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Characterization of *Andrographis Paniculata* Extract Obtained by Microwave-Assisted Extraction (MAE) Method with Radiation Time

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Abstract: The purpose of this research was to extract *Andrographis paniculata* using the Microwave Assisted Extraction (MAE) method at 4 radiation (4 minutes, 6 minutes, 8 minutes, and 10 minutes) and characterize the extract on phenolic content, flavonoid content, andrographolide content, antioxidant activity, and functional group using Fourier Transform Infrared Spectroscopy (FTIR). Radiation times of the MAE method had a significant effect on phenolic content, flavonoid content, andrographolide content, and antioxidant activity of *Andrographis paniculata*. Radiation time for 10 minutes of MAE gave higher phenolic, flavonoid, andrographolide, and antioxidant activity of *Andrographis paniculata*. The IR spectra of the Andrographis paniculate extract increased as the radiation time increased. The optimum radiation time was at 8 minutes with the result of phenolic content at 57.2 mg GAE /g, flavonoid content at 113.07 ppm, andrographolide at 763.47 µg/ml, antioxidant activity at 71.66% and the IR spectra at 1031.92 cm^{-1}, 2945.3 cm^{-1}, 3346.5 cm^{-1}.

Keywords: Andrographolide; Antioxidant activity; Flavonoid; IR spectra; Phenolic

Introduction

Medicinal plants have become the focus of significant research in efforts to identify bioactive compounds that can provide health benefits (Sarker et al., 2012). One plant that has attracted the attention of researchers is Andrographis paniculata (AP), which is known to have potential anti-inflammatory, antiviral, immune system-boosting, and antimicrobial properties (Singha et al., 2018). Extraction of active compounds from this plant is a key step in developing bioactive products based on AP which can be used as supplements and nutraceutical foods.

One promising extraction method is the Microwave Assisted Extraction (MAE) Method. This method utilizes microwave energy to increase the extraction efficiency of bioactive compounds from plant material (Rao et al., 2018). Compared to conventional extraction methods, MAE is known to produce higher-quality extracts in a shorter time (Azwanida, 2015).

Apart from the extraction method, the time factor also plays an important role in the extraction process. Different radiation times can affect extraction kinetics, leading to variations in the amount and type of compounds extracted (Dzah et al., 2017). Therefore, it is important to understand how different radiation times affect the extraction results of bioactive compounds from AP.

However, research that considers the effect of different radiation times on AP extraction using the MAE method is still limited. More in-depth information on the effect of radiation time on the extraction yield and profile of compounds extracted from these plants will

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provide valuable insights for optimizing the extraction process (Doldolova et al., 2021).

Therefore, this study aims to investigate the effect of different radiation times on the extraction of bioactive compounds from AP using the microwave-assisted extraction (MAE) Method. The results of this research are expected to provide better information on how to optimize the extraction of active compounds from this plant for pharmaceuticals, health applications, and nutraceutical foods.

Method

Chemicals and Reagents

AP were obtained from Malang, East Java, Indonesia. The AP were dried using the oven at 50˚C for 24 hours, and ground to produce a powder with a particle size of 1777µm. All chemicals and materials for extract and analysis were of analytical grade (Merck, Germany) and included andrographolide standard, 50% methanol, propanol, Whatman No. 4 filter paper, Folin-Ciocalteu reagent, saturated sodium carbonate, gallic acid, potassium bromide (KBr) powder.

Andrographis Paniculata Extraction and Recovery

The AP was extracted by the MAE method (Rahayu et al., 2019)(Sharp Model R -222Y(S)) at a level power high (70°C) with a slight modification. AP powder was dissolved in methanol 50% at a ratio of 5 g powder to 100 mL methanol. It soaked for 24 hours. The solution was extracted in a microwave oven for 4 (T1), 6 (T2), 8 (T3), or 10 (T4) min (one minute on and two minutes off) to avoid overheating. The crude extract was filtered through Whatman no. 4 filter paper, and the ethanol was evaporated using a rotary evaporator at 55 mm Hg pressure and 50°C. The filtrate was concentrated using a rotary evaporator (IKA RV 10) to remove the solvent. The concentrated filtrate was then analyzed as described below.

Measurements of Phenolic Content

The Folin-Ciocalteu reagent method, as outlined in Maung and Chamba's study from 2012, was employed to assess the Total Phenolic Content (TPC). A solution of saturated sodium carbonate was prepared and left to stand for 24 hours. Standard concentrations of gallic acid, ranging from 25 to 200 ppm, were freshly made at room temperature for each analysis. A solution containing 0.5 mL of 1:10 diluted gallic acid in 4.5 mL of distilled water, 0.2 mL of Folin reagent (Merck), and 0.5 mL of saturated sodium carbonate was prepared and utilized to generate a standard sample analysis curve. The AT-1900 UV spectrophotometer was utilized to measure the absorbance of each sample at a wavelength of 725 nm. The TPC was computed using the absorption values and a linear regression equation, enabling comparison with the gallic acid standard. The findings were expressed as milligrams of gallic acid equivalents (GAE) per gram of dry sample weight (mg/g).

Measurement of Flavonoid Content

The method, as outlined in Asadi-Samani from 2016, prepared and dissolved it in a solvent such as methanol or ethanol until the appropriate concentration for testing is achieved. Transfer the sample solution into a test tube or beaker. Add the AlCl3/Potassium acetate reagent solution to the test tube or beaker containing the sample. Stir gently. Allow the mixture to react in darkness for approximately 30 minutes until a reaction with the flavonoids occurs. Centrifuge the mixture to separate the precipitate. Use a spectrophotometer to measure the absorbance of the solution at the appropriate wavelength (usually around 415 nm). Utilize a calibration curve (if using a standard solution) or correlate the sample absorbance with the total flavonoid concentration.

Andrographolide Content

Total andrographolide testing using Thin Layer Chromatography (TLC) refers to the method outlined by Sajeeb et al. (2015) with slight modifications. Take 5 mL of each sample. Transfer it into a 15 mL conical tube. Centrifuge the sample, then extract the supernatant. Dilute as necessary. Spot the supernatant on a silica gel stationary phase, using 1 µL, and include a reference compound of andrographolide. Elute with a mobile phase of chloroform-methanol (90:10) until the boundary. Scan the andrographolide spot at a wavelength of 233 nm. Calculate the andrographolide content.

Antioxidant Activity

The antioxidant activity assessment using the DPPH method is based on Molyneux's (Molyneux, 2004) protocol with slight modifications. Take AP extract and dissolve it in an appropriate solvent to achieve the desired concentration for testing. Dissolve DPPH in methanol solvent to obtain the appropriate concentration. Mix the extract solution with the DPPH solution in a test tube or beaker. Stir until well mixed. Allow the mixture to react in darkness for approximately 30 minutes or until the reaction is complete. Utilize a spectrophotometer to measure the absorbance of the solution at the appropriate wavelength (usually around 517 nm). Calculate the percentage of antioxidant inhibition.

FTIR Analysis

The chemical composition was identified utilizing the procedure outlined by Maoela et al. (2009), with minor adjustments. The UV-Vis spectrum was recorded using a GBS UV/VIS 920. A single milligram of AP extract underwent dehydration in a vacuum desiccator. Following this, it was finely ground and meticulously blended with 200 mg of KBr powder, which had been oven-dried and was of analytical reagent grade (Merck, DAC, USP). This powdered mixture was placed into a die and compacted into a clear disk.

Result and Discussion

The results of total phenolic extraction of AP with varying radiation times using the MAE method had a highly significant effect (P<0.01). Table 1 showed that the highest total phenolics in the results of this study were obtained at T3 with a radiation time of 8 minutes, resulting in 57.2 mg GAE /g extract. This research demonstrates that T3 is the optimum time to produce the highest total phenolics from AP. This is because radiation time is one of the factors that influence the results of total phenolic extraction using the microwave-assisted extraction (MAE) method. The radiation time determines the length of time the plant material is exposed to microwaves, which can accelerate the release of phenolic compounds from the plant matrix into the solvent. Too short a radiation time can reduce extraction efficiency; conversely, a radiation time that is too long can cause degradation of phenolic compounds due to excessive heat.

Table 1. The Phenolic Content of Andrographis Paniculata Extract

Treatment	Total Phenolic (mg GAE / g)
T1	45.4 ± 0.47^{a}
T2	52.8 ± 0.07^{b}
T3	57.2 ± 0.06^{c}
T4	52.0 ± 0.05^{b}

Superscripts ^{a,b,c,} indicate that varying radiation times using the MAE method had a very significant effect (P<0.01) on the total phenolics content of Andrographis paniculata

Furthermore, this conclusion is supported by several studies evaluating the effect of radiation time on the MAE method for the extraction of total phenolics from various plant materials, including Vitis vinifera leaves (Djemaa-Landri et al., 2020), Nerium oleander leaves (Ayouaz et al., 2020), and AP (Ismail I et al., 2022). The results of this research show that different radiation times yield different total phenolic extraction results. For instance, research by Djemaa-Landri et al. (2020) found that the optimal radiation time for extraction of total phenolics from grape leaves with a 50% ethanol solvent was 47 seconds, resulting in a yield of 23.77 mg GAE/g dry powder. Meanwhile, research by Ayouaz et al. (2020) found that the optimal radiation time for extraction of total phenolics from Nerium oleander leaves with an 80% methanol solvent was 5 minutes, with a yield of 25.752 mg GAE/g dry powder. In addition, research by Ismail et al. (2022) found that the optimal radiation time for extraction of total phenolics from AP leaves with a 70% ethanol solvent was 35 minutes, resulting in a yield of 58.67 mg GAE/g dry powder with 1104.53 (mg GAE/g extract).

Jaiyesimi et al. (2020) employed a maceration extraction method with an 80% methanol solvent to obtain free and bound phenolic extracts from AP leaves. The study revealed that free and bound phenolic extracts from AP could reduce fasting blood glucose levels, increase serum insulin levels, improve β-cell function, inhibit pro-inflammatory cytokines, and enhance antioxidant activity in diabetic mice. These studies collectively conclude that the radiation time in the MAE method yields varying results in total phenolics depending on the type of plant material and solvent used. The optimal radiation time must be adjusted to the characteristics of the plant material and solvent to maximize the yield of total phenolic extraction.

Table 2. The Flavonoid Content of Andrographis Paniculata Extract

Treatment	Flavonoid (ppm)
T1	90.92 ± 0.97^{a}
T2	111.30 ± 0.63^{c}
T3	113.07 ± 0.19^{d}
T4	98.32 ± 0.63^{b}

Superscripts ^{a,b,c,d} indicate that varying radiation times using the MAE method had a very significant effect (P<0.01) on the flavonoid content of Andrographis paniculata

The results of total flavonoids from Andrographis paniculata extract with irradiation time using the MAE method are presented in Table 2. Based on the statistical results, it has a very significant effect (P<0.01) on flavonoid content. This is believed to be because the irradiation time can accelerate the release of flavonoid compounds from the plant matrix into the solvent. If the irradiation time is too short, it may reduce the extraction efficiency, while if it is too long, it may cause the phenolic compounds to degrade due to excessive heat. This is consistent with Delazar et al. (2012) Microwave energy causes molecular movement through ion migration and dipole rotation. This very fast movement creates friction or accuracy, which ultimately produces heat energy in the material so that the cell walls and tissues of the material are damaged and active compounds can be extracted. The correct irradiation

time is required to produce the highest total flavonoids. The optimum total flavonoids obtained at T3 was 113.07 ppm. The irradiation time was 8 minutes.

The extraction of AP using 50% methanol and maceration for 24 hours at room temperature resulted in a total of 33.50 ppm of flavonoids (Adiguna et al., 2023). Ramonah et al. (2023) stated that the total results of bitter flavonoids extracted using 96% ethanol for 5 days were 10.257 ± 0.047 mg QE/g. Based on this research, it can be concluded that the total flavonoids of AP extract are influenced by several factors, including solvent, solvent concentration, and extraction method. Based on the comparison, it can be concluded that in this study, the MAE method with a time of 8 minutes produced the highest total flavonoids.

Andrographis paniculate contains 10 types of flavonoids, which generally include 5-hydroxy-7,8-dimethoxyflavone,5-hydroxy-7,8,2',3' tetramethoxyflavone, 5-hydroxy-7,8,2'-trimethoxyflavone, 7-O-methyl wogonin, and 2'-methyl ether (Chao et al., 2010; Niranjan et al., 2010; Syamsul et al., 2018). The largest phenolic content in red ginger is gingerol, shogaol, paradol, and gingerdion (Mošovská et al., 2015).

Table 3. The Andrographolide Content of *Andrographis paniculata* Extract

Treatment	Andrographolide (µg/ml)
T1	625.25 ± 1.51[a]
T2	704.92 ± 6.15[b]
T3	763.47 ± 1.35[d]
T4	714.89 ± 0.40[c]

Superscripts [a,b,c,d] indicate that varying radiation times using the MAE method had a very significant effect (P<0.01) on the Andrographolide content of Andrographis paniculata

The results of total andrographolide content of AP extract with radiation time on the MAE method are presented in Table 3. Based on statistical results, it gives a very significant effect (P <0.01) on total andrographolide. It is suspected that radiation time has an impact on the number of active compounds extracted, but excessive time can reduce the quality of the extract. The movement in the extraction process using electromagnetic waves will produce friction which produces heat energy in the material so that bioactive compounds can be extracted (Rahayu et al., 2019). The highest total andrographolide was produced in T3 with a radiation time of 8 minutes amounting to 763.47 µg/ml. Total andrographolide produced will vary influenced by several things including the solvent used, radiation time, AP parts extracted, AP form (fresh or dry), and extraction method. The dissolved andrographolide isolates with methanol and analyzed a total andrographolide of 0.8907 µg (Warditiani et al., 2014).

Andrographolide is a secondary metabolite of the AP plant that has functional health benefits including improving aim, anti-inflammatory, antioxidant, and anticancer. Andrographolide has a role as an anti-inflammatory in human skin. Andrographolide can restore skin barrier function and reduce inflammation in keratinocyte cells stimulated by lipopolysaccharide (LPS) or tumor necrosis factor (TNF)-α/interferon (IFN)-γ by preventing the activation of nuclear factor kappa B (NF-κB) and c-Fos and regulating the expression of filagrin, involucrin, and loricrin (Bayazid et al., 2021).

Andrographolide has a role as an analgesic for the development of herbal-based drugs. Andrographolide showed significant analgesic activity in rats induced with acetic acid. Andrographolide levels in ethanol extracts and ethyl acetate fractions of AP its analgesic activity (Tumewu et al., 2021). Andrographolide has a role as an antioxidant that can protect cells from oxidative stress. Andrographolide has high antioxidant activity with an IC50 value of 0.94 µg/mL and an FRAP value of 0.65 mM Fe(II)/g. Andrographolide can also increase the expression of antioxidant enzymes such as superoxide dismutase, catalase, and glutathione peroxidase (Li et al., 2022).

Table 4. The Antioxidant Activity of *Andrographis paniculate* Extract

Treatment	Antioxidant Activity (%)
T1	68.48 ± 0.35[b]
T2	70.68 ± 0.47[c]
T3	71.66 ± 0.12[d]
T4	66.79 ± 0.51[a]

Superscripts [a,b,c,d] indicate that varying radiation times using the MAE method had a very significant effect (P<0.01) on the Antioxidant activity of Andrographis paniculata

The results of the antioxidant activity of AP extract with different radiation time using the MAE method are presented in Table 3. Based on the statistical results, it has a very significant effect (P<0.01) on antioxidant activity. This is thought to be because the radiation time provides a different response to the degradation of bioactive compounds in AP, thus having an influence on the total oxidant activity. This is supported by Rahayu et al. (2019) who explained that very fast movements in the extraction process using electromagnetic waves will produce friction which produces heat energy in the material so that bioactive compounds can be extracted. The optimum antioxidant activity from the research results was at T3 with a radiation time of 8 minutes. This is in line with the values of total flavonoids, total phenolics and total andrographolide with the highest values at T3.

From the results of this research, it can be concluded that AP extract has quite high phenolic and flavonoid content and is positively correlated with antioxidant activity. The andrographolide compound, which is the main phenolic compound in this plant, also contributes to antioxidant activity. Therefore, AP extract can be considered as a potential source of natural antioxidants. This is supported by Zuraida et al. (2017) who states that flavonoids are a class of secondary metabolites produced by plants, including the large group of polyphenols. Flavonoids have the ability to scavenge free radicals and inhibit lipid oxidation. The antioxidant activity of phenol and flavonoid components by reducing free radicals depends on the number of hydroxy groups in the molecular structure (Zuraida et al., 2017).

Previous research by Martin et al. (2022) explained that the extraction of bitter with methanol solvent using the soxhletation method obtained antioxidant results of >200 ppm. The very large IC50 value for bitter leaf extract indicates that the extract is less effective because its inhibitory ability can be achieved by using large amounts. According to Pangestu et al. (2017), there are five categories of antioxidants, namely very strong (<50 ppm), strong (50-100 ppm), medium (101-150 ppm), weak (150-200 ppm) and very weak (>200 ppm).

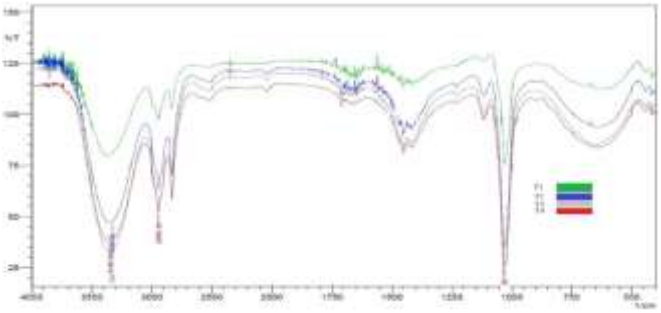

Figure 1. Functional groups of Andrographis paniculata extract

Explanation of the various symbol, see Table 5.

Table 5. Functional Group of *Andrographis paniculata* extract

Treatment				Group	Chemical Structure
T1	T2	T3	T4		
-	-	-	1031.92	C-O stretch C-N stretch	Alcohol Aliphatic amines
-	-	2945.3	2945.3	C-H stretch	Alkanes
-	3334.92	3332.99	3346.5	O-H, H-bonded	Phenols, hydrogen bound

Chemical Structure Fourier Transform Infrared Spectroscopy (FTIR)

The aim of testing the functional groups of AP extract using FTIR is to determine the chemical structure and active compound content of AP extract. The active compound responsible for the pharmacological activity of this plant is andrographolide. FTIR can detect functional groups contained in andrographolide, such as lactone, hydroxyl and carbonyl groups. Thus, testing the functional groups of AP extract using FTIR can provide information about the quality and purity of the extract.

Figure 1 and Table 5 showed peaks in the IR spectra at 1050-1300 cm^{-1} indicated C-O alcohol/ester/carboxylic acids groups, and peaks at 2850-2970 cm^{-1} indicated C-H alkanes groups. Peaks at 3200-3600 cm^{-1} indicated Phenols, hydrogen bound groups (Suharmiati et al., 2001). Jain et al. (2016) At peak 3749.62 cm^{-1} stretching mode display the presence of phenol compound. One of the groups that is the key to the existence of andrographolide is located at the peak 3200-3600 cm^{-1} which indicates a hydroxyl group with hydrogen bonds.

The IR spectra of AP extract using MAE with different radiation time gave the different spectra. The spectra that are key to the presence of andrographolide are at T2, T3 and T4 at 3200-3600 cm^{-1}. T1 did not appear peak at all. This is thought to be because different radiation times during the extraction process have an influence on the bioactive compounds extracted. This is consistent with Delazar et al. (2012), microwave energy induces molecular movement through ion migration and dipole rotation. This very fast movement causes friction which ultimately creates heat energy in the material, so that the cell walls and tissue of the material are damaged and active compounds can be extracted.

Conclusion

Radiation times of the MAE method had a significant effect on phenolic content, flavonoid content, andrographolide content, and antioxidant activity of AP. Radiation time for 10 minutes of MAE gave higher phenolic, flavonoid, andrographolide, and antioxidant activity of AP. The IR spectra of the AP extract increased as the radiation time increased.

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This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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Conflicts of Interest
The authors have declared that no competing interests exist.

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