



Environmentally Friendly Extraction of Salam Leaves Using the Ultrasound-Assisted Extraction and Evaluation of Their Antioxidant Potential

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ABSTRACT: Salam leaves (*Syzygium polyanthum*) have been found to contain phenolic compounds that exhibit significant antioxidant potential. The objective of this study was to ascertain the impact of varying methanol-water solvent ratios on the extraction yield, total phenolic content, and antioxidant activity of salam leaf extract through the utilization of the UAE method. The extraction process was executed using methanol–water solvent ratios of 9:1, 8:2, 7:3, 6:4, and 5:5 (v/v). The total phenolic content was determined using the Folin–Ciocalteu method. The antioxidant activity was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay based on IC_{50} values. The findings indicated that the extraction yield reached its zenith at a 9:1 ratio (12.4%) and attained its nadir at a 5:5 ratio (2.7%). TPC increased with higher water content, reaching a maximum of 12.264 ± 0.114 mg GAE/g extract at a 5:5 ratio. The strongest antioxidant activity was observed at 9:1 ($IC_{50} = 47.109 \pm 0.457$ ppm), while the weakest was at 5:5 ($IC_{50} = 74.300 \pm 0.945$ ppm). The findings demonstrate that UAE in combination with the optimisation of methanol:water solvent enables the extraction of phenolics and antioxidant activity to be tuned. The optimized UAE conditions offer a green, efficient, and practical approach for producing antioxidant-rich extracts from *S. polyanthum*, with potential applications in the food, nutraceutical, and natural product industries.

KEYWORDS: Environmentally friendly extraction; antioxidant; DPPH; UAE

1. Introduction

Syzygium polyanthum, commonly known as salam, is a medicinal and culinary plant that is widely used in Southeast Asia, particularly in Indonesia [1]. In traditional medicine, its leaves have long been used to treat various health disorders, including hypertension, diabetes, diarrhea, and inflammation [2]. The bioactivity of salam leaves is closely associated with the presence of secondary metabolites, including flavonoids, tannins, alkaloids, and phenolic compounds, which exhibit strong antioxidant properties [3]. Antioxidants play an essential role in preventing oxidative stress caused by free radicals, which are associated with the development of chronic diseases such as cancer, cardiovascular diseases, and diabetes mellitus [4].

In recent years, research has increasingly focused on natural antioxidants derived from plants because of growing concerns regarding the safety of synthetic antioxidants. Phenolic compounds are considered one of the most important groups of natural antioxidants because of their ability to donate hydrogen atoms or electrons to neutralize free radicals [5]. Consequently, the extraction process is a crucial step in obtaining bioactive compounds with high antioxidant potential [6]. Conventional extraction techniques, such as maceration and Soxhlet extraction, generally require large amounts of organic solvents, long extraction times, and high energy consumption. These limitations may reduce extraction efficiency and cause adverse environmental impacts [7].

In recent years, green extraction technology has emerged as an environmentally friendly alternative for recovering bioactive compounds from plant materials [8]. Green extraction techniques emphasize the use of safer solvents, lower energy consumption, shorter extraction times, and sustainable processing methods [9]. One of the most promising green extraction techniques is UAE. UAE uses ultrasonic waves to generate cavitation, which disrupts plant cell walls, enhances mass transfer, and improves the release of intracellular compounds into the extraction solvent [10]. According to the principles of green extraction proposed by Chemat et al. [10], environmentally friendly extraction processes should reduce the use of hazardous solvents, minimize energy consumption, shorten extraction time, and maximize the utilization of renewable resources. UAE satisfies several of these criteria, making it a promising technology for the sustainable recovery of plant-derived bioactive compounds. Compared with conventional extraction methods, UAE offers several advantages, including higher extraction efficiency, lower solvent consumption, shorter processing time, and better preservation of thermolabile compounds [11].

Numerous studies have demonstrated that UAE significantly enhances the extraction yield of phenolic compounds and antioxidant activity from medicinal plants [12]. However, studies on the green extraction of salam leaves using UAE remain limited, particularly regarding the relationship between extraction conditions and antioxidant properties. Furthermore, environmentally friendly extraction parameters for obtaining high phenolic content from salam leaves have not been thoroughly investigated.

Therefore, this study aimed to investigate the green extraction of salam leaves using UAE and to evaluate the antioxidant activity and total phenolic content of the resulting extracts. The findings of this study are expected to contribute to the development of sustainable extraction methods for natural antioxidant sources and to support the utilization of salam leaves as a potential functional ingredient in food, pharmaceutical, and nutraceutical applications.

2. Materials and Methods

2.1. Material.

Fresh salam leaves (*S. polyanthum*) were collected from Walikukun Village, Serang, Banten Province, Indonesia. The collected leaves were washed with distilled water before further processing. The chemicals used in this study included methanol, distilled water, Folin–Ciocalteu reagent, sodium carbonate (Na_2CO_3), gallic acid, and 2,2-diphenyl-1-picrylhydrazyl (DPPH), all purchased from Sigma-Aldrich. All reagents were of analytical grade.

2.2. Preparation of plant material.

Fresh salam leaves were thoroughly washed and air-dried at room temperature for several days until a constant weight was achieved. The dried leaves were ground using a laboratory blender and sieved to obtain a uniform particle size. The powdered samples were stored in airtight containers at room temperature until extraction.

2.3. *Ultrasound-assisted extraction.*

The extraction process was performed using UAE. A 1 g portion of salam leaf powder was mixed with 10 mL of a methanol–water solvent mixture at different ratios (9:1, 8:2, 7:3, 6:4, and 5:5, v/v), corresponding to a sample-to-solvent ratio of 1:10 (w/v). The extraction was carried out in an ultrasonic bath operating at a frequency of 40 kHz and an ultrasonic power of 200 W for 60 min [13]. The extraction temperature was maintained below 40°C to prevent degradation of thermolabile compounds. After extraction, the mixtures were filtered through Whatman No. 1 filter paper, and the filtrates were concentrated using a rotary evaporator under reduced pressure at temperatures below 50°C.

2.4. *Determination of Extraction Yield*

The extraction yield was determined gravimetrically using the following equation:

$$\text{Extraction Yield (\%)} = \frac{\text{Weight of Extract}}{\text{weight of dry sample}} \times 100\%$$

where the weight of the extract is the mass of the concentrated extract obtained (g), and the weight of the dry sample is the initial weight of the dry salam leaf powder used for extraction (g.)

2.5. *Determination of total phenolic content (TPC).*

The total phenolic content was determined using the Folin–Ciocalteu colorimetric method. Briefly, 0.5 mL of the extract solution was mixed with 2.5 mL of Folin–Ciocalteu reagent and incubated for 5 min. Subsequently, 2 mL of 7.5% sodium carbonate solution was added. The mixture was incubated in the dark at room temperature for 30 min, after which the absorbance was measured at 765 nm using a UV–Visible spectrophotometer [14]. Gallic acid was used as the standard for constructing the calibration curve [15]. The total phenolic content was expressed as milligrams of gallic acid equivalents per gram of extract (mg GAE/g extract).

2.6. *Determination of antioxidant activity using DPPH Method.*

The antioxidant activity of the salam leaf extracts was evaluated using the DPPH radical scavenging assay. A total of 2.4 mL of the extract solution at concentrations of 20, 40, 60, 80, and 100 ppm was mixed with 0.6 mL of DPPH solution. The mixture was vortexed and incubated in the dark at room temperature for 30 min. The absorbance was measured at 517 nm using a UV–Visible spectrophotometer. The IC₅₀ value was calculated from the linear regression equation obtained by plotting extract concentration against percentage inhibition. The IC₅₀ value represented the concentration required to inhibit 50% of DPPH radicals [16,17].

2.7. *Statistical analysis.*

All experiments were conducted in triplicate, and the results were expressed as the mean $\pm$ standard deviation (SD). Statistical analyses were performed using IBM SPSS Statistics version XX (or the software used), with statistical significance accepted at $p < 0.05$.

3. Results and Discussion

3.1. Environmentally friendly extraction.

The extraction of salam leaf samples was carried out using the UAE method. Methanol–water solvent mixtures with volume ratios of 9:1, 8:2, 7:3, 6:4, and 5:5 (v/v) were used as extraction solvents. The extraction results are presented in Table 1. The highest extraction yield was obtained from sample S1 using a methanol–water ratio of 9:1, with a yield of 12.4%. In contrast, the lowest extraction yield was obtained from sample S5 using a solvent ratio of 5:5, with a yield of 2.7%. These results indicated that increasing the proportion of water in the extraction solvent reduced the extraction yield of salam leaves. Sample S1, which contained the highest proportion of methanol, produced the greatest extraction yield. This result was likely attributable to the superior ability of methanol to penetrate plant cell walls and dissolve semipolar secondary metabolites present in salam leaves.

Table 1. Extraction yield of salam leaf extracts obtained using UAE.

Sample code	Methanol:Water (v/v)	Extract weight (g)	Extraction yield (%)
S1	9:1	0.124	12.4
S2	8:2	0.108	10.8
S3	7:3	0.096	9.6
S4	6:4	0.045	4.5
S5	5:5	0.027	2.7

The gradual decline in extraction yield from solvent ratios of 8:2 to 5:5 suggested that increasing the water content reduced the solvent's ability to extract semipolar compounds from salam leaves. Water, as a highly polar solvent, was more effective at dissolving polar compounds, whereas most secondary metabolites in salam leaves were more soluble in organic solvents such as methanol [18]. Consequently, methanol-rich solvent mixtures exhibited greater extraction efficiency than solvent mixtures containing higher proportions of water. The UAE process produced extracts with colours ranging from dark green to yellowish-brown, depending on the solvent composition. The extract obtained from sample S1 exhibited the darkest green colour, which was likely due to the higher extraction of chlorophyll in the methanol-rich solvent. The polarity of the extraction solvent significantly influenced the colour of the extracts. Increasing the proportion of water tended to enhance the extraction of polar compounds, including tannins, flavonoid glycosides, and other phenolic constituents, thereby altering the colour intensity of the extracts [19].

3.2. Total phenolic content (TPC).

The TPC assay of the samples was initiated by preparing a gallic acid standard curve. Various concentrations of gallic acid were prepared at 20, 30, 40, 50, and 60 ppm. The gallic acid standard curve along with its linear regression equation is presented in Figure 1.

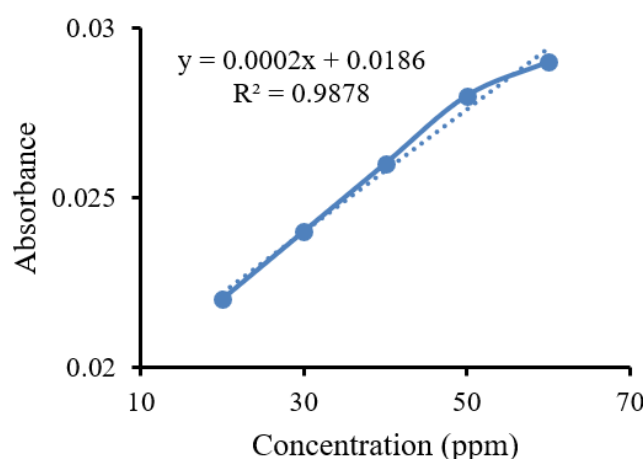


Figure 1. The gallic acid standard curve.

The TPC of the salam leaf extracts was determined using the Folin–Ciocalteu colorimetric method and was expressed as milligrams of gallic acid equivalents per gram of extract (mg GAE/g extract). The results showed that variations in the methanol-to-water solvent ratio influenced the extraction of phenolic compounds from the salam leaves. The total phenolic content measured for each treatment is presented in Table 2.

Table 2. The results of total phenolic content.

Sample	TFC mg GAE/g extract
S1 (9:1)	10.160±0.010
S2 (8:2)	11.1437±0.028
S3 (7:3)	11.625±0.012
S4 (6:4)	12.087±0.080
S5 (5:5)	12.264±0.114

The results demonstrated that increasing the proportion of water in the methanol–water solvent system enhanced the extraction of phenolic compounds from salam leaves. Sample S1, extracted using a methanol-to-water ratio of 9:1, exhibited the lowest total phenolic content (10.160 ± 0.010 mg GAE/g extract), whereas sample S5, extracted with an equal proportion of methanol and water (5:5), showed the highest total phenolic content (12.264 ± 0.114 mg GAE/g extract). The increase in total phenolic content with increasing water proportion suggested that the phenolic compounds present in salam leaves were predominantly polar and therefore more readily extracted by solvents with higher polarity. Water enhanced the solubility of several classes of phenolic compounds, including phenolic acids, flavonoid glycosides, and tannins [12]. Consequently, methanol–water mixtures provided a more suitable extraction medium for these compounds than highly concentrated methanol, resulting in improved extraction efficiency and greater recovery of phenolic compounds from the plant material.

During UAE, ultrasonic waves generated acoustic cavitation, resulting in the formation and collapse of microbubbles within the extraction solvent [20]. The resulting mechanical forces disrupted the plant cell walls, thereby facilitating the release and diffusion of phenolic compounds into the solvent. Furthermore, increasing the water content in the solvent likely enhanced the swelling of plant tissues and improved solvent penetration into the cellular matrix, which further promoted the extraction of phenolic constituents [18]. Although the overall extraction yield decreased as the water proportion increased, the total phenolic content increased significantly. This finding suggested that extraction yield did not necessarily correspond to the concentration of phenolic compounds in the extract. The higher extraction

yields obtained with methanol-rich solvents were likely attributable to the co-extraction of chlorophyll, lipids, and other semipolar or non-phenolic constituents. In contrast, solvent systems containing higher proportions of water exhibited greater selectivity toward phenolic compounds, thereby producing extracts with higher phenolic content despite their lower overall extraction yields.

3.3. Antioxidant test result.

The antioxidant activity of the salam leaf extracts was evaluated using the DPPH radical scavenging assay. The results demonstrated that variations in the methanol-to-water solvent ratio influenced the free radical scavenging activity of the extracts. Antioxidant activity was expressed as the IC_{50} value, which represents the concentration of extract required to inhibit 50% of DPPH free radicals. A lower IC_{50} value indicated stronger antioxidant activity. The relationship between extract concentration and the percentage of DPPH radical inhibition is presented in Figure 2, whereas the IC_{50} values of the salam leaf extracts obtained using different solvent compositions are summarized in Table 3.

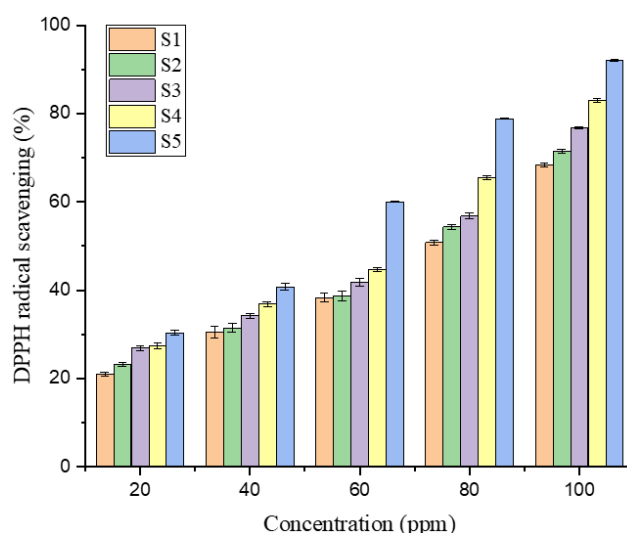


Figure 2. The relationship between concentration and DPPH radical scavenging of salam leaves extracts.

Table 3. The antioxidant activity (IC_{50}) values for each sample.

Sample	(IC_{50}) ppm $\pm$ SD
S1 (9:1)	47.109 $\pm$ 0.4574
S2 (8:2)	57.858 $\pm$ 0.3090
S3 (7:3)	64.432 $\pm$ 0.1777
S4 (6:4)	70.306 $\pm$ 0.5083
S5 (5:5)	74.300 $\pm$ 0.9453

The IC_{50} values presented in Table 3 showed that increasing the proportion of water in the methanol–water solvent system resulted in an increase in the IC_{50} value of the salam leaf extracts. Sample S1 (9:1 methanol) exhibited the lowest IC_{50} value (47.109 ± 0.457 ppm), whereas sample S5 (5:5 methanol) showed the highest IC_{50} value (74.300 ± 0.945 ppm). Because a lower IC_{50} value indicates stronger antioxidant activity, sample S1 exhibited the greatest antioxidant activity among all treatments, while sample S5 showed the weakest antioxidant activity.

The stronger antioxidant activity observed in sample S1 was likely attributable to the extraction of antioxidant compounds that were more soluble in methanol-rich solvent systems.

During UAE, ultrasonic waves generated acoustic cavitation, resulting in the formation and collapse of microbubbles within the extraction solvent. These mechanical forces disrupted the plant cell walls, facilitating the release and diffusion of antioxidant compounds into the solvent [19]. Although increasing the proportion of water may have improved the extraction of certain polar compounds by enhancing plant tissue swelling and solvent penetration, excessive water content appeared to reduce the extraction of antioxidant constituents responsible for DPPH radical scavenging activity.

The antioxidant activity observed in this study was not entirely consistent with the total phenolic content results. Although the total phenolic content increased with increasing water content in the solvent system, the IC_{50} values also increased, indicating weaker antioxidant activity. This finding suggested that the antioxidant activity of salam leaf extracts was influenced not only by the total phenolic content but also by the composition and bioactivity of individual phenolic compounds and other antioxidant constituents present in the extracts. Different phenolic compounds possess different radical scavenging capacities because of variations in their chemical structures, including the number and position of hydroxyl groups [22].

3.4. The advantages of environmentally friendly extraction.

The green extraction approach applied to *Syzygium polyanthum* leaves was supported by the use of UAE, which enabled efficient extraction using reduced solvent volumes and methanol–water solvent mixtures with increased water content. Increasing the proportion of water in the solvent system from 9:1 to 5:5 significantly enhanced the total phenolic content of the extracts, highlighting the potential of water as an environmentally friendly co-solvent for improving the recovery of phenolic compounds while reducing the use of organic solvents and minimizing the environmental impact of the extraction process. The improved recovery of phenolic compounds observed at higher water proportions was primarily attributed to the increased polarity of the solvent system, which enhanced the solubility of polar phenolic constituents. Furthermore, water facilitated the swelling of plant tissues and improved solvent penetration into the intracellular matrix. Simultaneously, acoustic cavitation generated during UAE disrupted the plant cell walls and accelerated mass transfer, thereby promoting the release of phenolic compounds into the extraction solvent [20]. The combined effects of solvent polarity and ultrasonic cavitation contributed to greater phenolic recovery while supporting a greener extraction process through reduced reliance on organic solvents.

Although increasing the water content improved the total phenolic content, the antioxidant activity determined by the DPPH assay did not follow the same trend. The methanol-rich solvent system (9:1) exhibited the lowest IC_{50} value and therefore the strongest antioxidant activity. This finding suggested that antioxidant activity depended not only on the total amount of phenolic compounds extracted but also on the composition and bioactivity of individual phenolic constituents and other antioxidant compounds present in the extracts. Different phenolic compounds possess different radical-scavenging capacities because of variations in their chemical structures and hydroxyl group substitutions [23]. Compared with conventional extraction techniques, the combination of UAE and methanol–water solvent systems represented a more sustainable extraction approach by reducing solvent consumption, lowering energy requirements, shortening extraction time, and minimizing environmental impacts. The findings demonstrated that integrating UAE with environmentally friendly

solvent systems could reduce dependence on organic solvents while improving the selective recovery of valuable bioactive compounds. Consequently, this extraction strategy showed considerable potential for the sustainable production of natural antioxidant extracts for applications in the food, pharmaceutical, and nutraceutical industries.

3.5. Statistical analysis.

The experimental data were analyzed using one-way analysis of variance (ANOVA), followed by Tukey's Honestly Significant Difference (HSD) post hoc test at a significance level of $p < 0.05$. The results demonstrated that the methanol–water solvent ratio significantly influenced both the total phenolic content and antioxidant activity (IC_{50}) of the salam leaf extracts. For antioxidant activity, the ANOVA revealed a highly significant effect of solvent composition ($F = 1163.76$, $p < 0.001$). Similarly, the total phenolic content was significantly affected by the solvent ratio ($F = 517.45$, $p < 0.001$). Tukey's HSD test further indicated that all solvent treatments differed significantly from one another ($p < 0.05$), confirming that variations in solvent composition had a substantial impact on the extraction efficiency of bioactive compounds.

4. Conclusions

This study demonstrated that the methanol–water solvent ratio significantly affected the extraction yield, total phenolic content, and antioxidant activity of *Syzygium polyanthum* leaf extracts obtained using UAE. The highest extraction yield (12.4%) and strongest antioxidant activity ($IC_{50} = 47.109 \pm 0.457$ ppm) were achieved using a methanol–water ratio of 9:1, whereas the highest total phenolic content (12.264 ± 0.114 mg GAE/g extract) was obtained at a ratio of 5:5. These findings indicate that solvent composition influenced different extraction responses and should be selected according to the desired bioactive properties. Overall, UAE combined with methanol–water solvent systems represents an efficient and environmentally friendly extraction approach with promising potential for the sustainable production of natural antioxidants from salam leaves.

Author Contribution

Panji Setya Amitra contributed to the conceptualization of the study, data collection, data analysis, manuscript writing, and funding acquisition. Alimuddin contributed to the methodology. Anton Irawan provided supervision. All authors have read and approved the final version of the manuscript.

Competing Interest

All authors should disclose any financial, personal, or professional relationships that might influence or appear to influence their research.

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