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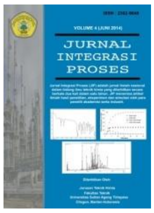

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MICROWAVE-ASSISTED EXTRACTION OF TANNIN FROM PAPAYA LEAVES *(CARICA PAPAYA LINN):* THE EFFECT OF SOLVENT RATIO AND MICROWAVE POWER

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Abstract

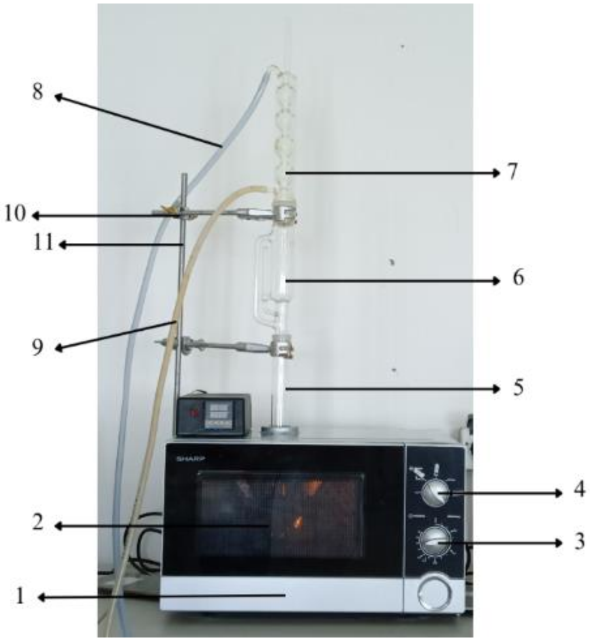
Papaya leaves *(Carica papaya Linn)* have many health benefits, such as antibacterial, anti-inflammatory, antidiarrhea, and others. Papaya leaves contain tannins, saponins, alkaloids, triterpenoids, steroids, and flavonoids. The common characteristic of papaya leaves is the green color, which indicates the presence of tannins. The study aims to discover the characteristics of papaya leaf extract, the effect of the solvent ratio, and the microwave power (MP) on the extraction of tannins from the papaya leaves. Papaya leaves are extracted using the microwave-assisted extraction (MAE) method at 100, 140, and 180 W for 30 minutes, with variations in the material to the solvent ratios 1:10, 1:20, 1:30, 1:40, and 1:50. Qualitative tannin testing was carried out through color observation and $FeCl_3$ tests. UV-Vis spectrophotometry was also used to determine the tannin levels obtained during extraction. The $FeCl_3$ test showed that papaya leaf extract contains tannins. The optimal concentration of tannins is produced at a ratio of 1:20, with a 180 W MP of 8.06 mg TAE/g and a yield of 0.81%. MAE can potentially increase the yield of tannins, which is potentially beneficial to health.

Keywords: Microwave-assisted extraction; Microwave power; Papaya leaves; Solvent ratio; Tannins

1. INTRODUCTION

As a country with a tropical climate, Indonesia boasts a diverse array of distinctive flora, including the papaya plant *(Carica papaya)*. The papaya plant is popular among Indonesians, making it easily found throughout all regions of Indonesia. The community extensively cultivates papaya due to its straightforward cultivation and maintenance. Besides, papaya flesh can also be consumed fresh or made into candy, jam, jelly, and pickles (Kumoro et al., 2020). About 75% of the world's papaya is produced by the ten countries crossed by the equator: Brazil (25%), Nigeria (15%), India (12%), Mexico (11%), Indonesia (10%), Ethiopia (4%), Congo (4%), Peru (3%), Venezuela (3%), and China (2%) (Hadolikar et al., 2021). Apart from the fruit, papaya seeds and leaves can be used as a medicine that can cure various health problems such as malaria, dengue fever, immunomodulation, jaundice, antiviral activity, increasing white blood cells and platelets, normalizing blood clotting, and improving the liver (Alhanif et al., 2021; Rahayu et al., 2021). In addition to various health benefits, papaya leaves contain several chemical compounds, such as flavonoids, steroids, saponins, alkaloids, triterpenoids, and tannins (Lim et al., 2021).

Tannin is a secondary metabolite compound found in various plants. Naturally, tannins can dissolve and impart color to water. The color produced by tannins varies depending on the source used. For example, the tannin color in papaya leaves ranges from blackish-green brown to reddish-brown (Tuntun, 2016). According to several studies conducted, tannins have functions such as antioxidants, antibacterial, corrosion inhibitors, antifungal, astringents, natural dyes, anti-cancer, anti-diabetes, and anti-diarrheal (Sudira et al., 2019).



Note:
1. Microwave
2. Three-necked round-bottom flask
3. Timer
4. Power controller
5. Connector
6. Soxhlet
7. Condenser
8. Tubing for directing cooling water to the bucket
9. Tubing for directing cooling water to the condenser
10. Clamp
11. Stative

Figure 1. The setup of microwave assisted extraction equipment

The solvent selection during the extraction process should be based on the material's characteristics, following the principle that polar solvents will dissolve polar compounds and non-polar solvents will dissolve non-polar compounds. The polarity of a solvent and solute can be quantified based on solubility parameters such as solubility based on Hansen and Hildebrand, as mentioned in previous research (Alhanif et al., 2023). Tannins are polar compounds and can be extracted using polar solvents (Asni et al., 2020). Ethanol is a suitable solvent for extracting tannin because it is safe to use, has a lower boiling point than tannin, is affordable, is available in abundant quantities, and can extract tannin effectively. Based on previous research, tannin extraction still has several disadvantages: the long time required, low yield, and excessive use of solvents. As a result, many researchers began to develop various methods (Nurwahyuwono et al., 2021; Ugo et al., 2019).

Several studies have been carried out related to tannin extraction from papaya leaves. Juárez-Rojop et al. (2014) succeeded in obtaining a tannin yield of 0.824% from extracting papaya leaves with a 96% ethanol solvent for 8 hours using the soxhlet method. Ugo et al. (2019) reported that extraction of tannin from papaya leaves using the maceration method with methanol solvent for 48 hours yielded a 3.1 mg TAE/g sample. Both methods are grouped in the conventional category, with the main disadvantages being long extraction times and low yields. Recently, modern extraction methods have also been used. Nurwahyuwono et al. (2021) Tantin extraction from papaya leaves was carried out using a methanol solvent using a stirred extractor. They obtained the highest tannin yield of 0.13% at a temperature of 60°C, a stirring speed of 300 rpm, and an extraction time of 150 minutes. Even though this method can extract in a shorter time than the maceration and soxhlet methods, the tannin yield obtained is lower than the previous two methods.

Therefore, in this research, tannin extraction from papaya leaves was carried out using other methods, such as microwave-assisted extraction (MAE). MAE is an innovative technology used to extract metabolites from plant materials. This method operates by utilizing microwave energy as a heating source, thereby enabling the extraction of compounds present in the material carried along with the solvent (Alara et al., 2021). Various material-solvent ratios and microwave power (MP) were used in this research to obtain the best conditions for tannin extraction from papaya leaves.

2. MATERIALS AND METHODS

2.1 Materials

The materials used include papaya leaves obtained from South Lampung papaya plantations, DI water and 96% ethanol from a chemical shop in Bandar Lampung, $FeCl_3$ and Na_2CO_3 anhydrous for analysis (Supelco), Folin-Ciocalteu reagent pro analysis (Merck).

2.2 Tannin Extraction and Analysis

2.2.1 Preparation

Papaya leaves harvested from the tree are washed clean and then dried in an oven at 50°C until they reach a constant weight. Next, the dried papaya leaves were crushed and sieved with a 50-mesh sieve.

2.2.2 Extraction

A total of 10 grams of fine papaya leaf powder and 100 ml of 96% ethanol were put into a three-neck flask. The three-neck flask containing papaya leaf powder and ethanol solvent is put into the microwave and connected to the connector. The MP was set at 100 watts, and the extraction process was carried out for 30 minutes. The microwave-assisted extraction R220MAWH set used can be seen in Figure 1. The extraction process was then repeated with variations in solvent volume (200, 300, 400, and 500 ml) and MP (140 and 180 watts).

2.2.3 Evaporation of papaya leaves extract

After the extraction, the papaya leaf extract is purified using a rotary evaporator R-100 with cold trap buchi. It is placed in a boiling flask and connected to the rotary evaporator apparatus. The rotation speed is set to 70 rpm, and the evaporation temperature is maintained at 55°C. The evaporation process is considered complete when the solvent no longer drips.

2.2.4 Tannins qualitative analysis

One ml of papaya leaf extract is placed into a test tube, and 1 ml of 1% $FeCl_3$ is added. Observe any changes where the formation of a brownish-green precipitate indicates the presence of tannin compounds in the extract.

2.2.5 Tannins content analysis

The tannin content analysis was conducted in several stages using a Scientific GENESYS 150-type UV-Vis spectrophotometer. The steps in the tannin content test include the preparation of a standard tannic acid solution, the determination of the maximum wavelength, the establishment of a standard curve, and the quantification of tannin content.

3. RESULTS AND DISCUSSION

3.1 Tannins Qualitative Analysis

In this study, tannin extraction from papaya leaves was carried out using the MAE method, with an extraction time of 30 minutes. The variations in the ratio of material to solvent used are 1:10, 1:20, 1:30, 1:40, and 1:50, while the variations in MP used are 100, 140, and 180 watts. The results of the papaya leaf experiment are presented in Table 1.

Based on Table 1, it can be seen that the results of extracting papaya leaves with a ratio of 1:10 produce a brownish-green or brown color. The volume of solvent and extraction time greatly influence the resulting color (Rosida, 2016). This characteristic indicates a Maillard reaction where the color changes to dark (like brown), which occurs due to the heating of the material. The Maillard reaction consists of three main stages. The initial stage begins with the formation of glycosylamine. sugar (a carbohydrate) reacts with amino acids to form glycosylamine compounds. The second stage occurs when the glycosylamine compound formed is dehydrated. This process causes glycosylamine to change into various furan derivatives, reductones, and other carbonyl compounds. The final stage involves the conversion of furan derivatives and carbonyl compounds formed in the second stage. This reaction produces various flavor (aroma) and color compounds that give distinctive characteristics to foods that undergo the Maillard process (Savitri et al., 2017).

Table 1. Color characteristics of tannin extract from papaya leaves

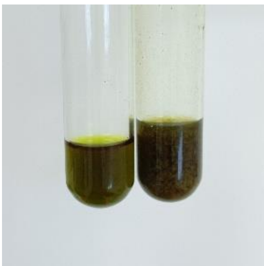
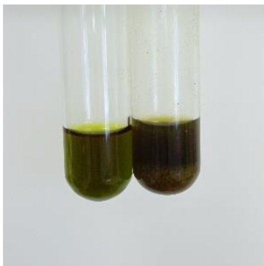
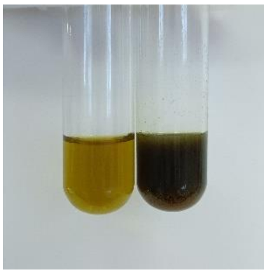
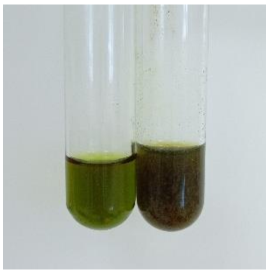
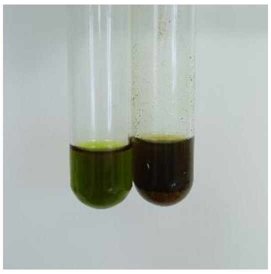
MP (Watt)	Material-Solvent Ratio (g/ml)	Tannin Extract Color
100	1:10	Greenish-brown
	1:20	Green
	1:30	Green
	1:40	Green
	1:50	Green
140	1:10	Brown
	1:20	Greenish-brown
	1:30	Green
	1:40	Green
	1:50	Green
180	1:10	Brown
	1:20	Greenish-brown
	1:30	Green
	1:40	Green
	1:50	Green

3.2 Qualitative Test for Papaya Leaves Extract

Identification of tannin compounds in papaya leaf extract was carried out using $FeCl_3$. The extract contains tannin if the addition of iron (III) chloride produces a greenish-brown precipitate (A'yun & Laily, 2015). The results of qualitative tests to identify tannin compounds in papaya leaf extract can be seen in Table 2.

The identification results showed the presence of tannin compounds, as indicated by forming a brownish-green precipitate after adding the $FeCl_3$ compound. The formation of a brownish-green precipitate is caused by the reaction between the tannin compound and iron (III) chloride, namely the formation of Fe^{3+} ions and the tricyanoferritricialiumFerri(III) compound (Halimu et al., 2017), as seen in Figure 2. The reaction to form complex compounds when tannin is reacted with $FeCl_3$ occurs due to the presence of an O atom with a lone pair of electrons that acts as a ligand, namely a simple molecule that can provide its electron pair to the central atom. In this reaction, the central atom is played by the Fe^{3+} ion. The Fe^{3+} ion binds to tannin, and two atoms are given as O elements in the 4' and 5' positions. Therefore, there are six pairs of unpaired electrons going to the central atom (Erginia & Pursitasari, 2014).

Table 2. Qualitative test results of papaya leaf extract

Material - Solvent Ratio	100 Watt Before-After Test	140 Watt Before-After Test	180 Watt Before-After Test	Alteration	Conclusion
1:10				Formation of a greenish-brown precipitate	Contains tannins
1:20				Formation of a greenish-brown precipitate	Contains tannins
1:30				Formation of a greenish-brown precipitate	Contains tannins
1:40				Formation of a greenish-brown precipitate	Contains tannins
1:50				Formation of a greenish-brown precipitate	Contains tannins

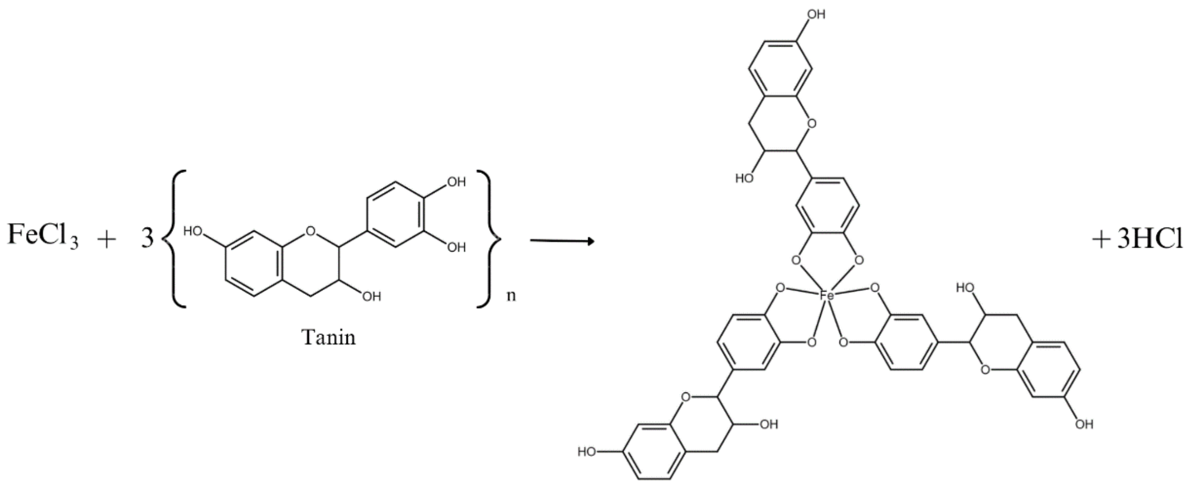


Figure 2. Reaction between tannin and $FeCl_3$

3.3 The Optimum Wavelength and Tannic Acid Standard Curve

The optimum wavelength of tannins was determined by identifying the absorbance at wavelengths between 500-800 nm using a UV-Vis spectrophotometer. The absorbance results for determining the optimum wavelength of a standard solution of tannic acid are shown in Figure 3. Based on Figure 3, the optimum wavelength for tannic acid is 746 nm. Therefore, a wavelength of 746 nm was used to determine the tannin content in papaya leaf extract. Next, a standard curve for tannic acid was created by measuring the absorption of standard solutions at various concentrations of 20, 40, 60, 80, and 100 ppm, as seen in Figure 4.

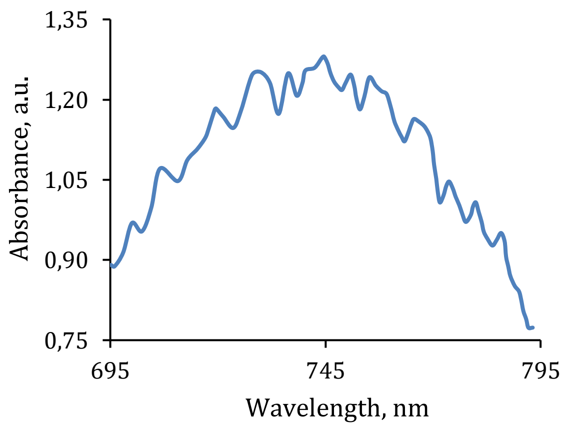


Figure 3. Optimal wavelength of standard tannic acid

Figure 4 produces a linear equation of the relationship between tannic acid concentration and absorbance measured at a wavelength of 746 nm. The linearity of the line is very good, with a correlation coefficient of 0.99426 (close to 1). These results show that the instrument used shows a good and accurate response and can establish a linear relationship between the absorption and the concentration of the measured solution. The concentration of a sample in a solution can be determined by measuring the absorbance at a certain wavelength using the Lambert-Beer law. Lambert-Beer's Law (Beer's Law) is a linear relationship between the absorption and concentration of a sample solution.

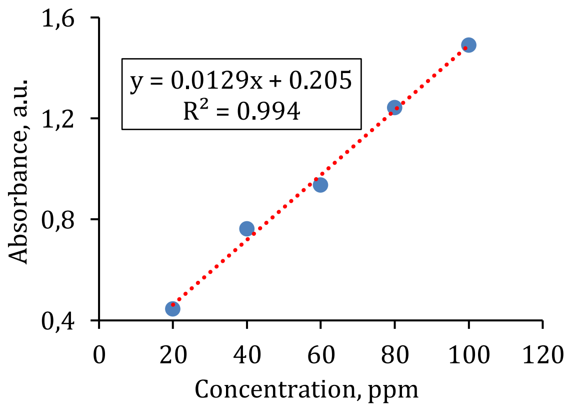


Figure 4. Standard tannic acid curve

3.4 Effect of Material-Solvent Ratio and Microwave Power on Tannin Concentration

The measurement of tannin content in papaya leaf extract at various solvent ratios and microwave power (MP) is shown in Figure 5. This study used a material-solvent ratio of 1:10-1:50, higher than previous studies. The aim is to obtain a higher extract than previous research, as reported by Indrasari & Buanasari (2022). They obtained optimum conditions for tannin extraction from Kedawung leaf using the MAE method at a solvent-material ratio of 1:40. Other results show that the extraction of tannins from coconut coir with ethanol solvent, a solvent-material ratio between 1:20 - 1:40 using the water-bath heating method and stirring obtained the highest yield of 11.08% at a ratio of 1:30, temperature 70°C, extraction time for 2 hours (Sirisangsawang & Phetyim, 2023).

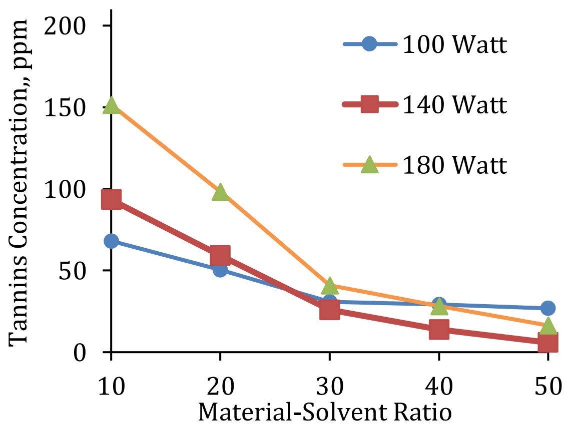


Figure 5. The effect of solvent ratio on the tannin content in papaya leaf extraction

However, based on Figure 5, the results show that the tannin concentration decreases as the solvent ratio increases. Increasing the volume of solvent affects the extraction time, where using a larger amount of solvent will result in a longer extraction time (Fajri & Daru, 2022). This phenomenon occurs because microwaves tend to be absorbed by the solvent, so only a small portion can be absorbed by the substance. In addition, the heat radiation emitted by the device cannot reach all dissolved substance particles with a higher amount of solvent. Therefore, in wave-based extraction (MAE and UAE), a high solvent ratio causes the extraction process not to run well (Indrasari & Buanasari, 2022). The results of this research are in line with research by Niawanti et al. (2022), which states that the yield of tannin from guava leaves decreases as the material-solvent ratio increases from 1:20 - 1:60. They obtained the highest tannin yield of 17.06% at a material-solvent ratio of 1:20, extraction temperature of 80°C, extraction time of 60 minutes.

The highest tannin concentration was obtained at 180-watt power, and a ratio of 1:10. Microwave power is correlated with the resulting temperature, where high microwave power causes an increase in solvent temperature. Increasing microwave power can also cause a decrease in surface tension and solvent viscosity, thereby increasing the solvent's ability to dissolve materials. This phenomenon aligns with this research, where the tannin concentration increases with increasing microwave power. Microwaves operate by emitting electromagnetic waves, and molecules in substances absorb energy from these waves to help break down cell walls (Nuri, 2014).

3.5 The Overall Tannin Content and Yield in Papaya Leaf Extraction

After obtaining the tannin concentration in the papaya leaf extract, the overall tannin content and yield (%) were calculated. Calculating the overall tannin content and yield requires data on the volume of papaya leaf extract, dilution factor, and concentration obtained from UV-Vis spectrophotometry results. The results of calculating tannin content, overall tannin content, and yield can be seen in Table 3.

Table 3. The overall tannin content and yield of papaya leaf extract

Power	Material to Solvent Ratio	Concentration (ppm)	Extract Volume (L)	Overall Tannin Content (mg TAE/g)	Yield (%)
100 watts	1:10	68.14	0.051	6.95	0.70
	1:20	50.39	0.065	6.55	0.66
	1:30	30.93	0.088	5.44	0.54
	1:40	29.22	0.092	5.38	0.54
	1:50	26.82	0.099	5.31	0.53
140 watts	1:10	93.33	0.019	3.55	0.35
	1:20	59.38	0.055	6.53	0.65
	1:30	25.81	0.074	3.82	0.38
	1:40	13.88	0.082	2.28	0.23
	1:50	5.89	0.091	1.07	0.11
180 watts	1:10	151.55	0.008	2.42	0.24
	1:20	98.30	0.041	8.06	0.81
	1:30	41.01	0.059	4.84	0.48
	1:40	28.22	0.070	3.95	0.40
	1:50	16.36	0.085	2.78	0.28

Table 4. Comparison of tannin extraction by various methods

Materials	Solvent	Extraction Methods	Optimum Conditions	Material-Solvent Ratio	Tannin Content (mg TAE/g)	Energy Consumption (kWh/g tannin)	References
Papaya leaves	Ethanol 96%	Microwave Assisted Extraction (MAE)	180 watts of MP; extraction time 30 minutes	1:20	8.06	11*	This study
	Methanol	Maceration	Extraction time of 48 hours	1:3	3.10	-	(Ugo et al., 2019)
	Ethanol 96%	Stirred extractor	Temperature 60°C; 300 rpm; extraction time of 150 minutes	N.R.	0.13	-	(Nurwahyuwono et al., 2021)
	Ethanol 96%	Soxhlet	Ambient temperature; extraction time of 8 hours	1:5	0.824	139**	(Juárez-Rojop et al., 2014)

Note: N.R. = Not Reported; *Equation from (Alara and Abdurahman, 2019); **(Zainutdinova et al., 2023)

Table 3 for 100 watts reveals that increasing solvent volume decreases overall tannin content and yield. The highest tannin content at 100 watts was found in a ratio of 1:10, with an overall tannin content of 6.95 mg TAE/g and a yield of 0.7%. At 140 watts, the overall tannin content and yield tend to fluctuate. At 1:10 and 1:20, the overall tannin content and yield increased, decreasing at 1:30, 1:40, and 1:50. In a ratio of 1:10, the tannin content and yield obtained were relatively low. This can be caused by the lack of solvent used to extract all the tannins in papaya leaves (Erfiza et al., 2016). In a ratio of 1:20, the tannin content and yield obtained were relatively high. This can be due to the amount of solvent sufficient to extract all the tannins found in papaya leaves, and the volume of solvent used is not excessive. As a result, extraction at this volume can effectively break down cell walls (Yulistiani et al., 2020). At a ratio of 1:30 to 1:50, the results show a decreasing trend. This shows that the volume used is too large, which can inhibit the absorption of microwave waves in the material and cause a lack of ability to damage cell walls effectively (Amaranti et al., 2021).

3.6 Comparison of Tannin Extraction by Various Methods

A comparison of the results of this research with previous research can be seen in Table 4. In general, it can be seen that the MAE method has advantages in producing high tannin yields in a shorter time compared to the maceration, stirred extractor, and soxhlet methods. The main advantage of the MAE method is its ability to quickly heat the sample solvent mixture, resulting in higher yields. This method is widely applicable for the rapid extraction of analytes, including thermally unstable substances like tannins (Kataoka, 2019). However, Figure 5 shows that increasing the material-solvent ratio reduces the obtained tannin concentration. Microwave power is the primary factor driving the increase in tannin extraction yield. In terms of energy consumption, the MAE method requires lower energy (11 kWh/g tannin) than the Soxhlet method (139 kWh/g tannin) (Alara & Abdurahman, 2019; Zainutdinova et al., 2023).

4. CONCLUSION

The qualitative test revealed tannin content in all solvent and microwave power variations, as evidenced by the formation of a greenish-brown precipitate in the $FeCl_3$ test results. The best conditions were obtained at a material-solvent ratio of 1:20 with an MP of 180 watts, producing tannins of 8.06 mg TAE/g and a yield of 0.81%. Increasing the solvent ratio affects the extraction time and inhibits microwave wave absorption into the material. The MP correlates with solvent temperature, and higher power causes an increase in solvent temperature. Therefore, the solvent's ability to dissolve the material and produce tannin increases. According to previous research, MAE has superior capabilities to conventional methods such as maceration, soxhlet, and stirred extraction. The data obtained may help optimize the tannin extraction process on a larger scale and have potential in the health sector.

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