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Journal of Biobased Chemicals

Department of Chemical Engineering
Universitas Jember

PREFACE

We would like to present our journal, Journal of Biobased Chemicals published by Department of Chemical Engineering, University of Jember, Indonesia. This is expected to enhance the findings and research about natural product and the derivatives, mostly in energy, chemicals and materials. We present the articles related to the products, process, and management for biobased chemicals.

This new journal was envisioned and founded to represent the growing needs of biobased chemicals researches as an emerging and increasingly vital field, now widely recognized as an ideal substitution of fossil based chemicals. The journal has objective to deliver and provide notable and standardize research and finding through journal reporting. The journal is intended as a window or a library for practitioners and researchers to share their works, to identify new issues, and to organize further research, while industrial users could apply the invention for scale-up, problem solving, and the application.

Hopefully, this edition would contribute a valuable thought for the readers and enhance a future research related to biobased chemicals product. Finally, we send the gratitude to all participants including authors, reviewers, and editors due to the contribution.

August 2022

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Journal of Biobased Chemicals

Journal of Biobased Chemicals provides any study on product, process, and management related to biomass valorization. The journal implements a regular system in terms of upload, review, and acceptance of the journal. Moreover, the journal is supported by an expert team in their own field to maintain quality of the publication.

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Hydrolysis of Mixed Sugarcane Bagasse and Rice Husk Using Cellulase Enzyme for Reducing Sugar

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Abstract. Reducing sugar can be produced from lignocellulosic raw materials. The content of polysaccharides such as cellulose, hemicellulose, and starch will be broken down into simpler carbohydrates. This study used a mixture of sugarcane bagasse and rice husks as lignocellulosic raw materials. The lignin content in the raw material must be removed through delignification or pretreatment so that enzymes can access cellulose and hemicellulose. This study used a physics-chemical pretreatment method, in which lignocellulosic material soak in 3% NaOH then heated with microwave and boiling water. The next process is enzymatic hydrolysis with variations of cellulase enzymes activity 0.434, 0.871, 2.61, and 3.49 FPU/g mixture of bagasse and rice husks. The cellulase enzyme used in this study was also derived from the fungus *Trichoderma viride*. Analysis of the sugar concentration resulting from hydrolysis used the DNS method with the 3.5-dinitrosalicylic acid reagent. The concentration of sugar from hydrolysis using a variety of enzymes with microwave heating pretreatment and boiling water pretreatment obtained the highest results which were the same at the addition of enzyme activity 3.49 FPU/g substrate at 24 hours, namely 4.077 g/L and 15.18 g/L. The optimum time for enzymatic hydrolysis is 12 hours and optimum enzyme activity is the addition of enzyme activity 2.61 FPU/g. The average concentration of sugar hydrolyzed by the addition of *Trichoderma viride* solution in pretreatment using microwave heating was 0.7611 g/L with a yield of 21.01 mg sugar/g substrate and with pretreatment in boiling water obtained 0.8679 g/L with a yield of 23.95 mg sugar/g substrate.

Keywords: *sugarcane bagasse, rice husk, enzymatic hydrolysis, lignocellulose, reducing sugar, and trichoderma viride*

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1. Introduction

Until now, many studies have used biomass waste as raw material to produce reduced sugar. Reducing sugar can be produced from the hydrolysis of lignocellulosic materials because the lignocellulosic structure can be converted into reducing sugars and has the potential to be further processed for the manufacture of butanol, acetone, ethanol, and other products with higher economic value [1]. Examples of reducing sugars are all monosaccharides (*glucose*, *fructose*, *galactose*), disaccharides (*lactose*, *maltose*) except sucrose and starch (*polysaccharides*) [2]. The content of cellulose and hemicellulose in lignocellulosic materials has the potential as a source of reducing sugar production. Polysaccharides will be crashed into simple sugar monomers such as reducing sugars [1]. Enzymatic hydrolysis can be chosen as a more environmentally friendly method than hydrolysis using acid to produce high concentrations of reducing sugars [1].

Biomass raw materials are available in abundance and are not used as food so that their use as alternative fuels or other economically valuable materials does not interfere with the availability of food. Biomass can be produced from plants, agricultural waste, and industrial waste [3]. Agricultural waste in Indonesia reaches 19.5 megatons per year for the main commodities, namely rice husks, cassava peels, sugar cane bagasse, coffee grounds, and cocoa husks (BPS Indonesia, 2018). In this study, mixed biomass from rice husks and bagasse agricultural waste is used because there is still no research that uses mixed raw materials of agricultural waste. In the manufacture of sugar reduction, raw materials through several steps are pretreatment, hydrolysis, and fermentation, so to produce reducing sugar through 2 steps namely pretreatment and hydrolysis [4].

Pretreatment is classified into several methods, are physical, physics-chemical, chemical, and biological pretreatment [5]. Some of the common pretreatment methods can be combined. Microwave heating is generally used in combination with other pretreatment methods, especially chemical treatments [6]. Pretreatment with boiling water heating is suggested as one of the leading pretreatment methods [5]. In this study, before pretreatment using a microwave and heated with boiling water, raw materials were soaked in NaOH 3%. The pretreatment methods with microwave heating and boiling water heating are more often recommended for use as well as a method that is suitable for laboratory scale, therefore the two pretreatment methods are compared with the effect of sugar concentration resulting from hydrolysis in this study.

Hydrolysis can be done chemically, biologically, and enzymatically. Enzymatic hydrolysis has several advantages compared to acid hydrolysis which provides high sugar results and relatively low maintenance costs because there are no corrosive materials [7]. Acid hydrolysis has the disadvantage that is not environmentally friendly. The hydrolysis method assisted by microorganisms can be compared with enzymatic hydrolysis in this study. From this study, it is expected that the hydrolysis results will obtain high sugar concentrations and optimum time for enzymatic hydrolysis.

2. Materials and Methods

2.1. Materials

The materials used in this study were bagasse taken from a sugar factory in Jember and rice husks taken from rice processing Wirolegi Jember, cellulase enzymes, *Trichoderma viride* culture was obtained from microbiology laboratory, FMIPA Universitas Jember, sodium citrate, citric acid, sodium hydroxide (NaOH), aquadest, dinitrosalicylic acid (DNS), potassium sodium tartrate ($KNaC_4H_4O_6.4H_2O$), sodium metabisulfite ($Na_2S_2O_5$).

2.2. Pretreatment

Twenty-five grams mixture of bagasse and rice husks were each soaked in 250 ml of 3% NaOH solution for 30 minutes. Raw materials that have been soaked can be heated in the microwave for 4 minutes and heated with boiling water for 15 minutes.

2.3. Hydrolysis

Enzymatic hydrolysis with the addition of cellulase enzyme activity, 5 grams of delignified sample was added to a flask then added 50 ml of citrate buffer solution pH 4.8. The cellulase enzyme used was *Viscozyme cassava* CL with an enzyme activity of 709 EGU/g. Each added enzyme activity of 0.434, 0.871, 2.61, and 3.49 FPU/g in a flask then hydrolyzed in an incubator shaker with temperature 50 ºC and speed 160 rpm for 24 hours. The sample was taken every 0, 6, 12, 24 hours.

3. Result and Discussion

3.1. Enzymatic Hydrolysis with The Treatment of Variations in Cellulase Enzyme Activity

Cellulase enzymes are biocatalysts that help support hydrolysis reactions. Sugar from the hydrolysis of polysaccharide components can be calculated from absorbance obtained after analysis using the DNS method. The higher absorbance, the higher sugar concentration obtained [8]. There is a component in the cellulase enzyme that can break the bonds in cellulose, namely endoglucanase (*endo*-β-1.4-D-*glucan*-4 *glucanohydrolase*) breaks down β-1.4-

glucanohydrolase bonds in the cellulose chain at random, exoglucanase (β-1.4-D-*glucancellobiohydrolase*) which breaks down cellobiose units from the end of the chain and β-glucosidase which breaks down cellobiose into glucose [9].

Table 1. Enzymatic hydrolysis sugar concentration by microwave heating pretreatment

Time (h)	Concentration (g/L)			
	M_1	M_2	M_3	M_4
0	0.7789	0.8011	0.8056	0.8323
6	2.880	3.133	3.151	3.258
12	3.089	3.365	3.961	3.970
24	3.245	3.383	4.063	4.077

Description: M_1: Addition of enzyme activity 0.434 FPU/g
M_2: Addition of enzyme activity 0.871 FPU/g
M_3: Addition of enzyme activity 2.61 FPU/g
M_4: Addition of enzyme activity 3.49 FPU/g

Based on Table 1, the sugar concentration increases with time increases. This is because the enzymes and raw materials collide with each other and react more so that the conversion is higher. The higher enzyme activity added, the higher sugar concentration obtained. This is because higher enzyme activity will hydrolyze more cellulose into sugar, also the higher enzyme activity, the reaction speed will increase [10]. The highest sugar concentration was obtained from the addition of enzyme activity 3.49 FPU/g (M_4) at 24 hours, namely 4.077 g/L. Most of the cellulase enzymes have optimum activity in the temperature range of 20 – 50 °C and the optimum pH range for cellulase activity is 4.5 – 7.0 [11]. If the temperature conditions increase to the optimum temperature, the rate of enzyme reaction will increase because the kinetic energy increases.

Table 2. Enzymatic hydrolysis sugar concentration by hot liquid water pretreatment

Time (h)	Concentration (g/L)			
	G_1	G_2	G_3	G_4
0	3.925	3.957	4.077	4.424
6	8.011	8.145	8.367	9.969
12	8.278	8.768	12.55	14.15
24	8.590	9.124	13.13	15.18

Description: G_1: Addition of enzyme activity 0.434 FPU/g
G_2: Addition of enzyme activity 0.871 FPU/g
G_3: Addition of enzyme activity 2.61 FPU/g
G_4: Addition of enzyme activity 3.49 FPU/g

Based on Table 2, the highest sugar concentration of 15.18 g/L resulted from the addition of the highest enzyme activity of 3.49 FPU/g, namely G_4 with a hydrolysis time of 24 hours. The

speed of the reaction also depends on the concentration of enzyme, where the reaction speed will increase as the concentration of the enzyme increases [8].

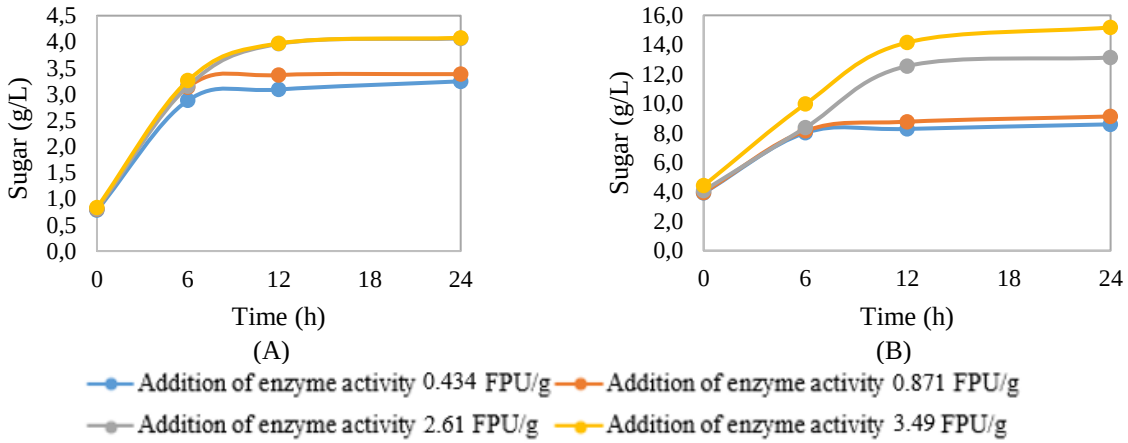

Figure 1. Graph of sugar concentration with cellulase enzyme variation treatment (A) microwave heating pretreatment (B) boiling water heating pretreatment

From Figure 1, it can be seen that the sugar concentration increased with each enzyme variation treatment. After hydrolysis for 24 hours the lowest sugar concentration was obtained from the addition of the smallest variation of the enzyme, namely 0.434 FPU/g with 3.245 g/L for pretreatment using microwave heating and 8.590 g/L for pretreatment using boiling water heating. The highest concentration from the addition of enzyme was 3.49 FPU/g with 4.077 g/L for pretreatment using microwave heating and 15.18 g/L for pretreatment using boiling water heating. From this statement, the addition of enzyme activity 2.61 FPU/g is quite an optimum enzyme activity, because the results of the sugar concentration are not much different from the treatment with the addition of enzyme activity 3.49 FPU/g.

Based on Figure 1, it can be concluded that the greater enzyme concentration, the more sugar concentration obtained and can increase the rate of hydrolysis to a certain concentration limit [12]. The increase in sugar concentration from the 12 - 24 hours of each enzyme variation treatment was not very significant or relatively constant because if it exceeded the optimum time, sugar inhibitors would form so that the sugar concentration produced was smaller or relatively constant [13]. It can be concluded that the optimum time for hydrolysis is 12 hours. The results of substrate hydrolysis will be constant with increasing enzyme concentration because the addition of enzymes is no longer effective [10].

3.2. Enzymatic hydrolysis with the addition of Trichoderma viride

The hydrolyzate sampling time was carried out on the 7th day of hydrolysis. The optimum operating temperature conditions for the growth of *Trichoderma viride* are at 20 ºC

– 36 ºC [14]. The operating temperature condition used in this study is 28 ºC, which is still included in the optimal temperature range so that the enzyme can work optimally in hydrolyzing cellulose to produce sugar concentrations. Cellulase enzymes produced from *Trichoderma viride* affect breaking the complex bonds of cellulose into simpler bonds, namely sugar.

Table 3. Sugar concentration and yield of hydrolysis addition of solution *Trichoderma viride* with microwave heating pretreatment

Repetition	Sugar concentration (g/L)	*Yield* (mg sugar/ g substrate)
1	0.9480	26.16
2	0.4273	11.79
3	0.9079	25.06

Based on Table 3, it can be seen that the average sugar concentration is 0.7611 g/L with an average yield of 21.01 mg sugar/g substrate for raw materials that are treated with microwave heating. One of the microbes that can produce cellulase enzymes is *Trichoderma sp*. *T. viride* can produce cellulase enzymes consisting of endoglucanase, exoglucanase and β-glucosidase [14].

Table 4. Sugar concentration and yield of hydrolysis addition of solution *Trichoderma viride* with boiling water heating pretreatment

Repetition	Sugar concentration (g/L)	*Yield* (mg sugar/ g substrate)
1	0.8412	23.22
2	0.8545	23.58
3	0.9079	25.06

From Table 4 it can be seen that the average sugar concentration obtained is 0.8679 g/L with a yield of 23.95 mg sugar/g substrate. The results of hydrolysis with pretreatment heated in boiling water obtained a higher concentration than hydrolysis with pretreatment using microwave heating. This can be interpreted that lignin content is reduced a lot so that there is a lot of decomposition of polysaccharide component by the cellulase enzyme from *Trichoderma viride* with pretreatment heated in boiling water.

There are 10 types of cellulosic enzymes produced by *Trichoderma viride* that work together to break down cellulose material [7]. Amorphous cellulose can be hydrolyzed by endoglucanase which is randomly soluble and crystalline cellulose can be degraded by cellobiohydrolase to produce cellobiose. These two types of enzymes work together to degrade

cellulose into cellobiose and other short cellooligosaccharides. β-glucosidase enzyme to hydrolyze cellobiose and other cellooligosaccharides produced by cellulase into glucose [7].

3.3. Effect of pretreatment for enzymatic hydrolysis

Hydrolysis by pretreatment heated in boiling water obtained a higher sugar concentration every hour based on Table 2 also obtained a higher sugar concentration based on Table 4 compared with the results of sugar concentration in Tables 1 and 3, namely hydrolysis where the raw material was pretreated with microwave heating. The pretreatment process with longer heating will damage most of the lignin structure, so enzymes can more easily access cellulose and hemicellulose so that the hydrolysis process runs more easily and a higher sugar concentration is obtained [15].

In the enzymatic hydrolysis process using lignocellulosic materials, pretreatment is an important step taken to increase the accessibility of cellulose-degrading enzymes [16]. The conversion of lignocellulosic biomass materials into sugars is carried out through a pretreatment process to open the biomass structure and release sugar groups from cellulose and hemicellulose and increase the porosity of the material [17]. Microwave heating is generally used in combination with other pretreatment methods. Microwave heating with a combination of pretreatment using an alkali has been widely studied, mainly because the results obtained by alkaline solvents are better and recommended and have been shown to produce high sugar yields and higher lignin removal compared to acidic solvents [6]. Boiling water heating pretreatment is suggested as one of the leading pretreatment methods [5].

There are several advantages of using a microwave as a pretreatment method, namely faster heating rate, shorter reaction time, and high energy efficiency. The main drawback of microwave heating is the non-uniform heat profile [6]. When lignocellulosic biomass is heated by microwave, selective heating of polar molecules is observed due to the effect of dipolar polarization. This selective heating also decreases the crystallinity of cellulose. In the presence of polar solvents, hot spots can cause rupture or explosion of some lignocellulosic structures [6]. The advantages of using boiling water heating are there is no need to reduce the particle size of the substrate, effective cost because there is no addition of other chemicals, not corrosive. The purpose of using boiling water heating is to trigger changes in the structure of lignocellulose to make cellulose more accessible to enzymes, hemicellulose on heating is maintained in the form of oligomers and the formation of monomers is minimized [5].

Based on Table 2 with the highest sugar concentration of 15.18 g/L and table 4 with an average sugar concentration of 0.8679 g/L, the highest sugar concentration was obtained from pretreatment of raw materials using boiling water heating for 15 minutes. This is because when soaking with 3% NaOH for 30 minutes, the lignin structure, the crystalline, and amorphous parts were damaged by NaOH solution, the solution also separated some of the lignin and hemicellulose and caused swelling of the cellulose [18]. Several studies have been conducted on the superiority of NaOH as a pretreatment solution for lignocellulosic materials. The strongest alkali catalyst that is effective in increasing the rate of enzymatic hydrolysis is NaOH solution compared to other alkali solvents [19]. The cellulose content after pretreatment increased. In addition to the NaOH solution, heating can also damage the lignin structure. The longer the delignification process uses heat, the more lignin is degraded.

4. Conclusion

Enzymatic hydrolysis with the method of adding variations in enzyme activity obtained a higher sugar concentration than hydrolysis with the addition of *Trichoderma viride*. the optimum time for enzymatic hydrolysis was 12 hours with the optimum variety of enzyme activity 2.61 FPU/g. Pretreatment by heating in boiling water which combined with 3% NaOH immersion obtained a higher sugar concentration because a lot of lignin was degraded, thereby increasing the accessibility of cellulose-degrading enzymes.

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Comparison of Pegagan (*Centella asiatica* (L.)) Extraction with Ultrasound-Assisted Extraction and Microwave-Assisted Extraction Methods Using Response Surface Methodology

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Abstract. Pegagan (*Centella asiatica* (L.)) is one of the herbaceous plants that grow, and flowers throughout the year. Pegagan has been used for hundreds of years, especially in the dermatology, and cosmetology industries. Pegagan (*Centella asiatica* (L.)) has distinctive bioactive components, namely triterpene ester glycoside compounds in the form of asiaticoside, and madecoside as well as triterpene group compounds in the form of asiatic acid, and madecasic acid. There are various extraction methods to produce the following compounds namely, conventional extraction methods, and modern extraction methods. The two methods have differences in the results obtained. Therefore, the difference in the extraction method will determine the result of the difference in the levels of a compound. So that this research was carried out on the effect of Pegagan (*Centella asiatica* (L.)) extraction using a comparison of the Microwave-Assisted Extraction (MAE), and Ultrasound-Assisted Extraction (UAE) methods on the total phenol content in the Pegagan (*Centella asiatica* (L.)) extracted. Analysis of the extracteded bioactive content was carried out by analyzing the total phenol content using the Folin-Ciocalteau reagent method and analyzing the data through the Analysis of Response Surface Design expert 11 to see the significance of the research variables. The results obtained from the study are in the form of a comparison of the optimal conditions for the total phenol content, namely, the comparison between the UAE, and MAE methods for *Centella asiatica* (L.). extracted with ethanol solvent which is more optimal by using the MAE method because in a short time it can produce 1130,7882 mg.

Keywords: *Ultrasound-Assisted Extraction, Microwave-Assisted Extraction, Centella asiatica* (L.), *Phenolic Test.*

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1. Introduction

Lately, there are many types of new modern medicines on the market, there is a global tendency to return to nature (back to nature). Some of the factors that encourage people to utilize natural medicines, among others, are the high prices of modern/synthetic medicines, and the many side effects contained in these drugs. Therefore, natural medicines are now increasingly popular, and in dem, and by the public, and their use is increasing not only in developing countries such as Indonesia, but also in developed countries such as Germany, and the United States. One of the plants that have medicinal properties is Pegagan (*Centella asiatica* (L.)) [1].

Pegagan (*Centella asiatica* (L.)) is one of the herbaceous plants that grow, and flowers throughout the year. Pegagan is often found in rice fields, between grasses, on slightly moist soil, and can also be found in the lowl, and to the hight. This plant grow in tropical Asia, and grow in various countries such as the Philippines, China, India, Sri Lanka, Madagascar, and Indonesia [2]. Pegagan is one type of plant that has been widely used by people in Asian countries. Pegagan contains various beneficial compounds that can be used as medicines, natural fungicides, and antimicrobials [3]. The Pegagan herb is used to treat abdominal pain, cough, bloody cough, wound healing dysentery, inflammation, aches, and pains, asthma, hemorrhoids, tuberculosis, leprosy, fever, diabetes,and appetite enhancer [1]. Several researchers have tested the content of Pegagan compounds, such as: Pegagan extracted contains compounds that are antimicrobial, and anti-fungal [4], as an antioxidant [5] and anticancer [6].

The components of the compounds contained in Pegagan are triterpenoids, including pentacyclic triterpenic acids, and glycosides, which consist of: asiatic acid, asiaticoside, mandecassic acid, m, andecassoside, brahmoside, brahmic acid, brahminoside, thankuniside, isothankuniside, centalloside, madasiatic acid, centic acid. Pegagan also contains a flavonoid compounds such as: quercetin, kaempferol, and astragalin. Hydrocotylin alkaloids, as well as phytosterols, stigmasterol, and sitosterol. Several other compounds are tannins, amino acids, B vitamins , and resins [3]. There are various extraction methods to produce the following compounds namely, conventional extraction methods, and modern extraction methods.

Conventional extraction methods include maceration, and reflux. Mean while, modern extraction methods include Microwave-Assisted Extraction (MAE), and Ultrasound-Assisted Extraction (UAE) according to [7], extraction using the MAE method on onion skin with a power of 800 watts for 6 minutes produces a flavonoid levels of 17.18% and extraction using the maceration method for 3 days at room temperature produces a flavonoid levels of 14.92%.

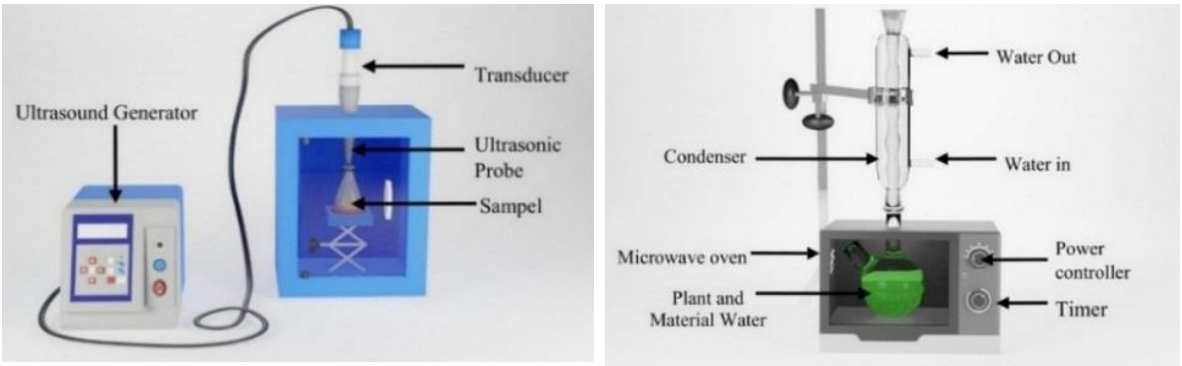
The results of another study conducted on white tea leaves showed that the optimum time, and temperature obtained for the UAE method was 40 for 19.5152 minutes with a flavonoid content of 0.39%.℃ [7]. In addition, research conducted on corn cobs showed that extraction using the reflux method with a temperature of 50 for 2 hours produced a phenolic content of 0.03%.℃ [8]. Therefore, the difference in the extraction method will determine the result of the difference in the levels of a compound.

Based on the problems and facts above, a study was conducted on the effect of Pegagan (*Centella asiatica* (L.)) extraction by using a comparison of the Microwave-Assisted Extraction (MAE) and Ultrasound-Assisted Extraction (UAE) methods on the total phenol content of the Pegagan (*Centella asiatica* (L.)) extracted. With this research, it is expected to find out the most optimum method to be able to determine the optimal conditions by producing the highest total phenol content in the Pegagan (*Centella asiatica* (L.)) extracted.

2. Materials and Methods

2.1. Materials

Pegagan leaves (*Centella asiatica* (L.)) were obtained from the Sumberarum area, Songgon District, Banyuwangi. The Pegagan leaves are dried in the sun for 2 days, the dried Pegagan leaves are crushed using a blender until they become powder, the Pegagan powder is sifted using a 40mesh sieve. Data processing using expert design.



(a) (b)

Figure 1. Tool Schematic (a) Ultrasound-Assisted Extraction (b)

In Figure 1 (a) this *Ultrasound-Assisted Extraction* method uses a probe-type Sonicator while for Figure 1 (b) *Microwave-Assisted Extraction* uses a microwave oven type Electrolux model EMM2308X, 23 liters, max temperature 250, dimensions 292.5 x 485 x 370 mm. Uses 800 W of power ℃.

The research procedure was carried out through 3 stages, including, (1) sample preparation, extraction using the method *Ultrasound-Assisted Extraction*, and *Microwave-*

Assisted Extraction, (3) Analysis of research results. Pegagan leaves were dried in the sun for 2 days with the determination of physical drying. Then mashed with a size of 40 mesh with a mass of 1 gram. Furthermore, the extraction was carried out with several variables including the UAE method of concentration variables of 25%, 50%, 75%, time variables 20 minutes, 40 minutes, and 60 minutes, power variables of 30 watts, 150 watts, and 270 watts. As for the MAE method, the variables are concentration variables of 25%, 50%, and 75%, time variables are 5 minutes, 10 minutes, and 15 minutes. Variable power of 150 watts, 300 watts, 450 watts. The extraction results were stored in an 8 ml vial at a temperature of 4℃.

2.2. Preparation of standart solution of gallic acid 100 ppm

Weighed 0.01 grams of gallic acid, then added 1 ml of ethanol, and added 1 distilled water until the volume became 100 ml.

2.3. Determination of the maximum wavelength of gallic acid

Take 1 ml of 100 ppm gallic acid mother liquor, put it in a test tube, and add 1 ml of Folin's reagent. Then the solution mixture was shaken until homogeneous, and allowed to stand, and at room temperature for 4-8 minutes. Added 4 ml of 10% Na_2CO_3 solution into a test tube shaken until homogeneous, and allowed to stand, and for 15 minutes at room temperature. Analyzed using UV-vis spectrophotometer with a wavelength range of 700 – 800 nm [9] .

2.4. Preparation of Gallic Acid Calibration Curve with Folin-Ciocalteu Reagent

Take 100 ppm gallic acid mother liquor, take 1 ml each, 3 ml, 5 ml, and 7 ml. Then diluted with distilled water to a final volume of 10 ml, so that a solution with a concentration of 10 ppm, 30 ppm, 50 ppm, and 70 ppm will be obtained. 0.2 ml of each solution was taken, and put into a test tube, and added 1 ml of Folin Ciocalteu reagent, shaken until homogeneous, allowed to stand for 8 minutes. Then 3 ml of 10% Na_2CO_3 was added, shaken until homogeneous, allowed to stand for 30 minutes at room temperature. Measure the absorption with the maximum wavelength that has been obtained previously. Then a calibration curve is made with the regression equation $y = ax + b$ [9].

2.5. Determination of total phenol content using the Folin-Ciocalteu method

Take 0.1 ml of extracted, add 9.9 ml of distilled water (dilution 100 times), and add 1 ml of Folin-Ciocalteu reagent then shake until homogeneous, and let stand for 8 minutes. Then add 3 ml of 10% Na_2CO_3 to the mixture, shake until homogeneous, and leave the solution for 1 hour at room temperature. Measure the absorption with a UV-vis spectrophotometer at its maximum wavelength. The content analysis was repeated 3 times so that the phenol content

obtained was as mg gallic acid equivalent/gram of fresh sample. Repeat 3 times so that the total phenol level is expressed as equivalent gallic acid (Acid Equivalent Gallic/AGE) [9].

3. Result and Discussion

3.1. Gallic acid standart curve

Determination of total phenol content begins with determining the standartgallic acid curve. The aim is to determine a linear regression equation which is then used in determining the total phenol content in the sample. The standartgallic acid curve was obtained by measuring the absorbance of a series of standard solutions of gallic acid with several concentrations of gallic acid, namely 10 ppm, 30 ppm, 50 ppm, and 70 ppm. Results From the measurement of the maximum wavelength of 765 nm. Based the results of making a standart curve using a UV-vis spectrophotometer, the absorbance measurement of the standartsolution of gallic acid from several concentrations was measured based on the maximum wavelength obtained, namely 765 nm. The following are the results of the standartgallic acid curve, and the straight-line equation that will be used to determine the total concentration of phenol.

Based the curve in figure 2, the equation of a straight line is obtained, namely y = 0.0078x + 0.2144. With the value of the correlation coefficient (R^2) = 0.9949. The correlation coefficient value shows the strength of the relationship between two variables. The strength and weakness of the relationship between the two variables, measured on an interval scale of 0-1. If the correlation coefficient value is close to 1, then the two variables have a very strong relationship [10] .

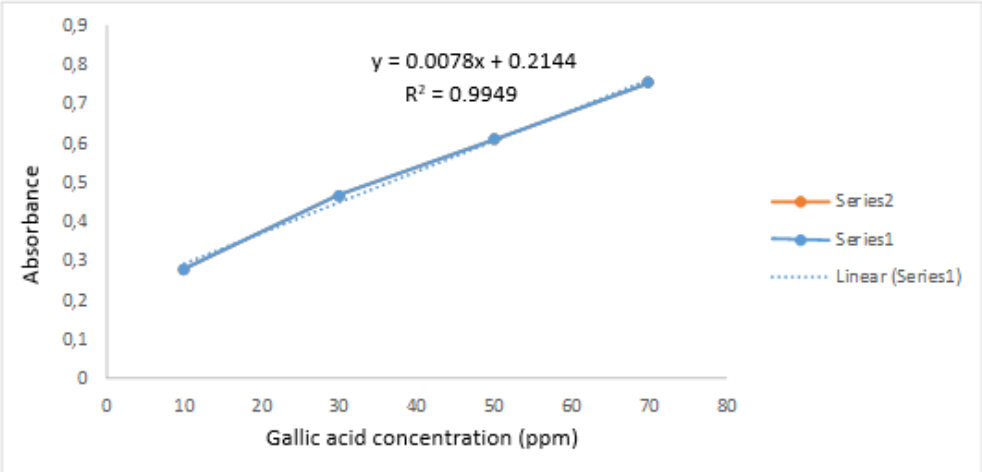


Figure 2. Gallic acid calibration curve *at 765nm wavelength.*

3.2. Extraction results of Pegagan (*Centella asiatica* (L.)) *Using the UAE method*

The results of absorbance and total phenol resulting from the research that has been done can be seen in Table 1 below.

Table 1. Total Phenol Content in Pegagan Plant Extracted (*Centella asiatica* (L.)) in UAE

No	Solvent Concentration (%)	Time (Min)	Power (Watts)	Absorbance (Average)	Total Phenol (mg.AGE/g)
1	50	40	150	0.675	589.87
2	50	40	150	0.704	627.05
3	50	40	150	0.656	566.15
4	75	20	150	0.507	357.13
5	75	40	270	0.621	521.28
6	50	40	150	0.682	598.85
7	75	40	30	0.432	278.97
8	25	40	30	0.442	291.79
9	25	60	150	0.531	405.90
10	50	20	270	0.732	662.95
11	50	60	270	0.867	836.67
12	25	40	270	0.662	573.21
13	50	40	150	0.707	631.54
14	50	60	30	0.561	443.72
15	75	60	150	0.563	446.92
16	25	20	150	0.544	422.56
17	50	20	30	0.603	498.21

It can be seen from the Table above that the highest total phenol content was 836.67 mg.AGE/g of the sample in the UAE variable power of 270 watts with a concentration of 50% for 60 minutes.

Analysis of response surface design with ANOVA (*Analysis of Variance*)

From Table 2 it can be seen from the significance of the variables used, the variable is said to be significant or significant if the p-value <0.05. The F-value of the model obtained from the calculation is 62.19. In the F distribution Table, it can be seen that the value of the F Table with the df model is 9, and the df error value is 4 at a probability of 0.05, the percentage point is 6.00 which means that the F-value is greater than the F Table. The hypothesis used in the F-value, namely, H_0 is the absence of a relationship between the independent, and dependent variables, while for H_1 is the presence of a relationship between the independent, and dependent variables. H_1 is accepted if the F-value is greater than the F-Table, while H_0 is accepted if the F-value is smaller than the F-Table. So that based on the existing F-value shows that F-value > F-Table, then this indicates a rejection of H_0 which means that H_1 is accepted. H_1 is a hypothesis which states that there is a significant or significant effect between the dependent variable, and the independent variable that has been tested, and analyzed.

Table 2. Results of ANOVA Analysis on Pegagan Plant Extracted UAE Method

Source	Sum of Square	df	Mean Square	F-Value	P-Value	
Model	3.217E+05	9	35742.48	62.19	<0.0001	Significant
A-Concentration	632.97	1	632.97	1.10	0.3289	
B-Time	3800.18	1	3800.18	6.61	0.0369	
C-Power	1.426E+05	1	1.426E+05	254.35	<0.0001	
AB	1955.85	1	1955.85	3.40	0.1076	
air conditioning	382.40	1	382.40	0.6653	0.4415	
BC	13019.95	1	13019.95	22.65	0.0021	
A2	1.553E+05	1	1.553E+05	270.26	<0.0001	
B2	16.93	1	16.93	0.0295	0.8686	
C2	136.33	1	136.33	0.2372	0.6411	
Residual	4023.21	7	574.74			
Lack of Fit	1083.21	3	361.07	0.4912	0.7072	Not Significant
Pure Error	2940.00	4	735.00			
Total Cast	3.257E+05	16				

In addition, the F-value is inversely proportional to the value of the p-value, in Table 2. The P-value is said to be significant or significant if the P-value <0.05. The value of the P-value can be seen as 0.0001, which means that the value is smaller than the set probability of 5% or 0.05. So that the research model of *Centella asaitica* (L.) extracted has a significant or significant effect on the total phenol content of the extracted.

The results of the analysis in Table 2, it also shows that the UAE power variable, and extraction time have a significant effect on the total phenol content with p-values of 0.0001, and 0.03695. For the interaction between variables on the response, the interaction of time on power has a real or significant effect on the total phenol content with a p-value of 0.0021. However, the concentration variable has a p-value of 0.3289, which means that the p-value is greater than 0.005 so that the concentration variable in the Pegagan extracted with the UAE method does not have a significant or significant effect the total phenol content, this is because the extraction power is too high so that can damage the compounds in the material. This is by following per under research conducted by [11], the use of sonication in the extraction process can cause vibrations that have the potential to cause heat so that it can damage the extracted content which causes the acquisition of the total phenol content to below so that it can affect the significance of the model which means it has no real or insignificant effect on the total phenol content. For the inaccuracy of the test or the Lack of Fit value, which means that the deviation or inaccuracy of the model. Lack of Fit testing is required if there are repeated observations. The hypotheses used in the Lack of Fit test are:

H_0 = There is no Lack of Fit in the research model

H_1 = There is a lack of fit in the research model

The Lack of Fit test is carried out to determine whether the research model is appropriate or not [12]. In Table 2, based on the ANOVA Table, it is found that Lack of fit has a P-value of 0.7072 or more than 0.05 so that H_0 is acceptable or which means there is no lack of fit in the research model. So it can be interpreted that the research model used is suitable for predicting the total phenol content in the conditions of the extraction process.

From the ANOVA analysis, the R square value of 0.9876 or 98.76% is more than 75% which indicates that the model is by following per under the research results. An adjusted R^2 value of 0.9718 indicates that there is a significant relationship between ethanol concentration, extraction time, and UAE power on response. The value of R square is declared according to the model if it is more than 75% [13]. So it can be said that the equation of the model can be used to predict the actual results of the study. Based on the regression equation obtained, it can be concluded that the effect of concentration, time, and power variables can affect the total phenol concentration. The regression equation is as follows:

$$\text{Total Fenol} = 602.692 - 8.895\text{A} + 21.795\text{B} + 135.178\text{C} + 22.1125\text{AB} - 9.7775\text{AC} + 57.0525\text{BC} - 192.07\text{A}^2 + 2.00525\text{B}^2 + 5.69025\text{C}^2$$

The equation shows that the extraction time response will increase directly proportional to power, the interaction between concentration, and time, the interaction between time, and power, the interaction between time, and power, and the interaction between power, and power. This is indicated by a positive constant. The response of total phenol concentration will decrease with increasing concentration, the interaction between concentration and power, and the interaction between concentrations. This is indicated by a constant which is negative.

Optimization of total phenol content using response surface methodology with the UAE method

The results of the extraction and testing of Pegagan (*Centella asiatica* (L.)) at various conditions of time, power, and concentration are presented in Table 4.1 shows that the value of total phenol content of Pegagan extracted was the highest (836.67 mg.AGE/g sample). The results of the measurement data were analyzed statistically using the help of Design Expert ver.11 software (response of the contour plot, and surface response in each variable as well as the optimum condition of total phenol content of Pegagan (*Centella asiatica* (L.)). In this study, the variables that used, among others, solvent concentration, time, and power.

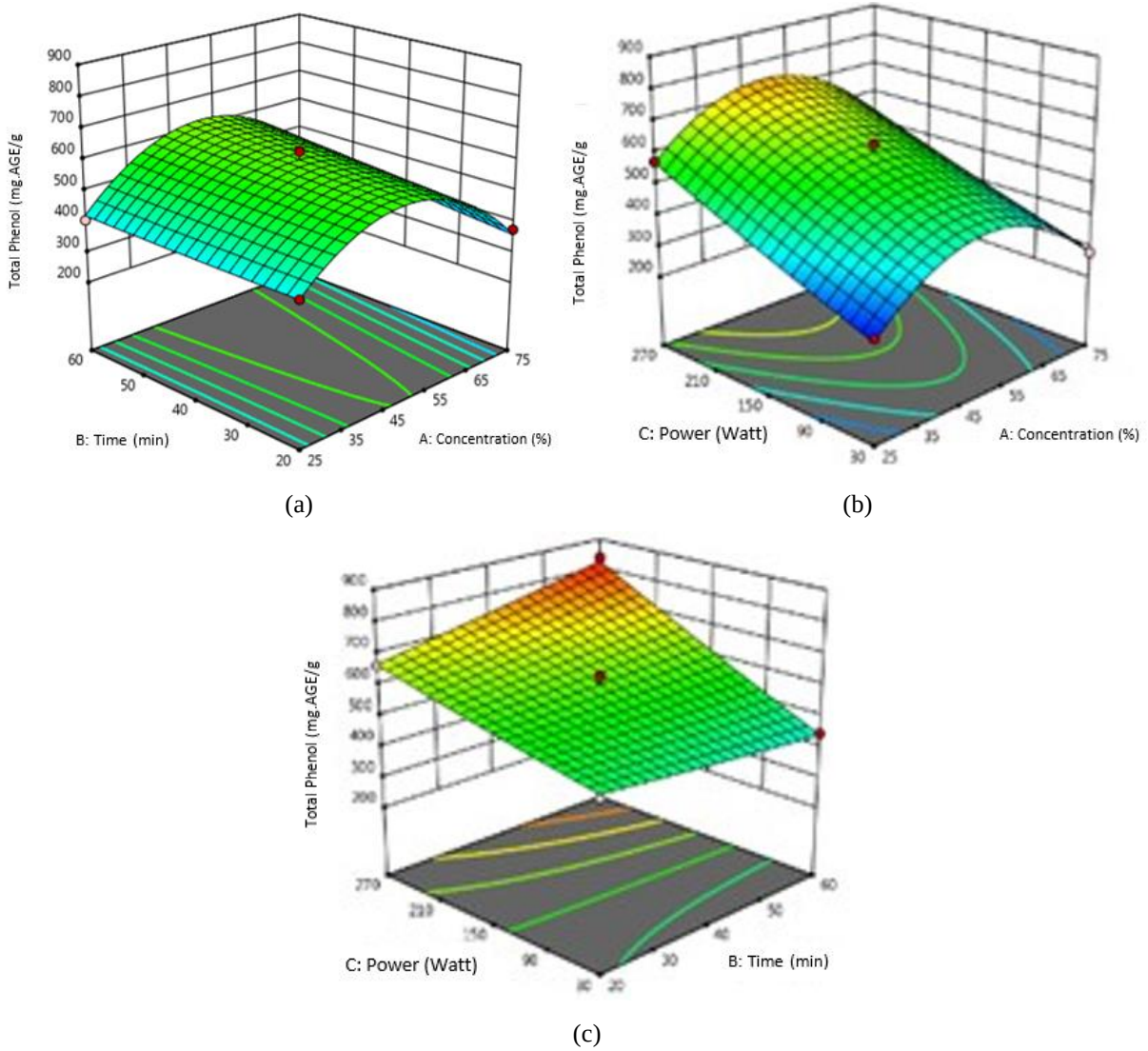


Figure 3. (a) Effect of concentration, and time, (b) Concentration , and I, (c) Time, and power on total phenol bioactive compounds using the UAE method

Pay attention to the graph in Figure 3. point (a) The graph shows the effect of the variable concentration of solvent, and extraction time on the levels of total phenol bioactive compounds. From the graph, it can be observed that at the time of the extraction for 20 minutes with ethanol concentration of 25%, and 150 watts of power produced a total phenol content of 422.56 mg.AGE/g, the total phenol content produced increased with increasing time. The increase in extraction time can result in longer contact between the material, and the solvent. The longer the extraction time, the higher the levels of compounds extracteded. This is by following per under research conducted by [14] which states that the longer the time, the more levels of bioactive compounds obtained. The longer the sonication time, the longer the contact of the mixture with the microbubble so that the more compounds contained in the sample cells are diffused with the solvent [15].

However, at a certain point when the ethanol concentration is 50%, and the power is 150 watts for 40 minutes with the optimum total phenol content of 631.54 mg.AGE/g after being at the optimum point the total phenol content produced or obtained begins to decrease. The decrease in phenol levels occurred at the highest point or optimum point due to the high temperature which was possible due to the increase in extracted time so that it was possible to experience the degradation process of bioactive compounds. Degradation is a process of termination or breaking of bonds in bioactive compounds so that the number of bioactive produced decreases. Steam distillation is one example of factors that can affect the chemical composition of bioactive compounds because heat , and water vapor can damage the molecular structure , and hydrolyze double bonds [16].

The effect of ethanol concentration showed that at the time of extraction using a solvent concentration of 25% for 20 minutes with a power of 150 watts resulted in a total phenol content of 422.56 mg.AGE/g, the higher the ethanol concentration, the higher the total phenol content obtained. This is because the solvent diffusion process into natural materials is getting better. Extraction of polyphenolic components from natural ingredients using solvents consists of two stages, namely the initiation, and diffusion stages. At the initiation stage, the natural material particles will absorb the solvent so that the particles experience bubbles. The diffusion stage is characterized by the diffusion of the solvent to a deeper part, and the polyphenol component will also be extracteded. This is by following per under research conducted by [17], indicating that the higher the concentration of ethanol, the more metabolites extracteded, either patterned or semipolar. The high concentration of ethanol gives a higher total phenol value.

However, at a certain point when the ethanol concentration is 50% and the power is 150 watts for 40 minutes with the optimum total phenol content of 631.54 mg.AGE/g after being at the optimum point the total phenol content produced or obtained begins to decrease. This indicates that there is a saturation point limit for the variable solvent concentration at the specified operating conditions. The decrease in phenol content occurs at the highest point or optimum point due to differences in the concentration of ethanol that can affect the solubility of phenolic compounds in the solvent. The higher the concentration of ethanol, the lower the polarity of the solvent. This is by following per under research [18], in *Centella asiatica* (L.) extracted which decreased in total phenol with treatment concentrations above 50%.

The effect of the power used by extraction using the UAE method can be seen in Figure 3 point (b) that the optimum conditions at the time of the extraction for 40 minutes with an

ethanol concentration of 50%, and a power of 150 watts resulted in a total phenol content of 627.05 mg.AGE/g. The greater the power used, the higher the phenol content obtained. So that the results of this study are by following per under the results of research from [19] which shows that the addition of power in the UAE can provide an increase in the total phenol content. The greater the power given, the greater the ultrasonic waves used in the sample. The magnitude of the vibration of the ultrasonic wave will make it easier for the solvent to diffuse on the surface of the material. The high power provided will accelerate the damage to the surface of the material so that the extracteded compounds will be more easily obtained so that the total phenol content is greater. However, it should be noted that too high a power can potentially damage the content of compounds present in the extracted. An increase in power will result in a decrease in the total phenol content. This is because the operating conditions with a power of 270 watts have exceeded the optimum point. The optimum point in this study is in operating conditions with a power of 150 watts. This is by following per under research [20], The operating conditions that have passed the saturation point will not increase the extraction yield even though the solvent continues to be added.

Figure 3 point (c) show the relationship between extraction power, and extraction time to the total phenol produced. In the graph, the optimum condition for operating power is 150 watts with a concentration of 50% for 40 minutes to get a total phenol of 631.54 mg.AGE/g sample. The greater the power in the UAE, and the time it takes, the greater the total phenol obtained. This is because the longer the extraction time, the more compounds are extracteded, and the power required is also greater so that when the power is large, the ultrasonic wave used for the sample is also greater because the magnitude of the vibration of the ultrasonic wave will facilitate the solvent diffusion. on the surface of the material. This is by following per under research [21], high power because the power acts as a driving force to break the structure of plant cell membranes so that the oil can diffuse out, and dissolve in the solvent. Thus, adding power will generally increase the phenol content, and speed up the extraction time.

The results of the optimization of Pegagan extraction conditions with the UAE method

Optimization of total phenol extraction the Pegagan plant (*Centella asiatica* (L.)) using the UAE method can be seen in Figure 5. shows that the optimal value for the total phenol concentration-response was obtained at 270 watts of power, 50.2525% concentration, and within 60 minutes with a value of 824.422 mg.AGE/g sample obtained when the desirability value reaches 0.97804.

Figure 4. Graph of optimization plot with UAE method

3.3. Extraction results of Pegagan (Centella asiatica (L.)) using MAE method

The measurement results of the total phenol content in the Pegagan extracted can be seen in Table 3.

Table 3. Total phenol content in pegagan plant extracted by MAE method

No.	Power (Watts)	Solvent Concentration (%)	Time (minutes)	Average Absorbance	Total Phenol (mg.AGE/g sample)
1	150	75	10	0.681	597.56
2	450	25	10	0.673	587.31
3	450	50	5	0.64765	553.97
4	300	75	5	0.571	456.54
5	150	50	15	0.687	605.90
6	300	50	10	0.715	641.79
7	450	50	15	0.857	823.85
8	300	75	15	0.951	943.72
9	300	25	15	0.687	605.90
10	300	50	10	0.83	789.23
11	450	75	10	1.191	1251.41
12	150	50	5	0.558	440.51
13	150	25	10	0.584	473.21
14	300	25	5	0.575	462.31
15	300	50	10	0.689	607.82
16	300	50	10	0.697	618.08
17	300	50	10	0.686	604.62

It can be seen from Table 3. that the highest total phenol content is 1251.41 mg.AGE/g of sample at 450Watt MAE power variable with 75% concentration for 10 minutes. The results of the analysis in this study can be seen in Table 5.

Analysis of response surface design with ANOVA (*Analysis of Variance*)

Statistical analysis was carried out to prove whether the variables used for the *Centella asiatica* (L.) extraction process affects the resulting product, and the variable can be said to be significant if the p-value of the Analysis of Variance (ANOVA) method has a value of less than (5%) or < 5%. Using the Analysis of Variance (ANOVA) method because can test the difference from the average of more than two independent groups. This is related to the research variables which amounted to more than two, namely concentration, power, and extraction time. Each variant will be compared to determine the presence of significance in the data.

Table 4. Results of ANOVA analysis on pegagan plant extracted on MAE method

Source	Sum of Square	df	Mean Square	F-Value	P-Value	
Model	5.912E+05	9	65683.91	5.83	0.0150	Significant
There is	1.511E+05	1	1.511E+05	13.40	0.0081	
B-Concentration	1.569E+05	1	1.569E+05	13.92	0.0073	
C-Time	1.421E+05	1	1.421E+05	12.60	0.0093	
AB	72832.52	1	72832.52	2.46	0.0386	
air conditioning	2729.54	1	2729.54	0.2421	0.6378	
BC	29513.52	1	29513.52	2.62	0.1497	
A2	4312.19	1	4312.19	0.3825	0.5559	
B2	7807.82	1	7807.82	0.6925	0.4328	
C2	25783.13	1	25783.13	2.29	0.1742	
Residual	78919.19	7	11274.17			
Lack of Fit	54636.05	3	18212.02	3.00	0.1581	Not Significant
Pure Error	2483.15	4	6070.79			
Total Cast	6.701E+05	16				

From Table 4 it can be seen from the significance of the variables used, the variable is said to be significant or significant if the p-value < 0.05.

The F-value of the model obtained from the calculation is 5.83. In the F distribution Table, it can be seen that the value of the F Table with a model of 9, and a df error of 4 at a probability of 0.05 then the percentage point is 6.00 which means that the F-value is greater than the F Table.

In addition, the value of the F-value is inversely proportional to the value of the p-value, in Table 4.2. If the F-value has no significant effect on the variable, then the P-value has a significant value or has a significant effect on the variable. The value of the P-value can be seen as 0.0150 which means that the value is smaller than the set probability of 5% or 0.05. So that the analysis model of the *Centella asaitica* (L.) extracted has a significant or significant effect on the total phenol content of the extracted.

From the results of the analysis in Table 4, it also shows that the MAE power, extraction time, and solvent concentration have a significant effect on the total phenol content with p-values of 0.0081, 0.0093, and 0.0073. Related to the inaccuracy of the test or the value of Lack of Fit, which means that the deviation or imprecision of the model.

In Table 4 it can be seen that the P-value on the Lack of Fit is 0.1581, and the F-value on the lack of fit is 3.00. This value is greater than the probability of 0.05 so that H_0 is accepted, and declared insignificant, meaning that there is a suitability of the model or a discrepancy in the research model. The model is said to be appropriate if the p-value lack of fit is inversely proportional to the p-value of the model, so based on the data from Table 4, it can be interpreted that the model is appropriate.

From the ANOVA analysis, the R square value was 0.8822 or 88.22% which indicated that the model was by following per under the research results. An adjusted R^2 value of 0.7308 indicates that there is a close relationship between ethanol concentration, extraction time, and MAE power on the response. The value of R square is declared according to the model if it is more than 75% [13]. So it can be said that the equation of the model can be used to predict the actual results of the study. Based on the regression equation obtained, it can be concluded that the effect of concentration, time, and power variables can affect the total phenol concentration. The regression equation is as follows:

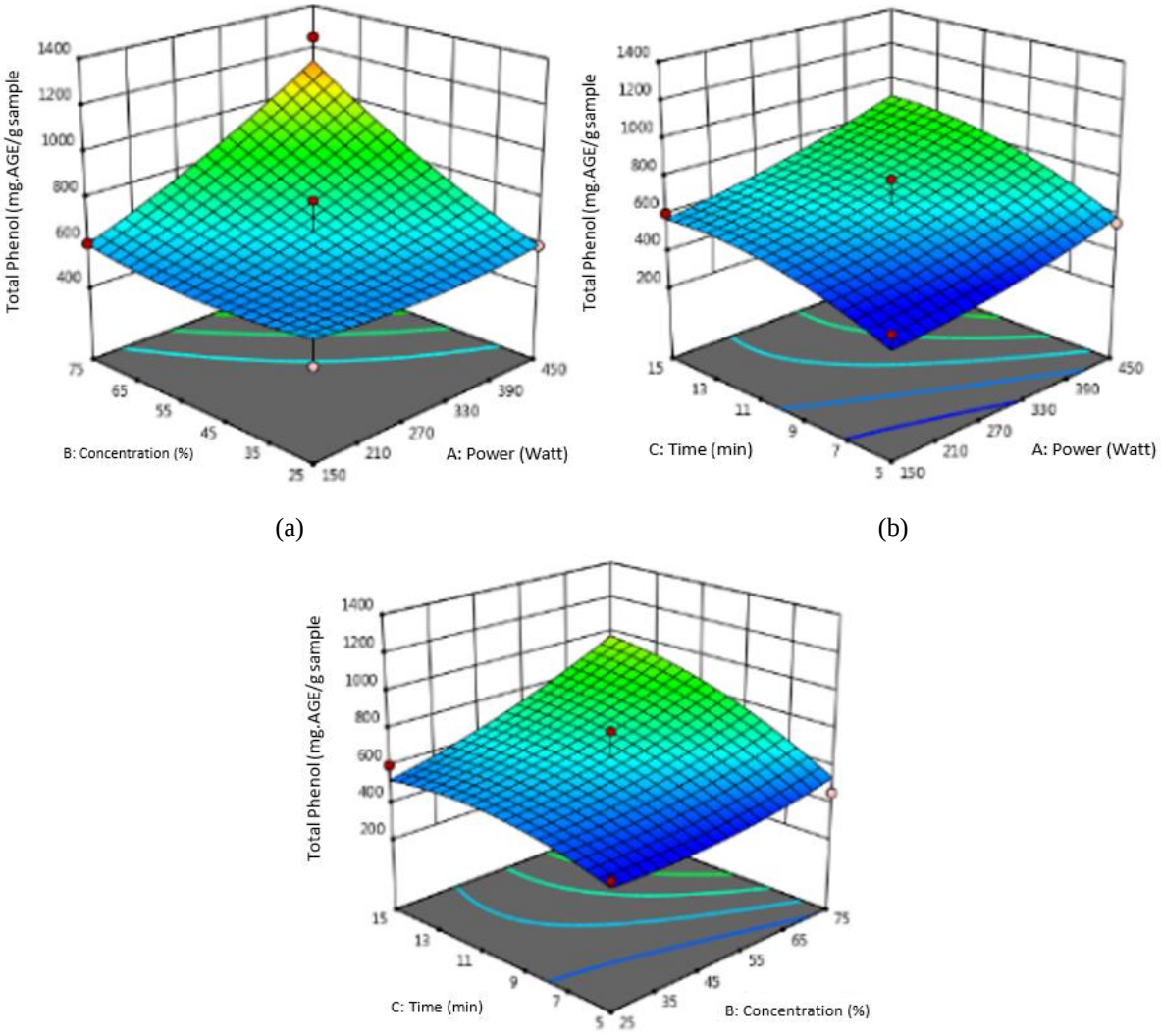
$$\text{Total Fenol} = 652.308 + 137.42A + 140.063B + 133.255C + 134.937AB + 26.1225AC + 85.8975BC + 32.0022A^2 + 43.0622B^2 - 78.2527C^2$$

The total phenol concentration response will decrease with increasing time. This is indicated by a constant which is negative.

Optimization of Total Phenol Content Using Responses Surface Methodology (RSM) with MAE Method.

Pay attention to the graph in Figure 5 point (a) the graph shows the effect of variable concentration of solvent, and operating power on MAE on total phenol bioactive compounds. From the graph, it can be observed that at the time of the lowest extraction with operating conditions for 10 minutes in a microwave of 150 watts, and a solvent concentration of 25% ethanol produced a total phenol content of 473.21 mg.AGE/g sample. Along with the increase in power, and concentration, the total phenol content also increases. Thus, it will increase directly proportional to the increase in power, and concentration. This is by following per under research conducted by [22] the higher the ethanol concentration, the higher the total phenol

obtained. The increase in ethanol concentration is directly proportional to the total phenol obtained [23]. Similarly, the effect of power according to research conducted by [24] the higher the power on the MAE used, the hotter the resulting temperature, so that the energy produced in the MAE is radiation, and rotation, therefore the presence of microwave radiation, and rotating vibrations will cause the pressure on the cell wall to increase, then the cell swells and more bioactive compounds are released or obtained.



(a) (b)

(c)

Figure 5. (a) Effect of concentration and power, (b) Effect of power and time, (c) concentration and time on total phenolic bioactive compounds using MAE method

On the graph in Figure 5 points (b) shows the effect of variable operating conditions of microwave power, and extraction time on total phenol bioactive compounds. From the graph it can be observed that at the time of the lowest extraction with operating conditions of 150 watts of power for 5 minutes with a solvent concentration of 50% ethanol with a low total phenol

content of 440.51 mg.AGE/g sample. However, when the time, and operating conditions increase, the total phenol content obtained is greater with the optimum condition for the total phenol content of 789.23 at operating conditions of 300 watts of power, and extraction for 10 minutes with a concentration of 50%. Thus, it will increase directly proportional to the increase in power, and time. This is by following per under research conducted by [25], the increase in the extraction time used will increase the penetration of the solvent into the material so that it is easier for the solvent to pull chemicals out of the material, while the less extraction time used will make it more difficult for the solvent to penetrate the walls of the material, so that the increase in time will directly proportional. Likewise, the effect of power as shown in Figure 5 point (b) shows that the higher the power, the higher the total phenol content obtained. This is by following per under research conducted by [21], the higher the microwave power produced, the tendency to increase the total phenol obtained. This is because the greater the power, the operating temperature increases, and the rate of distillation (*evaporation*) becomes greater. The greater the power, the greater the energy received by the material to be converted into heat so that the total phenol produced is greater.

Pay attention to the graph in Figure 5 point (c) the graph shows the effect of the variable length of extraction, and the concentration of ethanol solvent on total phenol bioactive compounds. From the graph it can be observed that at the time of the lowest extraction with a solvent concentration of 25% ethanol, for 5 minutes, and 300 watts of power with a low total phenol content of 462.31 mg.AGE/g sample. at the operating conditions of 300 watts of power, and extraction for 10 minutes with a concentration of 50%. Thus, it will increase directly proportional to the increase in concentration, and time. This is by following per under the research conducted [26], which states that the total phenol will increase followed by an increase in extraction time until it reaches the optimum limit. If it exceeds the optimum limit, the total phenol will decrease because the compounds contained are degraded due to too long time which can cause the temperature to increase. Microwave heating will cause the extraction temperature to continue to increase along with the increase in extraction time which will cause the degradation of phenol compounds. Microwaves can also damage enzymatic compounds that can reduce the activity of the phenolase enzyme. Likewise, the increasing concentration of total phenol increases with increasing solvent concentration. The more the amount of ethanol solvent used, the more the target compound dissolved in the ethanol [27] .

Result of optimizing conditions for extraction of pegagan with MAE method

Figure 6. shows that the desirability (D) value reaches its maximum value when the factor value is on the red line. The graph in Figure 9 can be seen that the optimal value for the response to the total phenol concentration was obtained at 450 watts of power, 75% concentration, and within 10 minutes by obtaining a value of 1130.7882 mg.AGE/g sample obtained when the desirability value reached 0.8513.

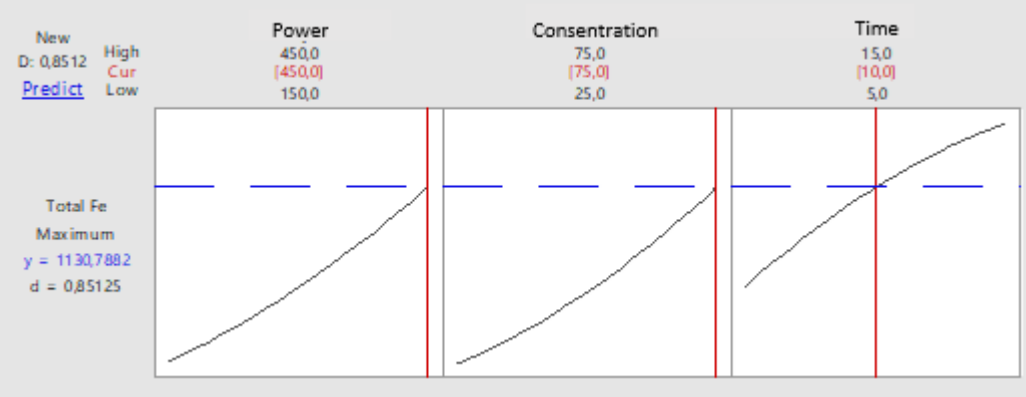


Figure 6. Graph of optimization plot with MAE method

3.4. Comparison of extraction results of Pegagan (*Centella asiatica* (L.)) *using the UAE method with extraction results using the MAE method.*

The results of the extraction process for the content of bioactive compounds are represented by analysis of the total phenol content. From the UAE method using ethanol solvent, the optimal value for the total phenol concentration response was obtained at the power the optimal value for the total phenol concentration response was obtained at 270 watts, 50.2525% concentration, and within 60 minutes obtained a value of 824.422 mg.AGE/g sample. obtained when the desirability value reaches 0.97804. While the results of the extraction process using the MAE method with ethanol solvent the optimal value in response to the total phenol concentration was obtained at 450 watts of power, 75% concentration, and within 10 minutes by obtaining a value of 1130.7882 mg.AGE/g sample obtained when the desirability value reached 0.8513. So it can be concluded that the extraction of Pegagan (*Centella asiatica* (L.)) using the microwave method gives the most maximum results. However, extraction using the UAE method also gives significant results, and the difference is not far from the MAE extraction method, so it can be said that the MAE method is the most optimal for producing bioactive compounds through testing for total phenol in the extraction of Pegagan. With a short time can produce a fairly high total phenol content of 1130.7882 mg.AGE/g. This study is quite high for the value of total phenol content compared to similar studies by comparing the UAE,

and MAE methods, because of the difference in which extraction method is in this study. extraction using the UAE method also gives significant results, and the difference is not far from the extraction of the MAE method, so it can be said that the MAE method is the most optimal for producing bioactive compounds through testing of total phenol in the Pegagan extracted. With a short time can produce a fairly high total phenol content of 1130.7882 mg.AGE/g. This study is quite high for the value of total phenol content compared to similar studies by comparing the UAE, and MAE methods, because of the difference in which extraction method is in this study. extraction using the UAE method also gives significant results, and the difference is not far from the extraction of the MAE method, so it can be said that the MAE method is the most optimal for producing bioactive compounds through testing of total phenol in the Pegagan extracted. With a short time can produce a fairly high total phenol content of 1130.7882 mg.AGE/g. This study is quite high for the value of total phenol content compared to similar studies by comparing the UAE, and MAE methods, because of the difference in which extraction method is in this study. With a short time can produce a fairly high total phenol content of 1130.7882 mg.AGE/g. This study is quite high for the value of total phenol content compared to similar studies by comparing the UAE, and MAE methods, because of the difference in which extraction method is in this study. With a short time can produce a fairly high total phenol content of 1130.7882 mg.AGE/g. This study is quite high for the value of total phenol content compared to similar studies by comparing the UAE, and MAE methods, because of the difference in which extraction method is in this study [28] this study used the maceration method for *Centella asiatica* (L.) extracted with a maximum wavelength of 765 nm to determine the total phenol content, with a sample weight of 150 g in powder form resulting in a total phenol content of only 3.67 mg.AGE/g sample for 30 minutes with a concentration of 3.67 mg.AGE/g sample for 30 minutes with a concentration 70% ethanol solvent. This has a big difference from the total phenol results obtained in the UAE method with a lower ethanol concentration of 52.77%, and the MAE method with a shorter time of 10 minutes, and both only use 1 gram of sample, but have the higher total phenol content was 1130.7882 mg.AGE/g for the MAE method while the UAE method had a total phenol content of 824.422 mg.AGE/g sample.

4. Conclusion

In the ANOVA analysis using the UAE method, variables that gave a significant response to the total phenol content were power, time, and ethanol concentration with an R

square value of 0.9876 or 98.76%. The optimum condition of the total phenol content using the UAE method is the optimal value for the response to the total phenol concentration obtained at 270 watts of power, 50.2525% concentration, and within 60 minutes by obtaining a value of 824,422 mg.AGE/g sample. In the ANOVA analysis using the MAE method, variables that gave a significant response to the total phenol content were power, time, and ethanol concentration with an R square value of 0.8822 or 88.22%.

So that the comparison between the UAE, and MAE methods for *Centella asiatica* (L.) extracted with ethanol solvent is more optimal, namely using the MAE method because in a short time of only 10 minutes it can produce 1130.7882 mg.AGE/g of total phenol sample, and also the concentration of ethanol. which is getting purer at 75% so that the MAE method is more efficient to use.

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Extraction of Antioxidant Compounds from *Sargassum* sp. Using Water as A Solvent and Ultrasound Assisted Extraction Method as A Derivation of Green Chemistry Principles

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Abstract. The content of bioactive compounds in *Sargassum* sp. already used in various fields. The Ultrasound Assisted Extraction (UAE) method and water solvent has met the principles of green chemistry so that it is used in this study as a method of extracting antioxidant compounds contained in *Sargassum* sp. The principle of green chemistry has the main goal of reducing or eliminating the impact of environmental damage. This research was conducted with several process variables, including the ratio of the sample mass to the volume of solvent (0.05 – 0.15 g/mL), time (30 – 40 minutes) and power (170 – 180 W) using the UAE method and water solvent (*aquadest*) on the total phenol value of *Sargassum* sp. result. Analysis of variance was carried out with the help of Design Expert software, Response Surface Method - Central Composite Design to determine the effect of the process variables carried out in the extraction process on the total phenol yield. The analysis of variance in this study shows the suitability between the research design and the results of the study which is indicated by an R^2 value of 0.9785. The highest results were obtained with a variable ratio of sample mass to solvent volume of 0.18 (g/mL), time of 40 minutes and power of 180 W with a total phenol yield of 212.8 mg GAE/g and antioxidant activity of 12.3%.

Keywords: *Sargassum sp., green chemistry, antioxidant, total phenol, Ultrasound Assisted Extraction (UAE), water solvent*

1. Introduction

Indonesia is a country that has the second longest coastline after Canada so that marine biodiversity in Indonesia is very high [1]. One of the existing species is seaweed which is commonly known as marine macroalgae. *Sargassum* species are brown macroalgae scattered

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around tropical oceans [2]. The content of bioactive compounds in *Sargassum* sp. already used in various fields, such as food, bioenergy, biofuels, pharmaceuticals, cosmetics, and textiles [3, 4, 5, 6]. *Sargassum* species in Indonesia have pharmacological potential due to the strong antioxidant activity of the phenolic compounds contained [7].

Bioactive compounds can be obtained by extraction. There are several extraction methods, one of which is Ultrasound Assisted Extraction (UAE). The UAE method is categorized as a non-conventional method [8]. The UAE method can extract bioactive compounds in a very short time and requires lower energy, solvents, and operating temperatures when compared to conventional methods [9]. Based on this effectiveness, the UAE method has a higher probability of application in the chemical and food industries [10, 11, 12, 13].

Extraction methods and the use of solvents that can reduce the impact of environmental damage are needed because the environment is currently experiencing a crisis of environmental damage in the last few decades. Green chemistry provides some methods that can overcome this. A method that can reduce the impact of environmental damage is the 'Green Extraction' method which is based on the design of an extraction process that will reduce or eliminate energy consumption, allow the use of alternative solvents and ensure safe and quality extracts, it was stated in green chemistry [14]. The UAE method is one of the methods of green extraction [13]. In addition to the extraction method, in green chemistry there is also the use of alternative solvents or referred to as 'Green Solvent'. Solvents indicated as green solvent are non-volatile organic compounds that have high solubility, low toxicity, are environmentally friendly, obtained from renewable resources at a reasonable price, and are easy to recycle [15, 16].

In this study, the extraction of antioxidant compounds from *Sargassum* sp. using the Ultrasound Assisted Extraction (UAE) method and the water solvent (*aquadest*). The method used is a derivation of the principle of green chemistry.

2. Materials and Methods

2.1. Materials

The materials needed in this study were *Sargassum* sp., aquadest, gallic acid, 2% Na_2CO_3 solution, $CuSO_4$ solution, sodium solution, potassium tartrate solution, Folin-Ciocalteu reagent, trolox, methanol solution, DPPH (1,1-*dipheny* l-2-*picrylhydrazyl*) and filter paper.

2.2. Sample preparation

The sample used in this study was *Sargassum* sp. obtained from Pesawsaran Regency, Lampung. The sample is then dried until there is no moisture content to prevent the growth of fungus on the sample. The drying process is carried out indoors so that the sample is not exposed to sunlight which can cause some damage to the sample. The dried samples were seen from the

constant weight obtained and sieved using an 80mesh sieve. The sample is weighed based on the value of the variation of the process variable given by the Design Expert.

2.3. Extraction

The prepared sample was then extracted using the UAE method. The mechanism of the UAE method is that ultrasound waves come into contact with a solvent containing a solid sample, then the formation of cavitation bubbles occurs which causes changes in pressure and temperature. This causes an increase in the mass transfer rate of the sample solid to the solvent [18]. Tools for the extraction of the UAE method were prepared and *Sargassum* sp. which has been weighed is put into a beaker glass. The solvent in the form of 100 mL of distilled water was put into a beaker glass and mixed with the sample. Extraction with the UAE method was carried out with a variety of treatment variables provided by Design Experts.

2.4. Total Phenol Analysis

The total phenol analysis method on the sample used was the Folin-Ciocalteu method. Calibration was carried out with different concentrations of gallic acid, namely 0.00, 0.25, 0.50, 0.75 and 1 mM. Then 200 µL extract of *Sargassum* sp. and 2.0 mL of solution A (10 mL of 2% Na_2CO_3 with 0.1 mL of CuSO4 and 0.1 mL of sodium and potassium tartrate) were mixed and after 4 minutes, 0.4 mL of 0.5 M sodium hydroxide were added. After 10 minutes 0.2 mL of Folin-Ciocalteu reagent (1:1 v/v with water) was added. The solution was allowed to stand for 30 min and absorbance was measured with a UV-Vis Spectrophotometer at 750 nm. The total phenol content was calculated as mg gallic acid equivalent (mg GAE) using a gallic acid calibration curve [19].

2.5. Antioxidant Activity Analysis

The DPPH test was carried out by taking 0.4 mL of *Sargassum* sp. extract, gallic acid antioxidant standard and trolox (50-400 g/mL) mixed with 3.6 mL of DPPH methanol solution (0.1 mM). The same amount of methanol (0.4 mL) was used as a blank (control) with 3.6 mL of DPPH solution. All samples were vortexed for 1 min and incubated in the dark for 30 min at 37°C. The decrease in absorbance of each sample was measured against methanol as a blank on a UV-Visible spectrophotometer at 517 nm. The percentage of DPPH inhibition or inhibition was calculated using equation 1 [19]:

$$\text{DPPH Inhibition (\%)} = \frac{A_{control} - A_{sample}}{A_{control}} x100\% \quad (1)$$

When: $A_{control}$: Absorbance control

A_{sample}: Absorbance sample

3. Result and Discussion

3.1. Effect of extraction process variables on total phenol content

The results of the total phenol test on *Sargassum* sp. using UAE method and water solvent can be seen in Table 1, it shows that the largest total phenol value is in the ratio of 0.18 (18 g sample: 100 ml solvent), 40 minutes time and power at 180 W is 212.8 mg GAE/g. The smallest total phenol value was found in the ratio of 0.016 g/mL, 40 minutes of time and power at 180 W of 42.7 mg GAE/g.

Table 1. Total Phenol from Treatment Variations Based on Response Surface Methodology Approach - Design Expert

No	Code	Factor 1	Factor 2	Factor 3	Response 1
		A: Ratio (g/mL)	B: Time (menit)	C: Power (W)	Total Phenol (mg GAE/g)
1	C17	0.18	40	180	212.8
2	C20	0.15	50	170	208.7
3	C3	0,.15	30	190	180.4
4	C13	0.15	50	190	177.2
5	C2	0.15	30	170	166.5
6	C6	0.1	57	180	158
7	C1	0.1	40	163	153.9
8	C5	0.1	40	180	151.5
9	C11	0.1	40	197	141.7
10	C9	0.1	40	180	136.7
11	C18	0.1	40	180	135.7
12	C16	0.1	40	180	134.1
13	C7	0.1	40	180	133.1
14	C12	0.1	40	180	131.9
15	C8	0.1	23	180	128.5
16	C4	0.05	50	190	103.3
17	C10	0.05	30	190	92.8
18	C14	0.05	50	170	91.1
19	C15	0.05	30	170	83.1
20	C19	0.016	40	180	42.7

The influence of process variables can be seen in Figures 1 – 3. In Figure 1, the variable that remains is variable C (power) of 180 W, in other words, it shows the relationship between variable A (ratio of sample mass to volume of solvent) and variable B (time) on total phenol yield. Figure 1 shows that the higher the ratio of the mass of the sample to the volume of the solvent, the higher the total phenol, whereas when the extraction time is carried out the longer it increases but at a certain time the total phenol yield does not show a big difference.

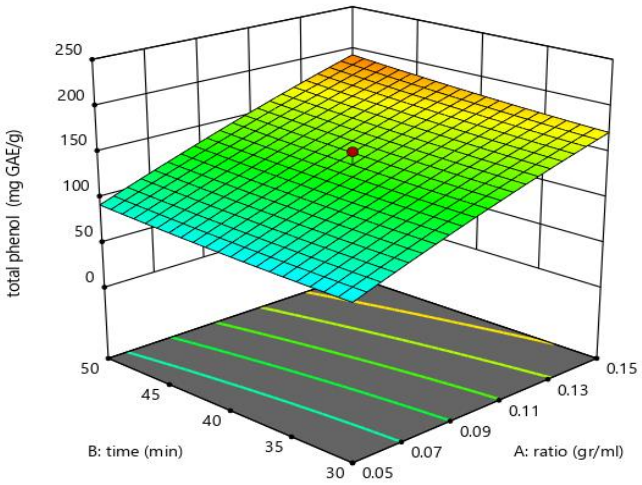

Figure 1. Relationship between total phenol with the ratio of sample mass to solvent volume (A) and time (B) at power (C): 180 W

The variable mass ratio of the sample to the volume of solvent has a significant impact. The larger the ratio value or the more samples contained in the extracted solution, the higher the total phenol yield. This happens because the difference in the concentration of a higher solute will increase the diffusivity and dissolution of the solute in the solvent which will increase during the extraction process. At a high ratio, the ultrasonic intensity applied to the sample is higher causing more effects of fragmentation, erosion and pore formation thereby increasing the yield. A high ratio also has an effect on increasing the contact area between the material and the solvent which can also increase the yield [9].

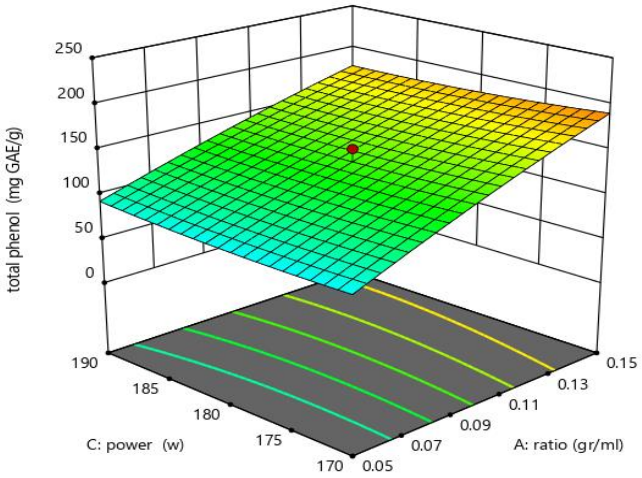

Figure 2. The relationship between total phenol with power (C) and the ratio of sample mass to solvent volume (A) at time (B): 40 minutes

In Figure 2, the variable that remains is variable B (time) for 40 minutes, in other words, it shows the relationship between variable A (ratio of sample mass to solvent volume) and

variable C (power). Figure 2 shows that the higher the ratio of the sample mass to the volume of the solvent gives a high total phenol, while the ultrasound power variable with increasing power gives a low total phenol yield but does not provide a large difference in the total phenol value. The time variable is quite influential or does not have such a large impact on the total phenol value. An increase in total phenol yield was also reported in the study [20] which states that the longer the extraction time will give a higher total phenol yield as well. However, in this study, several treatments with a longer extraction time actually gave smaller results. This is because increasing the ultrasound time initially increases the results and after that the results decrease or there is no increase in the results for a longer time. By the time increase, it will increase the exposure of the solute and the extraction medium and aid their release into the solvent. Giving ultrasound waves with a very long duration will cause structural damage to the solute so that it reduces the extraction yield [9]. High total phenol values can be obtained in a short time and if carried out for a long time will also allow damage to the extracted compounds.

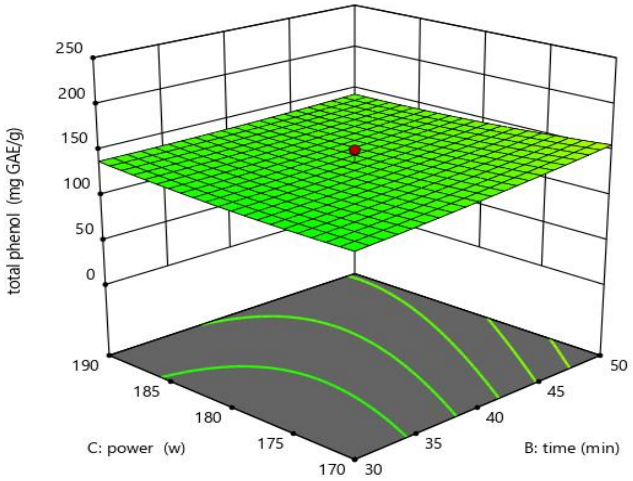

Figure 3. The relationship between total phenol with time (B) and power (C) on the ratio of sample mass to solvent volume (A): 0.1 g/mL

In Figure 3 the constant variable is variable A (sample mass ratio to solvent volume) of 0.1 g/mL in other words showing the relationship between variable B (time) and variable C (power). Figure 3 shows the variable treatment does not give a big difference to the total phenol value. The influence of the power variable is less influential in this study, it is intended that when the sample is given an increase in extraction power, the yield increases and then decreases. This is explained by the effect of cavitation bubble collapse which increases with increasing power which causes fragmentation, pore formation and mixing in the tissue so as to increase diffusivity and increase extraction yield [21]. Very high ultrasound intensity can reduce bioactive compounds. Very high power causes an increase in the number of bubbles formed.

The layer of cavitation bubbles around the probe tip (physical device used to connect electronic test equipment to the sample being tested) blocks energy transmission to the extraction medium (saturation effect) thereby reducing yield [9].

3.2. Analysis of Variance

The total phenol results from each variable variation were then analyzed using analysis of variance to determine the equation model that links the independent variables to the response variables. The analysis of variance model in this study is Central Composite Design – Quadratic. The results of the analysis of variance for the total phenol response can be seen in Table 2. It can be seen that the F-value of the model is 50.52 which implies that the model is significant, which means that the variables used have a significant effect on the total phenol yield of *Sargassum* sp.

Table 2. Results of analysis of total phenol variance from treatment variations based on the response surface methodology approach - design expert

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	32588.00	9	3620.89	50.52	< 0.0001	significant
A-ratio	30801.16	1	30801.16	429.74	< 0.0001	
B-time	840.10	1	840.10	11.72	0.0065	
C-power	19.26	1	19.26	0.2687	0.6155	
AB	52.53	1	52.53	0.7329	0.4120	
AC	195.03	1	195.03	2.72	0.1300	
BC	230.05	1	230.05	3.21	0.1035	
A^2	199.62	1	199.62	2.79	0.1261	
B^2	44.55	1	44.55	0.6216	0.4487	
C^2	163.37	1	163.37	2.28	0.1620	
Residual	716.74	10	71.67			
Lack of Fit	455.25	5	91.05	1.74	0.2788	not significant
Pure Error	261.49	5	52.30			
Cor Total	33304.75	19				

From Table 2, it can be seen that the p value of the model is < 0.0001 which indicates that the quadratic model used is influential or significant. The p-value less than 0.05 indicates a significant model term. A value greater than 0.1 indicates an insignificant model term, so it can be said that variables A and B (< 0.0001 and 0.0065) are significant models (provides a significant effect). This shows that the quadratic model can be used to predict the optimum

response conditions for total phenol from *Sargassum* sp. using the UAE method and water as a solvent with a variable ratio and time. The R^2 value obtained in this study is 0.9785.

Analysis of variance also provides a quadratic model equation that can be seen in equation 2 of the process variable to total phenol. The total phenol value is directly proportional to the variable mass ratio of the sample to the solvent volume and the extraction time is indicated by a positive constant, which indicates that when the variable value of the sample mass ratio to the solvent volume and extraction time increases, the total phenol value to be produced will also increase. The inversely proportional power variable is indicated by a negative constant which indicates that when the value of the power variable increases, the total phenol yield will decrease.

$$Y = 11.71 + 2.14\,A + 0.3264\,B - 0.023\,C + 0.058\,AB - 0.2179\,A - 0.1951\,BC - 0.4011\,A^2 + 0.091\,B^2 + 0.1624\,C^2 \quad (2)$$

When Y: total phenol (mg GAE/g)

A: ratio (g sample: mL solvent)

B: time (minutes)

C: power (W)

The statistical comparison between the actual data and the predicted data can be seen in the graph in Figure 4. This graph aims to determine the suitability between the given model and the actual data.

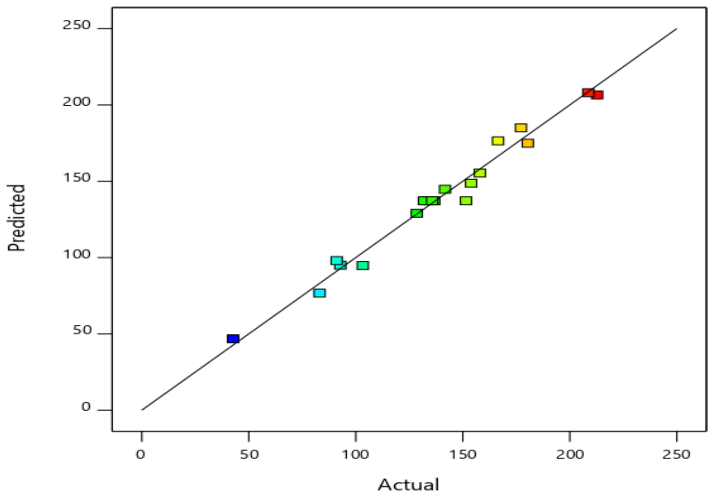

Figure 4. Prediction data plot vs. actual data

From Table 1 the results of the analysis of the total phenol contained in the extract of *Sargassum* sp. gives the highest value to the highest ratio variable of all ratio variations. Apart from Table 1, the response between the total phenol value and the extraction process variable can be seen in the p-value in Table 2 and the response visualization (3D Surface) in Figures 1

– 3. Table 2 shows the p value of variable A (ratio of sample mass to solvent volume) and variable B (time) is said to be significant, while variable C (power) is not significant. Likewise, the response visualization (3D Surface) shown in Figures 1 – 3 shows that the response variable A (ratio) is said to be significant, B (time) is quite significant and C (power) is not significant. The three considerations (result table, p value and 3D surface) on the total phenol value of *Sargassum* sp. giving the results that the most influential variable is the ratio treatment variable then the time variable is quite influential while the power variable is less influential.

3.3. Antioxidant activity

The results of the antioxidant activity test were carried out on the samples that had the highest total phenol test results. This was done because there was a relationship between total phenol content and antioxidant activity. The greater the total phenol content, the higher the antioxidant activity. The antioxidant activity test in this study was the DPPH method. This method is based on the ability of the tested extract to donate hydrogen which reacts with the DPPH radical, thereby neutralizing the free radical character and giving rise to the reduced form of DPPH (non-radical). Phenol compounds have a hydroxyl group and the function of the hydroxyl group is to act as a contributor to hydrogen atoms that react with free radicals through an electron transfer mechanism [7]. These conditions were obtained at the ratio variable 0.18 (18 g sample: 100 ml solvent), time 40 minutes and power at 180 W with a value of 212.8 mg GAE/gr. In the antioxidant test carried out on this sample, the results of DPPH inhibition were 12.3%.

3.4. Comparison with Other Methods

The total phenol yield of *Sargassum* sp. the highest in this study was 212.8 mg GAE/g. In another study regarding the total phenol yield of *Sargassum* sp. values obtained with maceration method and water solvent gave higher total phenol yield, namely 669.33 mg GAE/g with heating for 20 minutes and 352.5 mg GAE/g without heating for 24 hours [23]. Study of *Sargassum muticum* using the maceration method with water solvent also gave a high total phenol yield of 275.8 ± 4.98 5 mg GAE/g. There are variables such as the ratio of the sample to the solvent volume of 0.04 g/mL and 24 hours [22]. The difference in the total phenol yield can be seen from the differences in the extraction method used, the ratio of the sample to the solvent and also the extraction time.

Another study [7] produced a lower total phenol when compared to this study as much as 45 mg GAE/g. The study using the maceration method with heating and the help of a

magnetic stirrer and the ratio of the mass of the sample to the volume of water solvent is 1:20. Comparisons that can be made between study [7] and this is the extraction method. This study using the ultrasound assisted extraction (UAE) method, there was a treatment with a ratio of 0.05 (g/mL) and a time of 30 minutes and the higher total phenol yield was 83.1 mg GAE/g, while in [7] using the maceration method under the same conditions gave 45 mg GAE/g.

Study [20] also showed that the extraction results of macroalgae (one of them *Sargassum* sp.) using the UAE method gave higher yields when compared to conventional methods. Some of these studies show that the UAE method is more efficient than the maceration method. This is also in accordance with [24] which states that the UAE method provides higher polyphenol extraction results when compared to the conventional maceration method. The UAE method is more efficient or gives a higher total phenol yield when compared to the maceration method because in the UAE method ultrasound waves will provide effective interference on the sample cell wall so that it can facilitate the release of more polar bioactive [20]. In study [9] also stated that the UAE method can extract bioactive compounds in a very short time, low temperature and requires lower energy and solvents when compared to conventional methods.

Based on the studies that have been conducted on *Sargassum* sp., the variables that affect the total phenol value are extraction method, ratio, time, power (UAE method), temperature and type of *Sargassum*.

4. Conclusion

The influence of the variable ratio of sample to solvent on the extraction is very influential, it is shown that the total phenol yield in a high ratio variable will give a high total yield as well. As for time variable is quite influential with increasing time the total phenol yield shows an increase. And for power variable is less influential as the intensity of the total phenol yield tends to decrease. The highest value of total phenol from *Sargassum* sp. contained in the variable ratio 0.18 (g/mL), time of 40 minutes and power of 180 W with 212.8 mg GAE/g and antioxidant activity of 12.3%.

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RESEARCH ARTICLE

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Optimization of Extraction of Bioactive Compound from Pegagan Leaves Using Ethanol Solvent With Microwave-Assisted Extraction Method (MAE)

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Abstract. Treatment using natural ingredients in Indonesia is the main choice that is growing in society. This is because treatment with natural ingredients has relatively milder side effects compared to synthetic treatment. Therefore, further research is needed on natural ingredients that can be used as natural medicines, one of which is pegagan (*Centella asiatica* (L.)). Several studies have found bioactive compounds in pegagan that can be used as medicine by various methods. The author wants to know the optimal conditions for extracting pegagan bioactive compounds using the microwave-assisted extraction (MAE) method. This study used pegagan leaf size 40 mesh that had been dried. pegagan leaves were extracted using ethanol as a solvent with microwave power, solvent concentration, and extraction time as variables. Variable power 150 watts, 300 watts, and 450 watts. Variable solvent concentration 25%, 50%, and 75%. Variable extraction time for 5, 10, and 15 minutes. Analysis of the results of the study was carried out using total phenol analysis using the Folin-Ciocalten method. The research data obtained optimum operating conditions at 75% solvent concentration, 450-watt microwave power, and extraction time of 10 minutes with a total phenol content of 1251.410225 mg AGE/g sample.

Keywords: *extraction, pegagan, ethanol, bioactive compounds, microwave assisted extraction, optimum conditions*

1. Introduction

Recently, in Indonesia, treatment using natural ingredients has become the main choice that has developed in the community. This is because treatments made with natural ingredients

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have relatively milder side effects when compared to synthetic treatments. Therefore, a more in-depth research is needed on the natural ingredients used as natural medicines. One of the natural ingredients that can be used as medicine is pegagan (*Centella asiatica* (L.)) [1].

Pegagan (*Centella asiatica* (L.)) is one of the wild plants that grow in large numbers in various places such as plantations, fields, and in the yard. Pegagan has good prospects as a medicinal plant. Pegagan has been designated as a traditional medicinal plant since 1884 [2]. Pegagan is commonly used as a food additive, herbal tea, and component in cosmetics. In addition, this plant can also be used in the pharmaceutical world, namely as a therapeutic application [3]. Pegagan has long been used as a traditional medicine in the form of dry ingredients, fresh, or in the form of ingredients. This pegagan plant has a pharmacological effect that has been proven from several studies, such as pegagan used as a medicine in healing wounds, rheumatism, inflammation, hemorrhoids, asthma, leprosy, tuberculosis, fever, dysentery, and can increase appetite in Australia [4].

The pegagan plant has been used as a traditional medicinal herb in Asia for hundreds of years, such as traditional Chinese medicine and Ayurvedic medicine [5]. Pegagan geographically comes from China, Indonesia, India, Madagascar, Sri Lanka, and Malaysia, and grows in humid places. Because pegagan can be used in the health sector, this plant can reach the borders of Turkey, the West Indies, and North America [6]. *Centella asiatica* (L.) has many bioactive ingredients, such as asiaticoside, Asiatic acid, betulinic acid, thankunic acid, madeccasic acid, and madecassoside [7]. Asiaticoside in pegagan was identified as the most active major compound [8], so it can be used as a characteristic of the pegagan plant. *Centella asiatica* (L.) contains asiaticoside as an active constituent which plays an important role in increasing the stimulation of antioxidant levels that can assist in the wound healing process by helping the proliferation of fibroblasts and extracular matrix which have an important role in the wound healing process [9].

The bioactive compounds contained in *Centella asiatica* (L.) can be extracted by several methods. Based on several studies that have been carried out, the extraction of these bioactive compounds was carried out using the Soxhlet extraction method, maceration [10], subcritical water [11], viscozyme [12], and microwave-assisted extraction [13]. In its use, the pegagan plant is usually produced in the form of an extract first. Pegagan extract can be produced by maceration, fluidization, continuous filtration, and percolation. In general, the solvent specified in the extraction process is ethanol, water, ether, or a mixture of water with ethanol [3].

In the research conducted by [3] namely pegagan extraction using the microwave-assisted extraction method to determine the optimal conditions. This research used dry pegagan and ethanol as solvents. The results obtained in this study are the optimal conditions obtained with a ratio of 10 ml/g: 58% ethanol (solid/liquid ratio) at 300 W microwave power and in 3.4 minutes. The MAE process accelerates mass transfer and produces higher yields when using less solvent. The proportion of ethanol in water has a major influence in extracting the desired product quantity. Other studies have shown that the MAE method in extracting bioactive compounds from pegagan using ethanol as a solvent has a yield that is twice as large as using the Soxhlet extraction method [10]. The advantages of the microwave as an extraction method are time efficiency, reducing the use of organic solvents, and as an environmentally friendly extraction method [14]

In this study, the extraction of bioactive components from the *Centella asiatica* (L.) plant using the microwave-assisted extraction method will be carried out to study the optimization of the extraction of bioactive compounds from pegagan extract using ethanol as a solvent. Optimization results will be obtained using the Box-Behnken design (BBD) model and to determine the bioactive content contained in pegagan plants, a total phenolic analysis will be carried out.

2. Materials and Methods

2.1. Materials

The materials used in this study included 40 mesh size dried pegagan leaves, 96% technical ethanol, aquadest, Na_2CO_3, Folin-Ciocalteu reagent, and gallic acid.

2.2. Methods

Pegagan leaves were dried in the sun for 2 days with the determination of physical drying. Then mashed with a size of 40 mesh with a mass of 1 gram. Extraction was carried out with several variables, including at a solvent concentration of 25%; 50%; and 75%, 150 watts of microwave power; 300 watts; and 450 watts, and variable extraction time for 5 minutes; 10 minutes; and 15 minutes. The extraction results were stored in an 8 ml vial at a temperature of 4^oC.

2.3. Preparation of gallic acid solution 100 ppm

Weigh 0.01 grams of gallic acid, then add 1 ml of ethanol and add distilled water to a volume of 100 ml.

2.4. Determination of the maximum wavelength of gallic acid

Take 1 ml of 100 ppm gallic acid mother liquor, put it in a test tube, and add 1 ml of Folin's reagent, shake the two liquid mixtures until they are homogeneous, and allow to stand at room temperature for 4-8 minutes. Add 4 ml of 10% Na_2CO_3 solution into a test tube, shake, until homogeneous and allowed to stand for 15 minutes at room temperature. Then the solution was analyzed with a UV-vis spectrophotometer with a wavelength range of 700-800 nm.

2.5. Preparation of the gallic acid calibration curve for the folin-ciocalteu reagent

100 ppm gallic acid mother liquor, taken 1 ml each; 3 ml; 5 ml; and 7 ml. Then diluted with distilled water, to a final volume of 10 ml, so that a solution with a concentration of 10 ppm will be obtained; 30 ppm; 50 ppm; and 70 ppm. Each of these solutions was taken as much as 0.2 ml, put into a test tube, and added 1 ml of Folin-Ciocalteu reagent, shaken until homogeneous, allowed to stand for 8 minutes. Then 3 ml of 10% Na_2CO_3 was added, shaken until homogeneous, and allowed to stand for 30 minutes at room temperature. Measure the absorption with the maximum wavelength that has been obtained previously. Then a calibration curve is made using the regression equation $y = ax + b$.

2.6. Determination of total phenolic content by the folin-ciocalteu method

Take 0.1 ml of the extract, add 9.9 ml of distilled water (dilution 100 times) and add 1 ml of Folin-Ciocalteu reagent then shaken until homogeneous and allowed to stand for 8 minutes. Then add 3 ml of 10% Na_2CO_3 to the mixture, shake until homogeneous and let the solution stand for 1 hour at room temperature. Measure the absorption with a UV-vis spectrophotometer at its maximum wavelength. The content analysis was repeated 3 times so that the phenol content obtained was as mg gallic acid equivalent/gram of fresh sample.

3. Result and Discussion

The research was carried out in November 2020 s.d. December 2020 at the Basic Chemistry Laboratory and Bioprocess Laboratory, Chemical Engineering Study Program, Department of Mechanical Engineering, Faculty of Engineering, University of Jember. This study uses pegagan plants extracted to be taken bioactive compounds or components with the Microwave-Assisted Extraction method. The results of measuring the total phenol content in the pegagan plant extract can be seen in Table 1.

Tabel 1. Total phenol content in pegagan plant extract

No.	Power (Watt)	Concentration Pelarut (%)	Time (minute)	Absorbance Rata-Rata	Total Phenol (mgAGE/g sampel)
1	150	75	10	0.680	597.564102
2	450	25	10	0.672	587.307692
3	450	50	5	0.646	553.974359
4	300	75	5	0.570	456.538461
5	150	50	15	0.687	605.897435
6	300	50	10	0.715	641.794871
7	450	50	15	0.857	823.846153
8	300	75	15	0.950	943.717948
9	300	25	15	0.687	605.897435
10	300	50	15	0.830	789.230769
11	450	75	10	1.190	1251.41025
12	150	50	5	0.558	440.512820
13	150	25	10	0.583	473.205128
14	300	25	5	0.575	462.307692
15	300	50	10	0.688	607.820512
16	300	50	10	0.696	618.076923
17	300	50	10	0.686	604.615384

3.1. Gallic acid standard curve

Gallic acid standard curves were made using several concentrations of gallic acid, namely 10 ppm; 30 ppm; 50 ppm; and 70 ppm. The absorbance was measured with a maximum wavelength of 765 nm that had been previously obtained. The standard curve for gallic acid and the straight-line equation that will be used in determining the concentration of gallic acid can be seen in Figure 1.

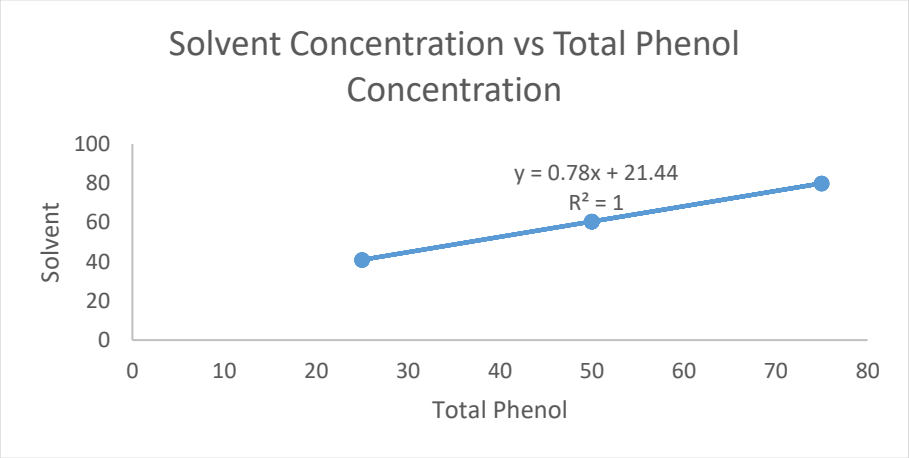


Figure 1. Gallic acid standard curve with various concentrations of 10, 30, 50, 70 ppm

From the curve, a straight line equation is obtained, namely y = 0.78x + 21.44, with R^2 = 1 which shows that the straight-line equation can be used to determine the total phenol content.

3.2. Analysis of total phenol by Response Surface Method (RSM)

Analysis of total phenol using the response surface method (RSM) was carried out to prove whether the variables used in the pegagan extraction process could affect the resulting product. The variable can be said to be significant if the p-value of the RSM analysis method has an alpha value (5%). P-value <0.05 indicates that the antioxidant activity produced is a response to the treatment variables which include solvent concentration, power, and extraction time. The F-value is inversely proportional to the F-table value, seen in Table 2.

Table 2. Results of Analysis of Variety (ANOVA) response of antioxidant activity

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Model	9	607455	67495	7.54	0.007
Linear	3	486103	162034	18.11	0.001
Power	1	151074	151074	16.89	0.005
Concentration	1	156944	156944	17.54	0.004
Time	1	178086	178086	19.91	0.003
Square	3	32114	10705	1.20	0.378
Power*Power	1	6743	6743	0.75	0.414
concentration * concentration	1	10973	10973	1.23	0.305
Time*Time	1	11390	11390	1.27	0.296
2-Way Interaction	3	105074	35025	3.92	0.062
Power* Concentration	1	72831	72831	8.14	0.025
Power*Time	1	2729	2729	0.31	0.598
Concentration *Time	1	29513	29513	3.30	0.112
Error	7	62620	8946		
Lack-of-Fit	4	61771	15443	54.57	0.004
Pure Error	3	849	283		
Total	16	670075			

The F-value is 7.54, the df value is 9 with the number of samples 17 at alpha 0.05, the F-table value is 2.49. So that the F-value is greater than the F-table which indicates that the model used has a significant effect on the response. The value of the P-value can be seen as 0.007 which means that the value is smaller than the set probability of 0.05. So that the analysis model of Pegagan (*Centella asiatica* (L.)) plant extract has a significant or significant effect on the total phenol content of the extract.

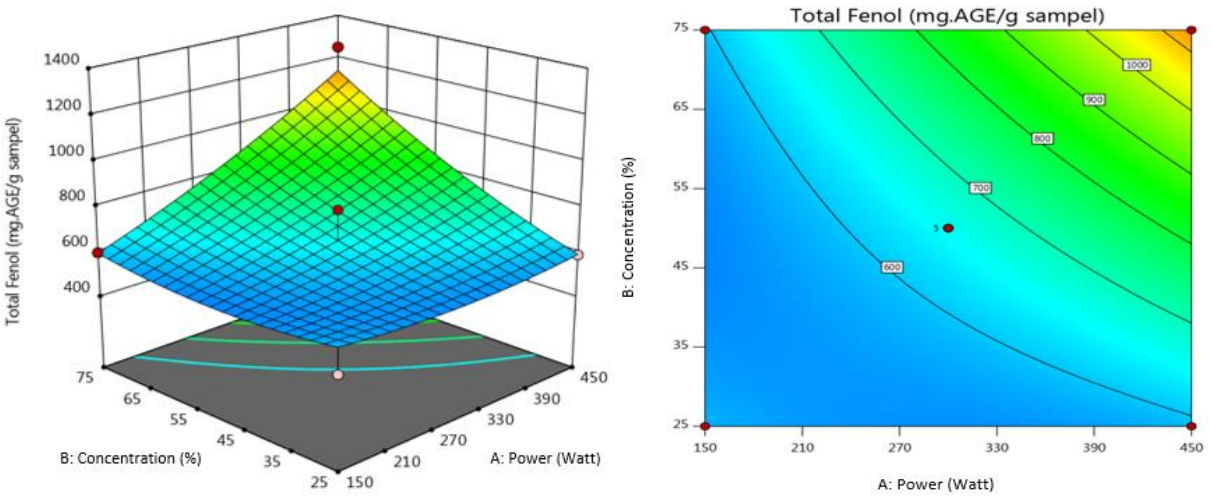
Tabel 3. Model Summery

S	R-sq	R-sq(adj)	R-sq(pred)
94.5819	90.65%	78.64%	0.00%

This analysis also obtained an R square value of 90.65% which indicates that the model used is by the results of the study. The value of R square can be stated according to the model if the value is more than 75% (Yingngam et al., 2020). The adjusted R-sq value of 78.64% indicates that there is a strong relationship between ethanol concentration, extraction time, and microwave power on the response.

3.3. Effect of variables (power, ethanol concentration, and radiation time) on the total phenol content

Figures 2, 3, and 4 are graphs showing the effect of each variable (concentration; power, time; power, concentration; time) on the total concentration of phenol. The figure shows that there are combinations of parameters that influence the response value through the presence of different colors. The lines that consist of the dots on the counterplot graph are a combination of 3 factors. The combination is formed from differences in the proportion of factors and produces the same response value for the total phenol content.



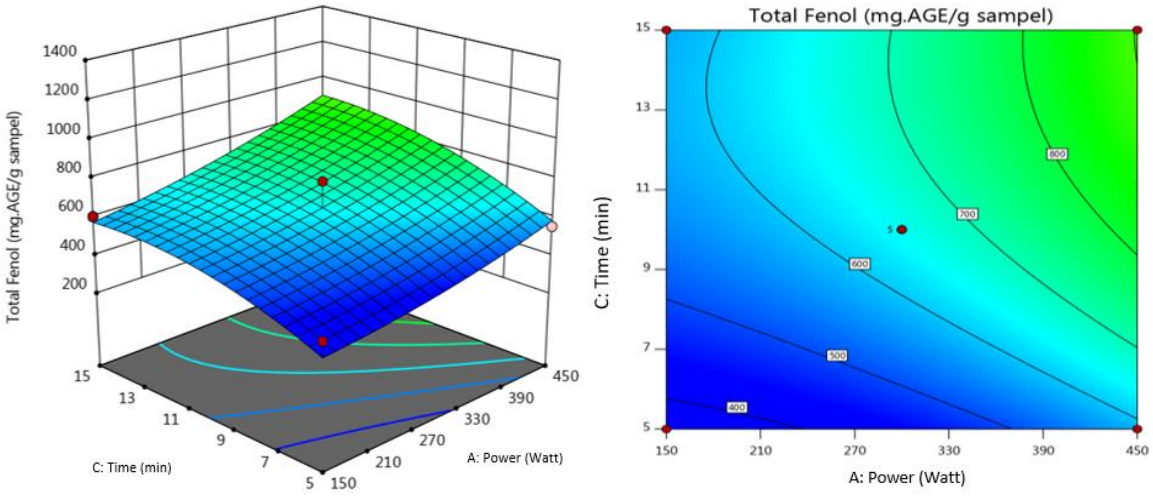
(a) (b)

Figure 2. Effect of Concentration and Power on Total Phenol of Pegagan Bioactive Compounds

The graph in Figure 2 point (a) can be observed that the extraction that produces the lowest yield at the operating conditions of the microwave power is 150 watts and the solvent concentration of ethanol is 25%. Thus, it can be seen that the total phenol content will increase with the increase in microwave power and solvent concentration. The effect of microwave

power in this study is following the research conducted by [15] which shows the higher the microwave power used will produce an increasing temperature by causing the energy generated in the microwave (radiation and rotation), so that there is microwave radiation and rotating vibrations which cause pressure on the cell wall to increase and the cell will swell, so that more and more bioactive compounds are produced.

figure 2 point (b), the X-axis shows the extraction power used, while the Y-axis shows the concentration of ethanol solvent used (%), and the lines in the contour indicate the response. The figure shows that with increasing power and solvent concentration, the total phenol concentration will increase. This can be seen from the change in the color of the area starting from the lowest in the blue area and rising to the orange area, namely the area with a higher total phenol concentration.



(a) (b)

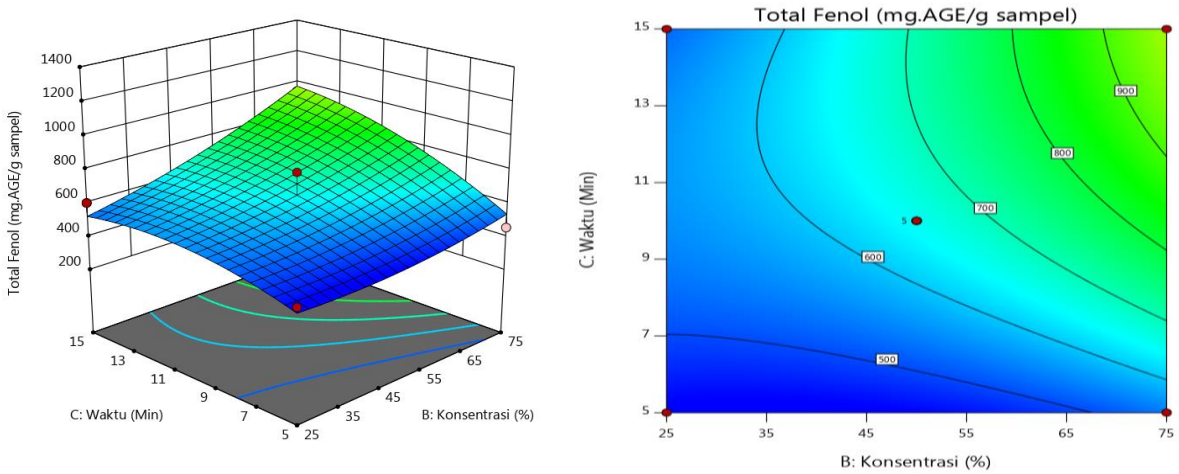
Figure 3. Effect of time and power interaction on total phenol of pegagan bioactive compounds

Figure 3 point (a) shows the response surface graph for the yield response, namely the relationship between extraction time and power to the total phenol produced. The graph in the figure shows that the extraction that produces the lowest yield is at the operating conditions of the 150-watt microwave power for 5 minutes. When the extraction time and microwave power are increased, the total phenol content produced will increase by producing the optimum total phenol content. This shows that the increase in total phenol content is directly proportional to the increase in power and extraction time.

The results obtained are by the results of research by [16] in extracting soursop leaves using the microwave-assisted extraction method, which results in an increased response of antioxidant activity with increasing extraction time. So that more and more target compounds can be extracted with ethanol solvent and MAE method, but the extraction time which increases

beyond the optimal extraction time will result in a decrease in the total phenol content. Microwave heating will cause the extraction temperature to increase with the increase in extraction time which causes the degradation of phenol compounds. In addition, microwaves can also reduce enzymatic activity which is the result of damage to the extracted compounds, the result of microwave heat will be an inhibitor of phenolase enzyme activity. And in [17] regarding extracts of phenolic compounds from rosella flower petals with microwaves which stated that an increase in the total phenol produced was in line with an increase in microwave power. This increase is due to the direct influence of microwave energy on biomolecules by ionic conduction and dipole rotation resulting in molecular motion and heating.

Figure 3 point (b) shows the response lines, where the outer line shows the lowest response value and the deeper line shows the higher response value. The figure shows that the total phenol concentration will increase with increasing extraction time and power. This can also be seen from the color change in the contour graph. This is also by the research conducted by [18] in determining the optimization of the total flavonoid content of brown algae by producing a significant effect of the interaction between power and extraction time, namely the flavonoid content increased with an increase in power of 300-450 watts and at 7-9 minutes.



(a) (b)

Figure 4. Effect of time and concentration interaction on total phenol of pegagan bioactive compounds

In figure 4 point (a) it is known that when the operating conditions of time and power increase, the total phenol content obtained increases. This shows that the increase in total phenol content is directly proportional to the increase in solvent concentration and extraction time. This is following the research conducted by [19], namely extraction with the MAE method on the antioxidant activity of corn silk extract by obtaining results showing that the higher the

concentration of ethanol solvent and the longer extraction time, the yield of corn silk extract will also increase.

Figure 4 point (b) also shows the response to the total phenol concentration which increases with increasing time and solvent concentration as evidenced by a change in color from the outermost (low) region to the deepest region, which shows a higher response value.

3.4. Regression Equation

Total phenol concentration = 932 – 2.30 power – 20.25 concentration + 25.24 time + 0.00178 power*power + 0.0819 concentration * concentration – 2.09 time*time+ 0.0360 power* concentration + 0.0348 power*time + 0.678 concentration *time

The regression equation above can be used to determine the response value of the total phenol concentration obtained if the solvent concentration, power, and extraction time are different. The coefficient of power, concentration, time shows the amount of increase or decrease in the value of the total phenol concentration. If the coefficient of power, concentration, and time are negative it will decrease the value of the total phenol concentration, whereas if it is positive it will increase the value of the total phenol concentration. In this equation, the value of the interaction coefficient between time and time has a negative value. This indicates that there is a maximum stationary point of the response surface [20].

The equation shows the interaction coefficient between power and time, power and concentration, and the interaction between concentration and time is positive, which means that the interaction between these variables can affect the response. The extraction time in the equation shows a positive value, where increasing the extraction time will increase the total phenol response until it reaches the optimal value. This is by [21], namely the longer the extraction time used, the longer the exposure time to microwaves in the sample, resulting in a high value of the antioxidant activity.

3.5. Optimization of total phenol

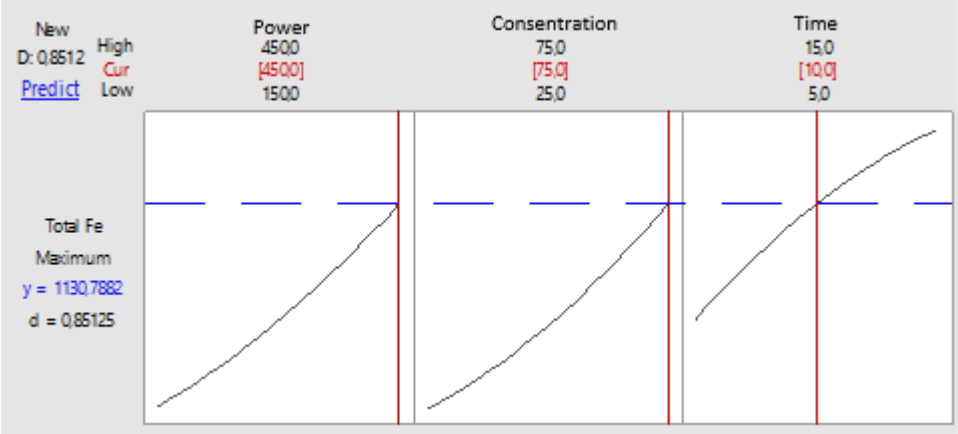


Figure 5. Graph of Optimization Value

Figure 5 shows that the desirability value reaches its maximum value when the factor value is in the red line. The desirability value is used to determine the accuracy of the optimal solution results, where on a scale of 1.00 - 0.80 it shows a very good number [22]. Judging from Figure 5, the desirability value obtained is 0.85125 which indicates that the variables used have a very good effect on the response. In the figure, the black line shows the desirability value for each response and the blue dotted line shows the response value at a certain desirability value. The graph in Figure 5 shows that the optimal value for the total phenol concentration-response was obtained at 450 watts of power, 75% concentration, and within 10 minutes by obtaining a value of 1130.7882 mgAGE/g samples obtained when the desirability value reached 0.85125.

4. Conclusion

The maximum total phenol content in the process of extracting gotu kola bioactive compounds using the MAE method resulted in a total phenol of 1251.410225 mg AGE/g sample. The operating conditions resulted in optimum total phenol at 75% solvent concentration, 450 watt microwave power, and within 10 minutes with an R-square value of 90.65%.

ACKNOWLEDGMENTS

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RESEARCH ARTICLE

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Essential Oil Extraction from Citronella (*Cymbopogon nardus* (*L.*)) Using *Solvent Free Microwave Extraction* Method (SFME)

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Abstract. Indonesia is one of the producing countries essential oils, essential oils are also a commodity that can generate foreign exchange for the country. Therefore, essential oils receive special attention from the Indonesian government. Indonesia generate 40-50 types of plants that produce essential oils and are traded in the world. Extraction using a microwave with the basic mechanism of microwave heating involves stirring polar molecules or ions that oscillate due to the influence of electric and magnetic fields called dipolar polarization. From the results of physical analysis, it can be seen that citronella essential oil obtained using the Solvent Free Microwave Extraction (SFME) method has met the standards and quality of citronella oil based on SNI 06-3953-1995, according to the parameters of color, specific gravity, and solubility in water. 80% ethanol shows citronella oil with good quality. The resultsiofiGC-MS (Gas Chromatography-Mass Spectrometry) analysis on the extraction of citronella essential oil using the Solvent Free Microwave Extraction (SFME) method obtained 2 components, namely citronella and geraniol, with citronellal percentages of 8.64% and 7.53%. Optimal operating conditions for the extraction of essential oils from citronella raw materials using the Solvent Free Microwave Extraction (SFME) method.

Keywords: *Extraction, Lemongrass, Microwave, GC-MS (Gas Chromatography-Mass Spectrometry)*

1. Introduction

Indonesia is a country that produces essential oils, essential oils are also a commodity that can generate foreign exchange for the country. Thus, essential oils receive special attention from the Indonesian government. Indonesia produces 40-50 types of plants that produce essential oils and are traded in the world. Until now, Indonesia has only produced several essential oils such as: clove essential oil, ylang leaf essential oil, patchouli leaf essential oil, vetiver essential oil, nutmeg essential oil, eucalyptus essential oil, citronella essential oil and

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sandalwood essential oil. In Indonesia, there are 6 types of essential oils, the most prominent of which are: nutmeg oil, vetiver oil, patchouli oil, eucalyptus oil, clove oil and citronella oil.

Lemongrass (*Cymbopogon nardus* (*L*.)) is one of the essential oil- producing plants from the Poaceae family and was known in Indonesia before World War II. Citronella contains about 1-2% essential oil on a dry basis and the composition of the essential oil depends on the diversity of treatments, habitats, and plant genetics [1]. According to Citronella oil is the name of citronella essential oil in the trading world. Citronella essential oil is included in one of the essential oil commodities that have considerable prospects among other essential oils [2].

The citronella plant has a characteristic lemon scent due to the main content of citronella, which is citral. Citral is a combination of netral and geranial isomers, usually used as raw material for ionine, beta carotene, and vitamin A products. Lemongrass essential oil has also been shown to have high anti-oxidant, anti-bacterial and anti-fungal properties [3].

The process of extracting essential oils usually uses the conventional method, namely the hydrodistillation method, previous studies have shown that this distillation method takes a long time and also requires a lot of solvents to get yield results, this is less efficient in terms of time and energy and less friendly to the environment [4]. Therefore, a method was developed in the process of extracting essential oils, namely by using the Microwave Assisted Extraction (MAE) method. The Microwave Assisted Extraction (MAE) method consists of Microwave Assisted Hydrodistillation (MAHD), Microwave Steam Distillation (MSD), Microwave Steam Diffusion (MSDf), and others.

Based on the above method, further development of the next Microwave Hydrodistillation (MHD) method was carried out, namely the Solvent-Free Microwave Extraction (SFME) method. In this method, the extraction process is carried out without using any solvent and also utilizing heat from the microwave [5]. This method also combines microwave heating and distillation with atmospheric pressure. The principle of this method is that it does not use water or organic solvents, so that the extraction process uses the water content contained in the plant, so during the extraction process the raw materials to be extracted will not come into contact with chemicals [4].

Based on the description above, this research uses citronella to extract essential oils using the extraction method, namely Solvent-Free Microwave Extraction (SFME), which has never been done before, so this research was carried out using the Solvent-Free Microwave Extraction (SFME) and analyzed the quality of citronella essential oil produced based on the

standard of SNI 06-2386-2006.

2. Materials and Methods

2.1. Materials

The materials used in this study were citronella leaves with a water content of 48-52% and 80% ethanol. For the schematic of the tool in the Solvent Free Microwave Extraction method, the main tool consists of a microwave. The specifications of this research equipment are Microwave, 100 ml beaker glass, 250 ml beaker glass, Vial bottle, Spatula, Analytical balance, 1000 ml round bottom flask, 1000 ml wide 2nd round flask, Oven, Clamps, Stative, Gass Chromatography-Mass Spectrometry (GC-MS) and Scanning Electron Microscope (SEM).

The research was carried out at the Basic Chemistry Laboratory and the Bioprocess Laboratory of the Chemical Engineering/Engineering Study Program, Department of Mechanical Engineering, Faculty of Engineering, University of Jember. Research activities are carried out approximately from November 2020 to January 2021.

2.2. Material preparation

The first step the raw material in the form of citronella leaves is taken fresh from the field, then the citronella leaves are cleaned of dirt that sticks so as not to interfere with the extraction process, then the citronella leaves are cut into sizes of 2-3 cm.

2.3. Extraction using the Solvent Free Microwave Extraction (SFME) method

The extraction process begins by weighing the raw materials according to the variables then installing the extraction tool then inserting the raw material for citronella into a distillation flask, for the next step to drain the water in the cooling water system then insert the flask into the microwave, after the flask is inserted the next step is to turn on the microwave and adjust the microwave power according to the variable, then record the distillation time starting from the first drop of distillate out, then stop the extraction process after the specified time according to the variable and for the last step to store the essential oil in a vial.

3. Result and Discussion

This study used citronella raw material obtained from Kemuning Lor Village, Arjasa District, Jember Regency and has been determined according to the variable, namely in fresh condition. Here as seen in the Figure below:

Figure 1. Fresh scented lemongrass plant

The variables used in this study are the ratio of the mass of raw materials to the volume of the distiller, microwave power and extraction time. The ratio of the mass of raw materials in this study is 0.05; 0.10 and 0.15 g/ml in fresh condition and put in a distiller flask with a volume of 1000 ml, the size of cutting the material is 2-3 cm long because the citronella leaves are surrounded by oil glands, oil pockets and vessels so that if not cut it will cause oil is not extracted optimally. The selection of this material mass ratio variable aims to predict yield results. For microwave power in this study using power 300 W, 450 W, and 600 W. The choice of microwave power in this extraction is due to the influence on the amount of heat energy that will be received by the raw materials [7].

Extraction of citronella essential oil in this study used the Solvent-Free Microwave Extraction (SFME) method from fresh raw materials. The yield produced using this method is large due to the influence of the water content in the fresh ingredients. This is because the extraction with fresh raw materials has a much smaller amount of water in the distiller. a small amount of water will accelerate the increase in temperature and with this rapid increase in temperature, it will accelerate the opening of the oil glands and cause a faster rate of increase in yield.

Solvent-Free Microwave Extraction method is an extraction method without the use of solvents by utilizing microwaves as a heater. the extraction process of the Solvent Free Microwave Extraction method occurs synergy between mass transfer and heat transfer from inside and outside due to internal overheating so that the extraction process is faster. In this method, the water contained in the material is refluxed into a distiller flask using a clevenger.

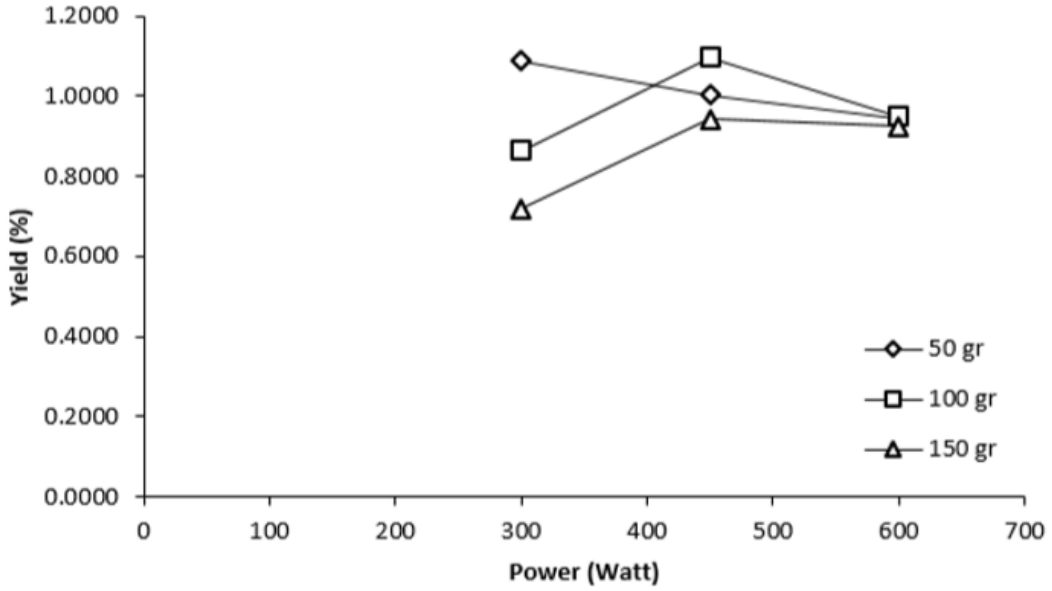


Figure 2. Graph of the effect of microwave power on oil yield fragrant lemongrass leaves

Power is the amount of energy delivered per unit time. In the extraction process, power has a major influence on the yield of citronella oil that will be produced. Microwave power is closely related to process temperature, where the greater the power to be used, the system temperature in the extraction process will increase faster [7]. The effect of power on temperature lies in the increase in temperature. The greater the power, the faster the temperature rise that occurs in the extraction process.

Based on Figure 2, it can be seen that the highest yield is at 450 watts at a mass ratio of 0.10 g/ml. Similar to research [9] on vetiver extraction, the highest yield value was obtained when the power was 450 watts in fresh raw material conditions. This is because the power is stable (power is not too low and high, so if low power causes heat transfer to be hampered and difficult to enter into the material, while for high power it can cause the material to burn quickly due to high heat transfer heat and damage the glands oil), then with a stable power can result in the material can be extracted properly. In this parameter, it can be seen that if the greater the microwave power used, the polar molecules in the material when exposed to microwave radiation will experience a faster rotation (oscillatory motion and collide with each other) and produce heat energy (*heat*) so that the target molecule can be extracted. out of the material. Yield reduction occurred at 300, 450 and 600 watts at a mass ratio of 0.05 g/mL. The cause of the decrease in yield is due to the small mass of raw materials used, while the small power also affects the small energy transfer in the sample and causes the yield to be not optimum due to material degradation. Supported by research, namely the extraction of pomelo peels using the

SFME method at a power of 300 watts and 450 watts the yield decreased by 5.3% due to damage to the oil glands at higher microwave power. Power that is too high can also cause raw materials to dry faster. According to [7] the water content in the plant also has an effect, so the higher the power used, the faster the boiling point. In this study, the optimum yield was obtained at 300 watts of power and 450 watts of power with a mass ratio of 0.10 g/ml. The water content in the plant also has an effect, so the higher the power used, the faster the boiling point. In this study, the optimum yield was obtained at 300 watts of power and 450 watts of power with a mass ratio of 0.10 g/ml. 2008) the water content in the plant also has an effect, so the higher the power used, the faster the boiling point. In this study, the optimum yield was obtained at 300 watts of power and 450 watts of power with a mass ratio of 0.10 g/ml.

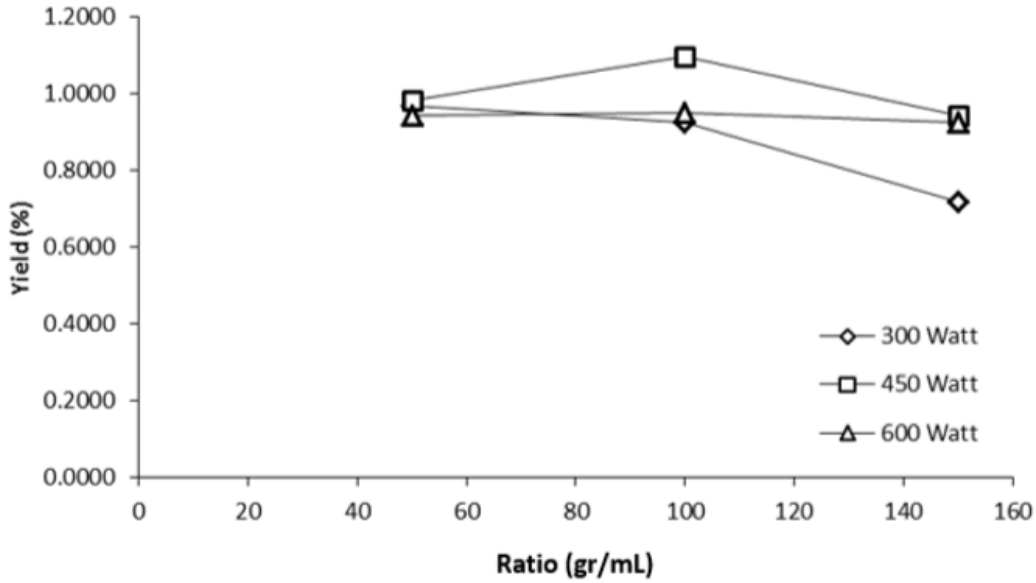


Figure 3. Graph of effect of ratio between material mass standard with distiller volume (f/d) against yield citronella oil

Extraction of citronella oil by solvent- free microwave extraction method using a mass ratio of 0.05; 0.10; 0.15 g/mL and for each variable will be placed in a distiller flask with a volume of 1000 mL. The effect of material mass per distiller volume (F/D) on yield can be seen in Figure 3.

Based on Figure 3, the optimum ratio is found at 0.10 g/mL. This is supported by research on the extraction of essential oil of eucalyptus leaves using the Solvent Free Microwave Extraction (SFME) method in fresh conditions, the optimum yield was obtained at a mass ratio of 0.10 g/ml. Furthermore, in the extraction of citronella essential oil, there was an increase in yield from a ratio of 0.05 gr/ml to 0.10 gr/ml at 450 watts of power. However, there

was a decrease in yield at a ratio of 0.15gr/ml. This is supported by research on the extraction of essential oils from stems, leaves, and peels of limes with the Solvent Free Microwave Extraction method on the effect of the ratio between the mass of raw materials and the volume of the distiller on the yield of fresh kaffir lime leaf oil. optimal at 0.25 g/ml, Broadly speaking, the yield increased from a ratio of 0.05 gr/ml to 0.25 gr/ml, but at a ratio of 0.3 gr/ml it decreased and was caused by several factors such as the amount of material in the distiller flask, the density of the material can cause the formation of pathways. "rat holes" steam which will affect the yield of essential oils, either increasing the yield or reducing the yield of essential oils. The material density factor is the ratio between the mass of the material and the volume capacity of the distiller flask. This can cause the steam generated by microwave heat to be difficult to penetrate in the material to carry the diffused oil molecules out of the material [6]. In addition, the ratio used relates to how dense (amount) of raw materials are included in the distiller flask, this causes the yield of citronella oil to decrease. For the lowest yield, based on Figure 2, which is 0.7181 at 300 watts of power, 150 grams of material and 60 minutes of time.

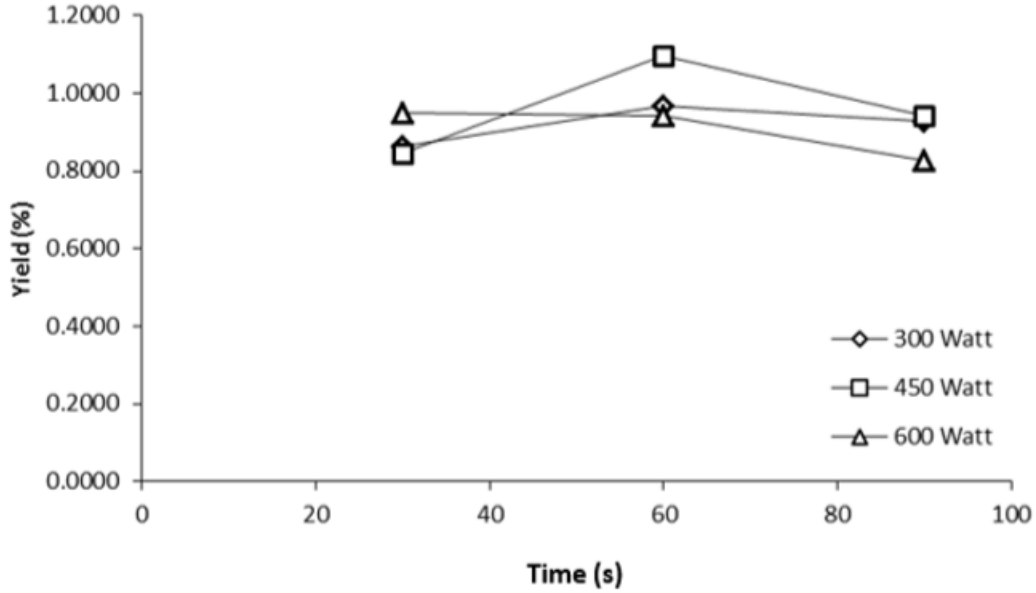


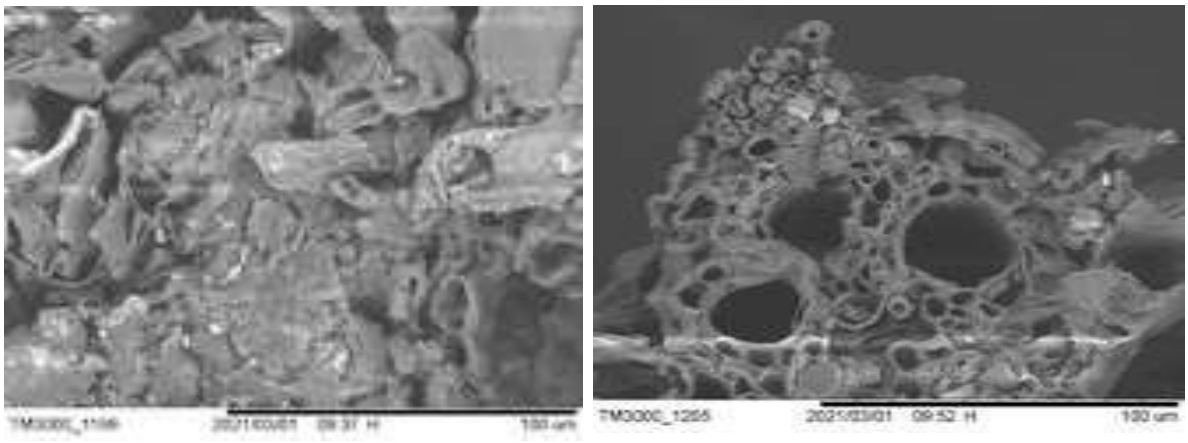
Figure 4. Graph of the effect of time on yield lemongrass oil

Figure 4. shows the relationship between extraction time and yield of citronella oil. The extraction of citronella oil using the Solvent Free Microwave Extraction method for 30 and 60 minutes, namely at 300 watts of 450 watts produces yields that tend to increase constantly with increasing extraction time. However, at 600 watts the yield decreases due to the high heat released causing the raw material to dry quickly. The lowest yield is at 90 minutes of 600 watts of mass 100 grams with a yield of 0.8259, this is because the high power and longtime cause

the raw materials to dry up to charred. According to with the longer extraction time, the increase in yield obtained becomes smaller or smaller.

The quality of essential oils is influenced by several factors such as raw materials, post-harvest handling, production and storage processes. Based on the results of this study, it is known that the quality of citronella essential oil is influenced by one factor, namely raw materials, the raw materials in this study are in different conditions, such as differences in material conditions from post-harvest to the extraction process, material conditions from fresh to slightly wilted. and this causes the content of the essential oil is different. From the results of the Gas Chromatography-Mass Spectroscopy (GC-MS) test, there are 84 components contained in the essential oil of citronella. There are two compounds with the highest % area, namely 6- octenal,3,7-dimethyl and geraniol, 6- octenal,3,7-dimethyl is another name for citronellal with a yield of 8.64% with a molecular weight of 154 and belongs to the class of oxidized monoterpenes. The second highest compound was geraniol with a yield of 7.53%, a molecular weight of 154 and was included in the oxidized monoterpene class.

In a previous study for the results of geraniol and sitonellal citronella essential oil using methanol as a solvent, namely 20.07% and 36.11%, respectively. These results are different due to several factors such as the method used and also the area of origin of the raw materials, such as weather factors and soil conditions during the citronella planting process. The GC-MS analysis test in this study used a random sample.



(a) (b)

Figure 5. SEM results of citronella leaves with 1000 times magnification (a) before extraction (b) after extraction

4. Conclusion

The effect of microwave power, the ratio of the mass of raw materials to the volume of the F/D distiller and the resulting extraction time as well as the suitability of the extraction

results to SNI, namely the increase in yield occurs at a power of 300 - 450 watts, but decreases at 600 watts with a raw material ratio of 0.10 g /ml, the amount of raw materials contained in the distillation flask causes an increase in the amount of yield, namely in the ratio of 0.05 g/ml – 0.10 g/ml, but there is a decrease in the ratio of 0.15 g/ml due to the density of raw materials, time extraction tends to increase in the variable 30-60 minutes, but there is a decrease in the extraction time of 90 minutes, the results of the GC-MS analysis show that there are 2 main components of citronella essential oil, namely citronella and geraniol with an abundance percentage of 8.64% and 7.53%, the analysis of the physical properties of citronella essential oil has good quality because it meets the Indonesian National Standards, namely the parameters of color, specific gravity, and 80% alcohol solubility. The optimum conditions for extracting citronella essential oil using the Solvent Free Microwave Extraction (SFME) method are as follows, the optimal power obtained when using 450 watts of power with a yield of 1.096%, the ratio between citronella and distiller (F/D) obtained the optimum conditions at the mass ratio 0.10 gr/ml, with a yield of 1.0969%, the optimal time for the extraction process is 60 minutes, with a yield of 1.0873%.

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