibacterial activities. The demand for natural antioxidants is rising due to concerns over synthetic alternatives. **Objectivity:** To determine the total phenolic content (TPC) and antioxidant activity of *S. myrtifolium* leaf extracts obtained with various solvents (ethanol 60, 70, 80, 96% v/v, ethyl acetate, n-hexane) and two extraction methods (maceration and ultrasonic-assisted extraction, UAE), and to assess the effects of solvent polarity and extraction method. **Methods:** Leaves were extracted by maceration and UAE. TPC was measured via the Folin–Ciocalteu method (tannic acid equivalents, %) using UV–Vis spectrophotometry. Antioxidant activity was evaluated using the DPPH assay (IC_{50}). Phytochemical screening was also performed. **Results:** TPC (maceration): 60% ethanol = 18.76%, 96% ethanol = 13.64%, ethyl acetate = 8.33%, n-hexane = 0.83%. TPC (UAE): 60% ethanol = 20.02%, 96% ethanol = 14.04%, ethyl acetate = 10.13%, n-hexane = 0.92%. Solvent type/concentration and extraction method significantly affected TPC ($p < 0.05$). UAE 96% ethanol showed the strongest antioxidant activity ($IC_{50} \approx 16.03$ ppm), ethyl acetate had moderate activity (≈67–76 ppm), and n-hexane the weakest (≈153–163 ppm). UAE 60% ethanol had the highest TPC but not the strongest activity ($IC_{50} \approx 88.27$ ppm), suggesting antioxidant potency depends on phenolic composition. **Conclusion:** UAE with 60% ethanol produced the highest TPC, while UAE with 96% ethanol exhibited the strongest antioxidant activity. Further profiling is needed to clarify the phenolic composition–activity relationship.

Keywords: Syzigium myrtifolium walp, phenolic, solvent concentration, UAE, Maceration

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INTRODUCTION

Indonesia, recognized as one of the world's megabiodiversity countries, possesses a vast array of biological resources comprising thousands of species and numerous local varieties, which represent valuable assets for the conservation and sustainable utilization of natural resources[1]. Indonesia ranks among the top countries in the world in terms of biodiversity richness, even achieving the second position in global biodiversity rankings[2]. With approximately 40,000 endemic species, around 6,000 of which are classified as medicinal plants[3]. An example is *Syzygium myrtifolium* Walp. (commonly referred to as red shoot), a species belonging to the Myrtaceae family, distinguished by its phenotypic characteristic of reddish young leaves that gradually turn green as they mature[4]. In Indonesia, the utilization of *Syzygium myrtifolium* Walp. remains suboptimal, as the plant is more commonly used as an ornamental rather than as a medicinal resource, despite its rich content of bioactive compounds with potential pharmacological activities[5]. According to Suryati[6], the ethyl acetate fraction of the ethanolic extract from *Syzygium myrtifolium* Walp. leaves contains secondary metabolites such as phenolics, triterpenoids, steroids, flavonoids, alkaloids, and coumarins. Phenolic compounds exhibit diverse bioactivities, including anti-inflammatory, antidiabetic, immunomodulatory, anticancer, antimicrobial, and antioxidant effects[7].

Phenolic compounds are well known for their strong antioxidant activity, enabling them to protect body cells from damage caused by free radicals a major contributing factor to degenerative diseases such as cancer, cardiovascular disorders, and neurodegenerative conditions[8,10]. Antioxidant compounds can prevent oxidation and play a crucial role in mitigating the harmful effects of free radicals associated with cardiovascular, cancer, and degenerative diseases[9,11]. They exert this activity by abstracting an electron or a hydrogen atom, thereby converting phenol molecules into relatively stable phenoxyl radicals and terminating oxidative chain reactions through either electron transfer or hydrogen atom transfer mechanisms[12]. In a study using maceration with ethanol, the red shoot leaf extract exhibited a remarkably strong antioxidant activity with an IC_{50} value of 2.195 ppm and a total phenolic content of 371.833 mg GAE/g extract[13]. Phenolic compounds, which are polar in nature, are effectively extracted using polar solvents such as ethanol especially in ethanol-water mixtures as these solvents enhance the solubility of phenolics, resulting in higher total phenolic content (TPC) compared to extracts obtained with non-polar solvents like hexane[14]. Conversely, the use of pure water as a solvent results in the lowest recovery of phenolic compounds due to its limited ability to solubilize certain types of phenolics[15].

The process of isolating specific compounds from a sample is referred to as extraction[16]. Extraction represents a critical initial step in the separation of phenolic compounds from plant materials. This process involves the penetration of a solvent into the cellular matrix, dissolution of phenolic compounds, and their subsequent diffusion into a liquid phase

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separated from the solid residue[16]. The efficiency and stability of the extracted bioactive compounds are significantly influenced by both the extraction method and the solvent used, as these factors determine the yield and integrity of the target compounds[17]. Extraction is considered effective when the compound of interest is readily soluble in the solvent, following the principle of 'like dissolves like', which emphasizes the similarity in polarity between the solute and the solvent[18]. Therefore, solvent selection plays a crucial role in determining the final outcome of extraction, including the yield and total phenolic content of the resulting extract. This study employed three solvents with varying polarities: ethanol (60%, 70%, 80%, and 96%), ethyl acetate, and n-hexane.

The choice of solvent significantly influences the extraction capacity of bioactive compounds from plants. Highly polar solvents (such as ethanol, methanol, and aqueous-organic mixtures) are generally more efficient in extracting phenolic compounds than non-polar solvents[19]. This study is consistent with Syukur, who reported a total phenolic content of 106.45 mg GAE/g in the ethanol extract of *Illicium verum*, compared to 52.30 mg GAE/g in the n-hexane extract[20]. Solvents significantly influence the effectiveness of active compound extraction from *Murraya koenigii* leaves[20]. In a maceration study using 95% ethanol, 96% methanol, and 99% acetone, the ethanolic extract exhibited the highest total phenolic content compared to the other solvents, and also contained flavonoids, terpenoids, saponins, and glycosides[21]. Similarly, methanol extraction yielded the highest total phenolic content, approximately 27.2 mg GAE/g dry weight, along with DPPH antioxidant activity reaching up to 93%, demonstrating that solvent polarity strongly influences the efficiency of phenolic compound extraction[22].

This study corroborates the findings of Handayani[23], who demonstrated that the antioxidant activity of Nothopanax fruticosum leaf extracts increased with solvent polarity: the n-hexane extract showed an IC_{50} of 33.839 µg/mL, the ethyl acetate extract an IC_{50} of 12.604 µg/mL, and the 96 % ethanol extract the lowest IC_{50} of 2.222 µg/mL, indicating markedly stronger DPPH-radical scavenging as ethanol concentration rose. The use of various solvents in extraction not only affects the yield and phenolic content but also results in varying antioxidant activity depending on the plant material[24].

Different extraction methods can also lead to variations in the levels of compounds[25]. In this study, two extraction methods will be compared: conventional and modern techniques. Maceration is selected as the conventional method due to its practicality compared to other techniques. Recent reviews by BMC Chemistry[26] emphasize that "the choice of extraction method and solvent critically determines the efficiency of phytochemical recovery from plant matrices, influencing compound yield, selectivity, and extract quality". For instance, the use of Ultrasound-Assisted Extraction (UAE) with methanol yielded total phenolic content approximately twice as high (~49.14 mg GAE/g dry weight) compared to thermal extraction using isopropanol (~24.76 mg GAE/g dry weight), due to the ultrasonic cavitation effect enhancing mass transfer and solvent penetration[27].

In this study, the Ultrasound-Assisted Extraction (UAE) method was chosen due to its high efficiency, short extraction time, and low solvent consumption, all of which contribute to a reduced environmental impact[28]. The cavitation mechanism generated by ultrasound enhances mass transfer and disrupts plant cell walls, leading to higher total phenolic content and antioxidant activity compared to conventional methods such as maceration and Soxhlet extraction, and also resulting in lower IC_{50} values (~120 µg/mL versus ~150-204 µg/mL for maceration/Soxhlet)[29]. In a study Disca[30], both Ultrasound-Assisted Extraction (UAE) and Microwave-Assisted Extraction (MAE) applied to cocoa bean shells yielded higher total phenolic content and antioxidant activity than traditional methods like Soxhlet extraction or magnetic stirring. A combined UAE–MAE process conducted at 90 °C produced the highest radical scavenging activity and TPC values[30].

Based on this literature review, the present study is designed to comprehensively evaluate the effect of extraction methods maceration and Ultrasound-Assisted Extraction (UAE) along with solvent variables, including ethanol concentration (60%, 70%, 80%, and 96%) and solvent type (ethanol, ethyl acetate, and n-hexane), on the phenolic content and antioxidant activity (via the DPPH method) of *Syzygium myrtifolium* Walp. leaf extracts. By integrating both approaches, the primary objective is to determine the optimal extraction conditions that maximize total phenolic content while achieving the highest antioxidant potential.

OBJECTIVE

Based on the description above, research will be conducted to determine the total phenolic content of Syzygium myrtifolium Walp. leaf extracts prepared with ethanol at 60, 70, 80, and 96% via maceration and ultrasonic-assisted extraction methods, and to quantify these compounds using the Folin-Ciocalteu colorimetric assay followed by UV–Vis spectrophotometric analysis.

MATERIALS AND METHODS

This study was conducted from April to June 2024 in the Pharmacy Laboratory, Faculty of Mathematics and Natural Sciences, University of Pakuan, Bogor. The materials used included Syzygium myrtifolium Walp. leaf crude drug, technical 96% ethanol (Emsure®), ethyl acetate (Emsure®), n-hexane (Emsure®), distilled water (Rofa®), gallic acid (Sigma®) and tannic acid, Folin–Ciocalteu reagent (Merck®), 20% sodium carbonate solution (Pudak Scientific®), DPPH powder (Aldrich®), 2 N sulfuric acid (Emsure®), ascorbic acid (Emsure®), ferric chloride ($FeCl_3$), gelatin and zinc powder (Nitra Kimia®), sodium chloride (Emsure®), magnesium powder, Mayer's reagent, Dragendorff's reagent, and Bouchardat's reagent. The equipment employed comprised an analytical balance (Lab PRO®), rotary evaporator (IKA®), UV–Visible spectrophotometer (Jasco V®), alcoholmeter, maceration vessels, ultrasonic-assisted extraction unit (Branson®), oven (Samsung®), muffle furnace (Daihan Scientific®), blender (Cosmos®), micropipettes and volumetric pipettes (Accucare®/Pyrex®), graduated cylinders and beakers (Pyrex®), evaporation dishes and porcelain crucibles, desiccator (Normax®), and a 40-mesh sieve (ABM®).

Sample collection

The plant used in this study comprised all parts of the *Syzygium myrtifolium* Walp. They were collected from the Merdesa Botanical Garden, Bogor regency, West Java, Indonesia (lat -6.5600°, long 106.7255°), and their identities were verified at Herbarium Depokensis, University of Indonesia (UIDEP), Depok, Indonesia.

Extraction compounds

Maceration and ultrasound-assisted extraction (UAE) were each performed in duplicate using n-hexane, ethyl acetate, and ethanol (60, 70, 80, and 96%) at a 1:10 material solvent ratio. For maceration, 100 g of Syzygium myrtifolium Walp. leaf powder was soaked in 450 mL solvent at room temperature with stirring every 6 hours over 18 hours; the residue was then re-macerated sequentially with 300 mL and 250 mL of the same solvent, and combined filtrates were concentrated by rotary evaporation and water-bath to yield a viscous extract[31]. For UAE, the same powder (100 g) was mixed with 450 mL solvent in a sealed beaker, sonicated at 45 °C and 40 kHz for 20 minutes, rested for 30 minutes, then subjected to two further sonication cycles with 300 mL and 250 mL solvent; all filtrates were likewise concentrated to obtain the extract Finally, the crude extracts were evaluated organoleptically for aroma, form, and color[32].

Determination of Moisture Content

Moisture content was determined gravimetrically. Evaporation dishes were pre-tared by drying at 105 °C for 10 minutes. Precisely 2 g of powdered crude drug and 2 g of viscous red-shoot leaf extract were placed in the tared dishes and dried in an oven at 105 °C until a constant weight was achieved[31].

Determination of Ash Content

Approximately 2 g of powdered plant crude drug was accurately weighed and transferred into a preheated, tared silica crucible to ensure even distribution. The sample was then gradually heated in a muffle furnace at 600 °C, after which it was cooled and reweighed[31]. Ash content was calculated as:

$$Ash\ Content\ (\%) = \frac{(weight\ of\ crucible + ash) - (weight\ of\ empty\ crucible)}{weight\ of\ sample} x\ 100\%$$

Determination of Total Phenolic Content in the Samples

- **Preparation of 100ppm gallic Acid Stock Solution**

Fifty milligrams of gallic acid were dissolved in distilled water and brought to a final volume of 50 mL to yield a 1000 ppm stock solution. Then, 1 mL of this 1000 ppm stock was pipetted into a 10 mL volumetric flask and diluted to the mark with distilled water to obtain a 100 ppm working stock[33].

- **Preparation of 20% Na_2CO_3 Solution**

Twenty grams of sodium carbonate (Na_2CO_3) were dissolved in distilled water and gently heated until fully dissolved. The solution was allowed to stand at room temperature for 24 hours, then filtered and diluted with distilled water to a final volume of 100 mL[33].

- **Determination of Maximum Wavelenght**

0.6 mL of the 100 ppm gallic acid working solution (equivalent to 6 ppm) was transferred into a 10 mL volumetric flask. Then, 750 µL of Folin–Ciocalteu reagent and 7.5 mL of distilled water were added, the mixture was shaken and allowed to react for 5 minutes. Next, 750 µL of the 20% Na_2CO_3 solution was added, and the flask was filled to the mark with distilled water. After shaking for 30 seconds, the absorbance of the resulting solution was measured over the 400–800 nm range to identify the wavelength of maximum absorption[33].

- **Determination of Optimum Incubation Time**

A total of 0.6 mL of gallic acid solution (from the 100 ppm stock, equivalent to 6 ppm) was transferred into a 10 mL volumetric flask. Then, 750 µL of Folin–Ciocalteu reagent and 7.5 mL of distilled water were added, the mixture was shaken and allowed to stand for 5 minutes. Next, 750 µL of 20% Na_2CO_3 solution was added and the volume was adjusted to the mark with distilled water. The solution was shaken for at least 30 seconds, and its absorbance was measured at the determined maximum wavelength at 5-minute intervals over a time range of 0 to 60 minutes[33].

- **Determination of Gallic Acid Standard Curve**

The gallic acid standard curve was prepared by pipetting aliquots of the 100 ppm gallic acid stock solution into 10 mL volumetric flasks to obtain final concentrations of 2, 4, 6, 8, and 10 ppm. To each flask, 750 µL of Folin–Ciocalteu reagent and 7.5 mL of distilled water were added, the mixture was shaken and left to stand for 5 minutes. Then, 750 µL of 20% Na_2CO_3 solution was added, and the volume was adjusted to the mark with distilled water. After shaking for at least 30 seconds, the absorbance of each solution was measured at the previously determined maximum wavelength. The absorbance values were used to calculate the linear regression equation[33].

- **Determination of Total Phenolic Content**

For total phenolic content determination, 0.5 g of sample was accurately weighed and dissolved in absolute ethanol to a final volume of 50 mL. A 500 µL aliquot of this solution was transferred into a 10 mL volumetric flask, followed by the addition of 750 µL Folin–Ciocalteu reagent and 7.5 mL distilled water. The mixture was shaken and left to stand for 5 minutes. Then, 750 µL of 20% Na_2CO_3 solution was added, and the volume was adjusted with distilled water to the mark. After shaking for at least 30 seconds, the absorbance was measured at the maximum wavelength. The determination was carried out in quadruplicate[33].

- **Calculation of Total Phenolic Content**

Data analysis was performed using linear regression and correlation to construct the calibration curve of gallic acid. The absorbance values of the standard solutions were plotted against their concentrations to generate a standard curve. From this curve, the linear regression equation was obtained with the following form:

$$y = bx \pm a$$

Where:

- y = absorbance
- b = slope
- x = concentration
- a = intercept

This equation was then used to determine the total phenolic concentration in the sample. A valid calibration curve must meet the requirements of a correlation coefficient $(r) \geq 0.99$ and a coefficient of determination $(R^2) \geq 0.990$, indicating a strong linear relationship.

The formula to calculate the phenolic concentration is:

$$x = \frac{y - a}{b}$$

The total phenolic content of *Syzygium myrtifolium* Walp. leaves was then calculated using the following formula:

$$Phenolic\ Content\ (\%) = \frac{C(ppm)x\ Volume\ (mL)x\ fp\ x\ 10^{-6}}{Sample\ Weight\ (g)} x\ 100\%$$

- **Determination of DPPH Maximum Wavelength**

A total of 1 mL of DPPH solution was pipetted into a 10 mL volumetric flask wrapped in aluminum foil, then diluted to the mark with absolute methanol. The absorbance was measured using a UV-Vis spectrophotometer in the wavelength range of 450-550 nm[34].

- **Determination of Optimum Incubation Time**

A total of 1 mL of vitamin C solution (100 ppm) was pipetted into a 10 mL volumetric flask wrapped in aluminum foil, followed by the addition of 1 mL of 1 mM DPPH solution. The mixture was then diluted to the mark with absolute methanol. Absorbance was measured at the previously determined maximum wavelength at intervals of 10, 20, 30, 40, 50, and 60 minutes to determine the optimal and stable incubation time[35].

- **Preparation of Vitamin C Standard Solutions**

A series of vitamin C standard solutions with concentrations of 2, 4, 6, 8, and 10 ppm were prepared from a 100 ppm stock solution. Each was transferred into a 10 mL volumetric flask wrapped in aluminum foil, followed by the addition of 1 mL of DPPH solution and dilution to the mark with methanol. The solutions were incubated for the determined optimal time, and their absorbance was measured using a UV-Vis spectrophotometer at the maximum wavelength[35].

- **Preparation of Test Solution Variations**

A total of 50 mg of red shoot (*Syzygium myrtifolium* Walp.) leaf extract from each solvent was accurately weighed and dissolved in methanol in a 50 mL volumetric flask wrapped in aluminum foil. From this solution, 10 mL was pipetted into a 100 mL volumetric flask and diluted to the mark with methanol. Serial dilutions of the test solution were then prepared at concentrations of 5, 10, 15, 20, and 25 ppm by pipetting 0.5, 1.0, 1.5, 2.0, and 2.5 mL, respectively, into separate 10 mL volumetric flasks. To each flask, 1 mL of 1 mM DPPH solution was added, followed by methanol to the mark. The mixtures were homogenized and incubated at room temperature for 25 minutes. Absorbance was then measured using a UV-Vis spectrophotometer at the previously determined maximum wavelength[35].

- **Antioxidant Activity Test Using the DPPH Method**

The antioxidant activity using the DPPH method was evaluated by measuring the absorbance of the sample solutions, positive control (vitamin C), and blank at the maximum wavelength using a UV–Vis spectrophotometer. The percentage of radical inhibition was calculated using the following formula:

$$\%Inhibition = \frac{\boldsymbol{A}blank - \boldsymbol{A}sample}{\boldsymbol{A}blank} x\ 100\%$$

The resulting inhibition percentages were then plotted against the sample concentrations (ppm) with concentration on the x-axis and % inhibition on the y-axis. The data were analyzed using linear regression with the equation:

$$Y = a + bx$$

where a is the intercept, b is the slope, and the value of x at $y = 50$ corresponds to the IC_{50}, the concentration required to inhibit 50% of the DPPH radicals. This approach provides a rapid and reagent-efficient method for quantitatively assessing antioxidant potential, with IC_{50} serving as a critical parameter for evaluating the effectiveness of the tested compounds[36].

RESULTS

Result of Simplicia Preparation from Syzygium myrtifolium Walp. Leaves

The plant determination conducted at the Herbarium Depokensis, Universitas Indonesia (UIDEP), confirmed that the sample used in this study was *Syzygium myrtifolium* Walp., a member of the Myrtaceae family. After the wet sorting process, a total of 5500 grams of fresh *Syzygium myrtifolium* Walp. leaves were obtained. From this, 1715 grams of dried leaf simplicia powder were produced, resulting in a yield of 31.18%. This indicates that from every 100 grams of fresh *Syzygium myrtifolium* Walp. leaves processed, approximately 31.18 grams of simplicia powder were obtained.

The yield achieved in this study is higher than that reported by Syafriana & Wiranti[37], who obtained a simplicia yield of 27%. The difference is presumed to be due to the use of both red and green leaves in this research, whereas by Syafriana & Wiranti,[37] used only red leaves and this is aligns with their findings which showed that the drying of green leaves resulted in a higher powder yield (29%) compared to red leaves (27%)[37]. This may be attributed to the higher water content found in younger leaves, as their cells are still metabolically active and rich in water.

Result of Syzygium myrtifolium Walp. Leaf Extract Preparation

The extract of *Syzygium myrtifolium* Walp. leaves was prepared using 100 grams of powdered simplicia and 1000 mL of various solvents, including n-hexane, ethyl acetate, and ethanol at concentrations of 60%, 70%, 80%, and 96%, using both maceration and ultrasound-assisted extraction (UAE) methods. During the maceration process, periodic stirring was carried out to enhance the interaction between the powdered simplicia and the solvent, thereby improving the dissolution of bioactive compounds into the solvent medium[38]. The liquid extract resulting from the maceration of *Moringa oleifera* leaves was evaporated using a rotary evaporator at a water bath temperature of 60 °C to obtain a dry concentrate, with this controlled temperature preserving the stability of phenolic, flavonoid, and tannin compounds without degradation[39].

Organoleptic evaluation showed that the thick extract of *Syzygium myrtifolium Walp*. leaves obtained through both maceration and UAE methods had a dark brown color and a characteristic odor. These results were consistent with those reported by Cassiana[40]. The average extract yields (%) from each treatment are presented in Table 1. Based on the data, the extract yields from *Syzygium myrtifolium* Walp. leaves using maceration and UAE methods showed significant differences. The highest yield was obtained from maceration using 60% ethanol, while the lowest was with n-hexane.

Suryanita[41] reported a yield of 12.458% for the ethanolic extract (96%) of *Syzygium myrtifolium Walp*. leaves using maceration, while Imrawati[42] reported a 70% ethanolic extract yield of 18.24%. In contrast, the yield at the same ethanol concentration in this study reached 32.35%. According to Wakeel[43] ethanol extracts generally result in higher yields compared to those using n-hexane or ethyl acetate. This is because ethanol, being a polar solvent, is more effective at extracting a wide range of secondary metabolites, whereas n-hexane, a non-polar solvent, is less efficient for this purpose.

Furthermore, the yields of thick extracts using maceration were as follows: 34.95% with 60% ethanol, 30.95% with 80% ethanol, 28.05% with 96% ethanol, 23.37% with ethyl acetate, and only 1.96% with n-hexane, indicating the latter's very low extraction efficiency. Compared to previous studies[41], these results show better extraction efficiency, suggesting that the methods used in this study were more effective at extracting active compounds. Meanwhile, extract yields using the UAE method were 28.1% with 60% ethanol, 1.62% with n-hexane (the lowest), and 25.35% with 70% ethanol. These results also demonstrated better performance than previous studies, further indicating the effectiveness of the chosen extraction approach in isolating bioactive compounds.

Both maceration and UAE methods showed a decrease in yield with increasing ethanol concentration. This indicates that 96% ethanol is less efficient in extracting active components from *Syzygium myrtifolium* Walp. leaves compared to lower concentrations, in both maceration and UAE methods. Lohvina[44] reported that the lower the solvent concentration, the more optimal the extraction obtained. This

Table 1. Extraction Yields (%) of *Syzygium myrtifolium Walp*. Leaf Extracts

Extraction Method	Sample	Mean Yield (%) ± SD
Maceration	60% Ethanol Extract	34,95 ±1,3435
	70% Ethanol Extract	32,35 ±1,4849
	80% Ethanol Extract	30,95 ±1,7678
	96% Ethanol Extract	28,05 ±1,0607
	Ethyl Acetate Extract	23,37 ± 0,60
	n-Hexane Extract	1,96 ± 0,62
UAE	60% Ethanol Extract	28,1±1,5556
	70% Ethanol Extract	25,35 ±1,4849
	80% Ethanol Extract	24,8 ±1,2728
	96% Ethanol Extract	22,5 ±0,9899
	Ethyl Acetate Extract	21,35 ± 0,63
	n-Hexane Extract	1,62 ± 0,53

is evident in 50% ethanol, which yields higher levels of phenolics and flavonoids compared to 80% ethanol. The higher the polarity of the solvent, the better its extraction capability, as it can penetrate plant cell walls, cause the protoplasm to swell, and dissolve cellular components according to their solubility. The relationship between the polarity of the solvent and the extracted material affects the solvent's effectiveness in dissolving compounds[45].

Overall, the maceration technique consistently produced superior results compared to the UAE method across all tested solvent concentrations. This observation highlights the significant influence of extraction methods on yield, where higher yields correlate with greater amounts of extract[46]. This aligns with the research by Utami[32] who reported that conventional extraction methods, such as maceration, yield higher extracts compared to more modern techniques like ultrasound-assisted extraction (UAE).

The duration of the extraction process is a crucial factor that can affect the yield. Longer extraction times generally lead to increased yields until reaching an optimum point, after which the yield tends to decrease slightly[47]. In the maceration method, the powdered simplicia is soaked in solvent for three days, allowing for maximal diffusion of active components into the solvent. In contrast, although the UAE method can enhance solvent penetration through ultrasonic waves, the shorter extraction duration only 20 minutes results in suboptimal diffusion of active components.

Characterization Results of Crude Drug and Extracts *of Syzygium myrtifolium Walp*.

Determination of Moisture Content

Moisture content was determined gravimetrically as a non-specific standardization parameter. This procedure establishes the minimum permissible moisture level in the raw material. Excess moisture in the crude drug can promote microbial growth, induce chemical degradation of active constituents, and reduce sample shelf life. In extracts, moisture content critically influences overall stability, quality, and storage duration[31]. The mean moisture contents of the crude powder and the leaf extracts of *Syzygium myrtifolium Walp.* are presented in Table 2.

The crude powder exhibited the highest moisture content compared with all extracts obtained by maceration and ultrasound-assisted extraction (UAE). Nevertheless, its moisture level remained below the 10% maximum limit set by the Kemenkes RI (2017)[48] thereby ensuring preservation of quality and efficacy. Among ethanol extracts, 60% v/v ethanol yielded the highest moisture (both by maceration and UAE), whereas 96% v/v ethanol produced the lowest. Non-polar solvents such as n-hexane gave exceptionally low moisture contents (1.6718% for maceration and 1.7251% for UAE). These results indicate that increasing ethanol concentration reduces extract moisture content regardless of extraction method, and that the relatively high water content in the 60% ethanol extract accounts for its elevated moisture level compared to other solvent systems.

Table 2. Average Moisture Content of Samples

Extraction Method	Sample	Mean Moisture (%) ± SD	Quality Standard (%) Kemenkes RI, 2017[48]
	Powdered Crude Drug	6,7904 ± 0,2242	
Maceration	60% Ethanol Extract	6,0038 ± 0,0169	
	70% Ethanol Extract	5,8318 ± 0,0556	
	80% Ethanol Extract	5,5408 ± 0,5056	
	96% Ethanol Extract	5,0314 ± 0,1291	
	Ethyl Acetate Extract	3,3836 ± 0,1480	
	n-Hexane Extract	1,6718 ± 0,0108	≤ 10%
UAE	60% Ethanol Extract	5,8329 ± 0,1798	
	70% Ethanol Extract	5,7117 ± 0,1520	
	80% Ethanol Extract	5,4811 ± 0,3645	
	96% Ethanol Extract	4,9771 ± 0,3183	
	Ethyl Acetate Extract	3,6951 ± 0,0769	
	n-Hexane Extract	1,7251 ± 0,0501	

Comparing extraction techniques, UAE consistently produced extracts with lower moisture content than maceration at equivalent solvent concentrations. Utami[31] attributed this to cavitation effects during UAE, which enhance mass transfer efficiency without increasing water uptake. Li[49] further reported that ultrasonic waves yield more stable extracts, as lower moisture mitigates both active-constituent degradation and microbial proliferation.

Overall, both maceration and UAE generated moisture contents well below the 10% pharmacopeial threshold. Moreover, all extract values were lower than the 9.4% moisture reported *for S. myrtifolium* leaves by Isnaeni[50]. demonstrating the effectiveness of these extraction protocols in reducing water content. According to Golmakani[51]. Optimal moisture control is crucial for efficient extraction of phenolic compounds. Excess water can facilitate solubilization but may also accelerate degradation via enzymatic or chemical reactions. Spray-drying or similar pretreatment methods reducing moisture to around 5-7 % have been shown to raise phenolic recovery and antioxidant potential compared to samples with higher moisture (7.6-9.3 %) or overly dry residues (1–3 %).

Determination of Ash Content

Ash content testing is an important step in determining the optimal extraction method and solvent concentration for crude drugs[31]. Higher ash content indicates increased minerals and other inorganic substances, which can affect the quality and purity of the resulting extract[31]. The mean ash contents of the crude drug powder and the leaf extracts of *Syzygium myrtifolium Walp.* are shown in Table 3. Based on the data, the highest ash content was found in the crude drug powder compared to the extracts produced by both extraction methods. This is because the crude powder still contains all inorganic substances (minerals) present in the raw plant, whereas the extracts contain fewer inorganic substances due to most remaining in the crude powder during extraction. Both the ash content of the crude powder and the ethanol extracts of *Syzygium myrtifolium Walp.* comply with the maximum ash content requirement of not exceeding 10%. Kemenkes RI[48] Maintaining ash content below 10% aims to ensure that the quality and safety of the herbal material are preserved[52].

Based on solvent concentration, among both extraction methods (maceration and UAE), the highest ash content was found in the extracts prepared with 96% ethanol, while the lowest ash content was found in the extracts prepared with 60% ethanol. However, when compared with other solvents such as ethyl acetate and n-hexane, the ash content in the n-hexane extracts was much lower 1.7245% for maceration and 1.4992% for UAE. Highly polar solvents (96% ethanol) are more efficient at dissolving salts and minerals, and due to the "like dissolves like" principle, semi-polar/non-polar components trapped in lipid matrices, resins, or pigments are also extracted[53]. This increases the total ash content. Conversely, non-polar n-hexane extracts only small amounts of inorganic substances, yielding the lowest ash content but also producing relatively purer extracts free from mineral contaminants.

Therefore, the pattern of increasing ash content with rising solvent polarity reflects a balance between the efficiency of organic compound extraction and the co-extraction of inorganic components. This

Table 3. Average Ash Content of Samples

Extraction Method	Sample	Mean Ash Content (%) ± SD	Quality Standard (%) Kemenkes RI, 2017[48]
Maceration	Powdered Crude Drug	4,5350 ± 0,2172	≤ 10%
	60% Ethanol Extract	3,4396 ± 0,1159	
	70% Ethanol Extract	3,6029 ± 0,0973	
	80% Ethanol Extract	3,9861 ± 0,0646	
	96% Ethanol Extract	4,0849 ± 0,0367	
	Ethyl Acetate Extract	2,5422 ± 0,1165	
	n-Hexane Extract	1,7245 ± 0,0302	
UAE	60% Ethanol Extract	3,4792 ± 0,1010	
	70% Ethanol Extract	3,7227 ± 0,0319	
	80% Ethanol Extract	3,9564 ± 0,0803	
	96% Ethanol Extract	4,1674 ± 0,0775	
	Ethyl Acetate Extract	2,7159 ± 0,0582	
	n-Hexane Extract	1,4992 ± 0,2086	

study is consistent with Wilorianza[54]. who reported the highest ash content (9.5 % ± 0.21 % w/w) in Carica papaya seed extracts prepared with 70 % ethanol, compared to those obtained with 95 % ethanol (8.5 % ± 0.06 % w/w) using maceration. When comparing extraction methods, ultrasound-assisted extraction (UAE) tended to yield extracts with higher ash content than maceration at the same ethanol concentration, likely due to the enhanced efficiency of ultrasonic cavitation.

Isnaeni[55] reported an ash content of 4.5% for *Syzygium myrtifolium Walp.* leaf extracts substantially higher than values obtained using alternative extraction techniques. These discrepancies may arise from various factors, including plant species, environmental conditions, geographic origin, and post-harvest processing, all of which influence mineral composition[56]. Moreover, methodological differences in ash determination such as incineration temperature and duration can yield divergent results. According to Luo[57] low ash content indicates fewer inorganic or mineral constituents in the extract, implying a higher concentration of the desired bioactive compounds.

Phytochemical Screening Results

Phytochemical screening Phytochemical screening was carried out by observing visual color changes and precipitate formation after the addition of specific reagents. The main objective of this experiment was to identify the classes of secondary metabolites present in the samples. Tests were conducted on crude simplicia powder and all extracts of *Syzygium myrtifolium* Walp. prepared with different solvents, focusing on phenolics, tannins, flavonoids, alkaloids, and saponins. Both the crude powder and the ethanol and ethyl acetate extracts tested positive for all five classes of compounds. In addition, Syafriana[37] reported that the red shoot plant contains secondary metabolites such as alkaloids, flavonoids, tannins, triterpenoids, steroids, saponins, and phenolic compounds. However, in the present study, the n-hexane extract did not show the presence of flavonoids and saponins.

The development of a black coloration indicated the presence of phenolic compounds upon the addition of 1% $FeCl_3$. The black color arises from complex formation between Fe^{3+} ions and phenolic hydroxyl groups, in which Fe^{3+} binds to the free electron pairs on the oxygen atoms of the phenolic[58]. Tannins were identified in all samples by the formation of a white precipitate after adding 10% gelatin solution. Gelatin precipitates in the presence of tannins due to protein tannin coagulation[58]. Flavonoids were screened using magnesium turnings and concentrated HCl. A positive result was indicated by a red to orange hue of the test solution. Addition of HCl liberates hydrogen ions, and interaction with Mg generates nascent hydrogen; the resulting flavilium salts impart the characteristic red-orange coloration atoms[59].

Alkaloid detection employed three reagents: Mayer's, Dragendorff's, and Bouchardat's. All samples yielded a white precipitate with Mayer's reagent, an orange-brown precipitate with Dragendorff's reagent, and a brown precipitate with Bouchardat's reagent. The white precipitate with Mayer's reagent results from complexation of basic alkaloids with tetraiodomercurate(II) ions. Dragendorff's reagent produces an orange-brown precipitate through formation of tetraiodobismuthate(III) complexes. In contrast, Bouchardat's reagent forms a brown precipitate via covalent coordination between K^+ ions and alkaloid nitrogen atoms[59]. Saponins were confirmed by the presence of persistent frothing in each sample. Foam formation indicates the amphiphilic nature of saponins, which reduce surface tension in aqueous media and thereby stabilize bubbles[60]. These findings corroborate the work of Ahmad[61] who reported that both crude powder and ethanol extracts of *S. myrtifolium* contain phenolics, saponins, flavonoids, and alkaloids. They are also consistent with Astuti & Sari[62] who documented the presence of phenolics, flavonoids, tannins, and saponins in green leaf extracts of *S. myrtifolium* Walp.

Total Phenolic Content Determination

Total phenolic content (TPC) in the ethanol extracts of *Syzygium myrtifolium* Walp. leaves was quantified by UV-Vis spectrophotometry, exploiting the ability of conjugated phenolic structures to absorb in the UV-Vis region Table 4. A colorimetric assay was performed using Folin-Ciocalteu reagent and sodium carbonate (Na_2CO_3). In this redox reaction, phenolic compounds act as reducing agents while the Folin-Ciocalteu reagent (a phosphomolybdate-phosphotungstate mixture) is oxidized. Oxidized phenolics react with phosphomolybdate to form a blue molybdenum tungsten complex that absorbs at visible wavelengths.[63] Na_2CO_3 provides an alkaline medium necessary for efficient reduction and stabilizes the pH within the optimal range. Although gallic acid is commonly used as the calibration standard, this study employed tannic acid due to its high reactivity toward Folin-Ciocalteu reagent, yielding reliable calibration data[64].

The maximum absorption wavelength (λ max) of the tannic acid standard was determined by scanning solutions between 600 and 800 nm. A λ max of 760 nm (absorbance = 0.578), consistent with Molole[63], and was used for all subsequent TPC measurements of the leaf extracts. To establish the incubation period required for reaction completion, absorbance at 760 nm was measured over incubation times ranging from 5 to 75 minutes. The optimum incubation time was found to be 60 minutes, in agreement with[64].

A calibration curve was constructed using tannic acid standards at 6, 8, 10, 12, and 14 ppm. Linear regression of absorbance (y) versus concentration (x) yielded the equation: $y = 0.0454\,x + 0.0415$, R^2

Table 4. Total Phenolic Content of Leaf Extracts

Extraction Method	Sample	Mean Total Phenolic Content (%) ± SD
Maceration	60% Ethanol Extract	18,7624 ± 0,0921
	70% Ethanol Extract	16,4408 ± 0,0722
	80% Ethanol Extract	14,9874 ± 0,0844
	96% Ethanol Extract	13,6405 ± 0,0436
	Ethyl Acetate Extract	8,3334 ± 0,7440
	n-Hexane Extract	0,8305 ± 0,6547
UAE	60% Ethanol Extract	20,0180 ± 0,0727
	70% Ethanol Extract	17,6441 ± 0,0467
	80% Ethanol Extract	15,4134 ± 0,1427
	96% Ethanol Extract	14,0404 ± 0,0688
	Ethyl Acetate Extract	10,1349 ± 0,5084
	n-Hexane Extract	0,9219 ± 0,2237

= 0.9988. The high coefficient of determination indicates excellent linearity, confirming that absorbance increases proportionally with tannic acid concentration. Triplicate measurements at 760 nm ensured accuracy and reproducibility.

For each extract, a sample reagent mixture was incubated in the dark for 60 minutes before absorbance measurement at 760 nm. Concentrations were calculated from the tannic acid calibration curve. Among the solvents tested, 60% (v/v) ethanol being more polar dissolved polar phenolics most effectively, yielding the highest TPC, whereas n-hexane extracts exhibited the lowest TPC, by the principle of "like dissolves like." Ahmad[42] reported TPC values of 241 mg GAE/g for 60% ethanol, 101.3 mg GAE/g for ethyl acetate, and 7.2 mg GAE/g for n-hexane extracts of red shoot leaves. Phenolics, often present as glycosides, are more soluble in polar solvents such as ethanol[65]. (UAE) produced higher TPC than maceration, likely due to ultrasonic cavitation facilitating cell-wall disruption and rapid release of phenolics[66]. This observation concurs with Ibrahim[67], who demonstrated superior phenolic extraction by UAE over maceration.

Antioxidant Activity Results

Based on the data presented in Table 5, the antioxidant activity of the red shoot leaf extract obtained with ethanol as solvent was higher than that of extracts prepared with ethyl acetate or n-hexane, with 96% ethanol yielding the greatest activity. These results indicate that antioxidant activity increases with ethanol concentration. According to Sugihartini and Maryati[33], the IC_{50} value of ethanol extract from red shoot leaves of *Syzygium myrtifolium Walp.* falls within the "very strong" category, as concentrations below 50 ppm are sufficient to scavenge 50% of DPPH radicals. In the present study, the 96% ethanol extract prepared by UAE exhibited an IC_{50} of 16.0259 ppm, while the maceration extract showed an IC_{50} of 24.7693 ppm, both classifiable as very strong.

The pronounced antioxidant activity of the ethanol extract is attributable to its rich content of secondary metabolites, including phenolics, flavonoids, alkaloids, triterpenoids, steroids, and saponins. Among these, phenolic compounds are particularly effective DPPH scavengers, acting as reducing agents or hydrogen donors to neutralize free radicals. Generally, higher total phenolic content correlates with stronger antioxidant activity, although other constituents also contribute to the overall effect[33]. Therefore, the greater antioxidant potency observed in UAE extracts compared to maceration extracts can be explained by the enhanced extraction efficiency of phenolic compounds via ultrasonic cavitation, which facilitates cell-wall disruption and increases phenol yield, thereby augmenting free-radical neutralization.

CONCLUSION

This study highlights the 60% ethanol extract obtained through Ultrasonic Assisted Extraction (UAE) as yielding the highest total phenolic content (20.0180%) in *Syzygium myrtifolium Walp.* leaves. Comparative analysis across various ethanol concentrations (60%, 70%, 80%, and 96%) and extraction methods (maceration and UAE) revealed a significant influence of both solvent concentration and extraction technique on phenolic yield ($p < 0.05$). The maceration method showed decreasing phenolic levels with increasing ethanol concentration, while UAE consistently yielded higher levels. These findings underscore the importance of optimizing extraction parameters, with UAE using 60% ethanol emerging as the most effective strategy for maximizing phenolic content in *Syzygium myrtifolium* Walp., reinforcing its potential as a source of natural antioxidant compounds.

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