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Extraction Total Phenolic Content of Ketapang Leaves (Terminalia catappa) using Ultrasonic

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ABSTRACT

Ketapang leaves (Terminalia catappa) is a plant which has many pharmacological activities one of them is antioxidat activity. Phenolic compound is one that represented the antioxidant activity. The concentration of phenolic compounds was influenced by the method and condition of extraction. This study was conducted to determine the effect of ultrasonic extraction method, extraction time and the ratio of ethanol : water to total phenolic content in Ketapang leaves (Terminalia catappa). Extraction is completed by ultrasonic extraction method using 96% ethanol-water as solvent with solvent ratio variation (50:50, 60:40, 70:30) in time (5 minutes, 10 minutes, 20 minutes and 40 minutes). Measurment data from total phenolic content of the extracts Ketapang leaves (Terminalia catappa) shows that the extraction with 20 minutes with 96% ethanol-water 50:50 generate the highest total phenolic content, equal to 4862,352941mg GAE / g.

Keywords: *Phenolic, ketapang leaves, ultrasonic.*

1. INTRODUCTION

Ketapang plants (Terminalia catappa L) is one of the tribal plant members of Combretaceae scattered almost throughout the territory of Indonesia so easy to be cultivated (Riskitavani & Purwani, 2013). During this time people only know ketapang plant as a shade plant in gardens and roadside because it has broad leaves and bushy, branched like an umbrella being arrayed (Sahala & Soehihardjo, 2012). The ketapang plants (Terminalia catappa L.) have many benefits, especially their function as traditional medicinal plants (Patricia et al., 2016). In Asian countries, ketapang leaves are usually used for the treatment of dermatitis, hepatitis and diarrhea. Ketapang leaves also contain phenolic compounds that act as antioxidants (Baratelli et al., 2012). The content of ketapang leaves phenolic compound resulting from the extraction of maceration for four days that is equal to 111,396 ppm (Sahala & Soehihardjo, 2012).

Plants with a high content of phenolic compounds are known to have strong antioxidant activity (Lumingkewas et al., 2014). The antioxidant activity of phenolic compounds play an important role in the absorption and penetralkan free radicals or decompose peroxide (Margaretta et al., 2011). The concentration of phenolic compound from ketapang leaves can be influenced by the method and condition of extraction. Extraction methods continue to be developed to shorten extraction time, obtain more extracts, and less solvent volume, and have better activity (Utami et al., 2015).

One method of extraction is using ultrasonic method, ultrasonic method is method using ultrasonic wave with frequency greater than 20 kHz (Rafsanjani & Putri, 2015). Ultrasonic has a faster and more efficient capability in the extraction process compared to conventional extraction methods such as maceration extraction and soxhlet (Wahyuni & Widjanarko, 2015). Ultrasonic waves are capable of increasing solvent

diffusion in a substance, in which the cavitation bubble effect is generated not only around the particles but also directly to the center of the substance (Fuadi, 2012).

The composition of the extraction results other than depending on the extraction technique and the time of contact, the type of solvent used is also very influential (Mustapa et al., 2015). In principle a material will readily dissolve in the same solvent polarity (Ujic et al., 2016). Phenolic compounds are generally polar in that they are more soluble in polar solvents (Margaretta et al., 2011). Rafsanjani & Putri (2015) reported that the highest total phenolic content was produced with ethanol solvent of 2673.06 µg / g and the lowest was on ethyl acetate solvent at 1817.25 µg / g. This is because the extraction of phenol compounds will increase as the addition of solvent polarity.

Research conducted by (Katarzyna and Swigło, 2016) showed that the extraction of phenolic compounds with a binary solvent (alcohol-water) more efficiently than with pure alcohol solvent. Other studies have also reported that the use of ethanol-water binary solvent ratio (75:25) yielded more total content of phenol in the amount of 164.20 mg GAE / g compared with a ratio (100: 0) is equal to 152, 03 mg GAE / g (Sun et al., 2015). The use of ethanol-water solvents was also supported by the results of a total phenol analysis of the leaves of the gods, showing that the ethanol-water solvent (60:40) yielded the highest total phenol content of 94.24 mg GAE / g (Krisyanella et al., 2012).Based on the description above, the research conducted test total phenolic content of the leaves of Ketapang (Terminalia catappa) with ultrasonic extraction method with the effect of extraction time and solvent ratio of 96% ethanol: water.

2. METHODS

Material

The materials used in the research of total phenolic content of leaf extract (Terminalia catappa) assisted ultrasonic waves are: ketapang leaves, aquades, 96% ethanol (Merck), folin ciocalteu (Merck), sodium carbonate (Na2CO3) (Merck) , Gallic acid (Merck).

Equipment

The tools used in the test of total phenolic content of the ketapang leaf extract (Terminalia catappa) assisted ultrasonic waves: aluminum foil, blender (Sanex MX-T2GN), 100 ml bottle, stirring rod, mouthpiece (Pyrex), glass Beker 100 Ml (Pyrex), 250 ml glass beaker (pyrex), watch glass, filter paper, 100 ml measuring flask (Pyrex), 250 ml measuring flask (Pyrex), analytical balance, 25 ml volume pipette (Pyrex), 5 ml volume pipette (Pyrex), 1 ml volume pipette (Pyrex), dropper dropper, spatula, Genesys 10 Uv spectrophotometer, reaction tube (Pyrex), ultrasonic cleaner.

Experimental procedure

Sample Preparation

Ketapang leaves (Terminalia catappa) obtained from campus environment Faculty of Engineering University of Sultan Ageng Tirtayasa, Cilegon-Banten. Ketapang leaves used are the leaves that have grown a green, not a leaf that is young or old (Sahala & Soehihardjo, 2012). Leaf fresh ketapang washed first to remove the dirt on the leaves, then cutting samapai size 1-2 cm. The leaves of ketapang are dried by air-dried at room temperature (Krisyanella et al., 2012). Ketapang leaves that have dried and then made powder by blend until smooth, powder then filtered to get a fine powder (Patricia et al., 2016).

Ultrasonic Wave-assisted Extraction

Extraction was performed using a modified ultrasonic method as quoted from (Sari et al., 2012). Three grams of finely pressed leaves were inserted into 100 ml beaker and dissolved with solvent 96% ethanol: water (50:50, 60:40 and 70:30) by the ratio of sample mass and solvent volume 1:10 (w / v) And covered with aluminum foil. The prepared samples were extracted with the help of ultrasonic waves with variations of extraction time of 5 minutes, 10 minutes, 20 minutes and 40 minutes, the extract formed was filtered using filter paper to separate the residue from the filtrate.

Determination of Maximum Wavelength

This maximum wavelength measurement is used in the solution of gallic acid at a concentration of 100 ppm. 100 ppm gallic acid solution of 0.1 ml was poured into the reaction tube, then 1 ml of 50% Folin Ciocalteu solution and vortex for 1 min. The soluble solution added 2 ml of 2% sodium carbonate (Na 2CO3) solution. The mixture is kept in a dark room for 30 minutes. The maximum wavelength of gallic acid are scanned using spectrophotometer UV-Vis in the range of 700 nm - 800 nm.

Making Curve Standard Gallic Acid

The manufacture of calibration curve of gallic acid is done by preparing solution of gallic acid with different concentration (0, 25, 50, 100, 250, 500 mg / L). A total of 0.1 ml of different gallic acid concentrations were poured into the reaction tube, then added 1 ml of 50% Folin Ciocalteu solution and vortex for 1 min. Tersubut solution was added 2 ml solution of sodium carbonate (Na2CO3) 2%. The mixture is kept in a dark room for 30 minutes. The calibration curve is made by connecting the concentration of gallic acid with absorbance and expressed in mg of galic acid per gram of extract mass (mg GAE / g).

Total Phenolic Content Analysis

The total phenolic content was determined using the modified Folin Ciocalteu method as quoted from (Ismail et al., 2012). Extracts of 0.1 ml were poured into the reaction tube, then 1 ml of 50% Folin Ciocalteu solution and vortex for 1 min. The soluble solution added 2 ml of 2% sodium carbonate (Na 2CO3) solution. The mixture is kept in a dark room for 30 minutes. Absorbance of the extract solution was read at maximum wavelength with the spectrophotometer UV-Vis.

Experiment Variables

The variables changed in this study included ultrasonic assisted extraction time (5 minutes, 10 minutes, 20 minutes and 40 minutes) and solvent ratio 96%

ethanol:water (50:50, 60:40 and 70:30). The fixed variables in this study include the mass of ketapang leaf sample (3 grams), the ratio of the sample mass to the volume of solvent (1:10) and the type of solvent (ethanol: water). This research was done by UV-vis spectrophotometer analysis to see the total phenolic content of ketapang leaves by making standard curve of gallic acid at maximum wavelength

3. RESULTS AND DISCUSSION

Simplicia Preparing

Part of the plant used in this study are the leaves of Ketapang plants (Terminalia catappa). Ketapang leaves used are the leaves that have grown a green, not a young leaf. This is based on research reported by Aziz and Jack (2015) to the leaves of Nypa fruticans that is mature leaves have higher total phenolic content than young leaves. During the period of growth, the plants synthesize secondary metabolites and bioactive compounds with different amounts that are affected by the morphology and age of leaves (Felicia et al., 2016).

The ketapang leaves used in the extraction process are dried first. The drying process is carried out by winding in room temperature. The purpose of drying is to prevent the growth of mold growth or microorganisms and the decomposition of active compounds by enzymatic reactions and hydrolysis processes because high water content, so that the resulting sample is not easily damaged so it can be stored for a relatively long time, can also affect the extraction process undertaken. The lower the moisture content of materials it is increasingly easier for the solvent to extract the active components of the desired compound (Diniatik et al., 2015). This is supported by research conducted by (Verawati et al., 2016) that the total phenolic content of the dried leaves with maceration process produces greater levels of 356.7619 mg GAE / g extract, compared with fresh leaves only produce 293, 3015 mg GAE / g extract. The drying is carried out by means of the temperature at the room temperature, because based on the research (Masduqi et al., 2014) indicates that the drying process with aerated at room temperature results in a higher total phenolic content of 1656.3 ppm, compared with the drying process in the sun or Using an oven that successively yielded only 1179.7 ppm and 1274.4. This occurs because the draining with the wind at room temperature has a lower temperature than drying using the oven and in the sun. Phenolic compounds are easily oxidized and sensitive to heat treatment, so that in the presence of sun-drying or oven process it can decrease the content of phenolic compounds (Masduqi et al., 2014).

Ketapang Leaf Extraction

Ketapang leaves have phenolic compounds that are useful as natural antioxidants. The extraction of phenolic compounds from ketapang leaves is done by ultrasonic extraction method because it is a very simple extraction process and faster extraction time so it becomes an efficient alternative compared to conventional extraction technique (Fuadi, 2012.). The solvent used in the extraction process is a mixture of ethanol: water at a ratio of 50:50, 60:40 and 70:30. Selection of solvent ratio ethanol: water in ultrasonic extraction is based on the results of several studies. The use of ethanol-water binary solvent ratio (75:25) has been done by (Sun et al., 2015) to produce a total content of phenolic higher at 164.20 mg GAE / g. The use of ethanol-water in total phenolic analysis of leaf god with a ratio of 60:40 resulted in total phenolic content of the highest of 94.24 mg GAE / g (Krisyanella et al., 2012). Research conducted by (Rivai et al., 2013) also showed that the total phenolic content of soursop leaves with ethanol-water solvent at 50:50 ratio and resulted in the largest total phenolic content of 9.071 mg / g. Based on the results of such research, so this study aims to determine the best conditions in the comparison of ethanol: water 50:50, 60:40 and 70:30 on the extraction of leaves of Ketapang. Ultrasonic extraction process performed at the time of 5 minutes, 10 minutes, 20 minutes and 40 minutes. This variation of extraction time is intended to find the extraction conditions that produce the largest Total Phenolic Content (TPC).

Total Phenolic Content (TPC)

Determination of total phenolic content carried by the Folin-Ciocalteu method with comparative compound gallic acid. This method was chosen because it is a specific method, sensitive to the phenolic compounds and the use of reagents in small amounts and can react in a short time (Krisyanella, et al., 2012). The basis of this method is the reduction of the strength of the hydroxyl group of phenolic compounds. The presence of hydroxyl groups on the phenolic compounds can reduce complex compounds fosfomolibdat and fosfowolframat the Folin-Ciocalteu reagent into a blue colored complex compounds (Sahala & Soehihardjo, 2012). The intensity of blue color is determined by the number of phenolic compounds in the sample solution. The greater the concentration of phenolic compounds in the sample the more intense blue color that looks (Bouterfasa et al., 2016). In accordance with the principles of UV-Visibel that absorb color solution at a wavelength of 400-800 nm, the blue complex solution is what will set the value of absorbannya with a wavelength corresponding to the levels of the sample solution can be found (Krisyanella, et al., 2012). Total phenolic content test, the addition of Na2CO3 to form alkaline and accelerating the reduction reaction by the Folin-Ciocalteu of phenolic hydroxyl group in the sample (Gabriel et al., 2014).

The measurement of total phenolic content of this ketapang leaf extract using Folin-Ciocalteu reagent is done by three steps, namely the determination of the maximum absorbance of gallic acid, the manufacture of curve of atandar of gallic acid and the measurement of uptake in the sample.Determination of Maximum Wavelength Gallic Acid. Before measuring the absorbance of gallic acid solution and the extract solution sample, a maximum wavelength determination of gallic acid should be determined first. This is done to be able to determine at what wavelength the error acid gives the highest absorption. In determining the maximum wavelength is used gallic acid solution at a concentration of 100 ppm

and obtained a maximum wavelength of 752 nm in absorption 0.509.

Research conducted (Rahmawati et al., 2013) showed that the maximum wavelength of gallic acid with a concentration of 200 ppm was 755 nm. The value of the maximum wavelength of gallic acid based research is not much different from previous research.

Gallic Acid Calibration Curve

In this method gallic acid is used as compounds to express the equivalence of phenolic compounds in the sample to gallic acid, milligram equivalents of the inner gallic acid.

Table 1. Results of determination of phenolic total content of ketapang leaf extract

Ethanol :water	Time (min)	Absorbance (nm)	Concentration (ppm)	TPC (mg GAE/g ekstract)
50 : 50	5	0,275	88,4118	1768,23
	10	0,333	133,1176	2662,35
	20	0,52	243,1176	4862,35
	40	0,467	211,9411	4238,82
60 : 40	5	0,247	82,5294	1650,58
	10	0,257	88,4118	1768,23
	20	0,434	192,5294	3850,58
	40	0,375	157,8235	3156,47
70 : 30	5	0,182	44,29411	885,88
	10	0,296	111,3529	2227,05
	20	0,381	161,3529	3227,05
	40	0,194	51,3529	1027,05

Gallic acid is selected as a compound for reasons available in nature, easy to isolate, and has good stability (Sahala & Soehihardjo, 2012).

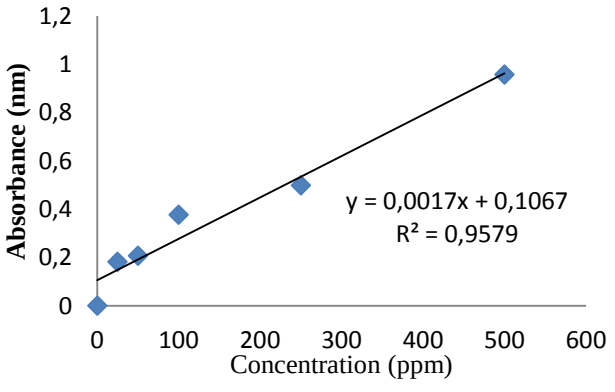

Fig.1. Gallic Acid Standard Curve

Preparation of standard curve of gallic acid is done by measuring the absorption given by the test solution with concentration of 0 ppm, 25 ppm, 50 ppm, 100 ppm, 250 ppm and 500 ppm at maximum wavelength of 752 nm. Based on the result of measurement of absorbance of standard solution of gallic acid at various concentrations then made standard curve of gallic acid with linear regression equation is y = 0,0017x + 0,1067, where x is concentration (C) ppm and y is absorbance (A). From the result of absorbance measurement, then calculated Total Phenolic Content (TPC) in test solution by using linear regression equation on standard curve. Total Phenolic Content (TPC) from ketapang leaves can be seen in table 1.

Determination of Absorption Samples

The extracted sample solution which has been reacted with Folin-Ciocalteu was measured uptake at a maximum wavelength of 752 nm. The results of the total phenolic content test can be seen in Table 1. From the obtained absorbance values, the total phenolic content of each sample can be calculated using the standard gained standard linear regression equation previously obtained. Based on the test results, it can be seen the operating conditions that have the highest total phenolic content.

Effect of Extraction Time Against Total Phenolic Content On Leaf Ketapang

The effect of ultrasonic extraction time on Total Phenolic Content (TPC), in ketapang leaf extract is seen in Fig. 2.

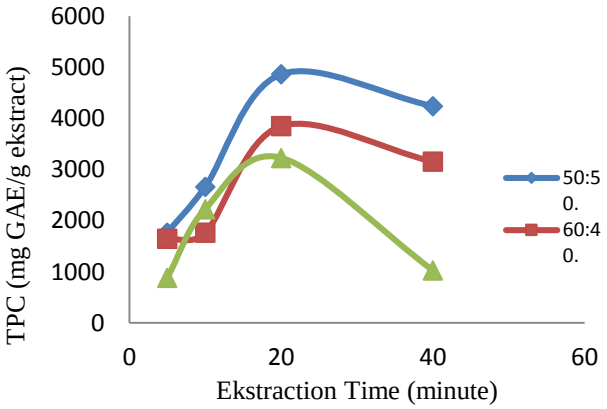

Fig.2. Effect of ultrasonic extraction time on TPC of ketapang leaf extract with solvent ratio ethanol: water 50:50, 60:40 and 70:30.

From Fig. 2 TPC expressed as mg gallic acid equivalents (GAE) per gram of leaf extract ketapan, and seen that the TPC is affected by the extraction time and the ratio of 96% ethanol: water is used. The higher the TPC gain along with the longer extraction time is up to 20 minutes for all the solvent ratio, but the total phenolic content decrease in 40 minutes. This is because the longer the time of extraction, the contact time between the leaves of Ketapang with the longer solvent so that more phenolic compounds (solutes) are absorbed into the solvent until the saturation point of the solution (Maslukhah et al., 2016).

The application of ultrasonic extraction on liquid solid extraction can increase the mass transfer caused by the rise of solvent penetration into the plant tissue by means of cavitation effects. The cavitation bubbles will form on the cell wall of plants due to the presence of ultrasonic waves. The effects of the outbreak of cavitation bubbles

can form or MicroJet shock wave with high pressure (200 MPa) and lead to increased cell wall pores (Wagterveld, 2013). Medium through which to experience the vibration caused by the ultrasonic waves. Vibrations were given ultrasound will provide an intensive agitation against the extraction process. The stirring process will increase the osmosis between the material and the solvent so that it will speed up the extraction process (Sari et al., 2012).

Extension of extraction time above 20 minutes, at solvent ratio ethanol: water 50:50, 60:40 and 70:30 resulted in decreased TPC gain (Fig. 3). This is because excessive extraction time can lead to increased temperature of the extract because the energy in ultrasonic is the intensity of the ultrasonic waves that propagate and bring energy to a surface area per unit of time. If the energy of the ultrasonic wave through the network, it will release heat energy, causing heating resulting in tissue temperature rise (Jos et al., 2011).

Rising temperatures can increase the phenolic level to a certain temperature then decrease along with the higher temperature increase, this is due to the decomposition of phenolic compounds (Maslukhah et al., 2016). The longer the ultrasonic extraction time will lead to higher extract temperature. This high temperature can be the cause of the reduced total phenolic content of the ketapang leaves, because the phenolic compounds are highly sensitive, unstable and highly susceptible to degradation. The most important degradation factors are temperature, oxygen and light content (Sari et al., 2012). According to the results of the study (Sari et al., 2012) phenolic content will be degraded at 60 ° C, and this corresponds to the results obtained, which at the time of extraction time of 40 minutes extract temperature reached 60oC and TPC value in ketapang leaves decreased. Research conducted by (Wardhani et al., 2013) showed that TPC from seaweed extraction would increase with increasing temperature, but too high temperature (above 55 °C) could cause decomposition of some phenolic compounds thereby reducing TPC value.

Table 2. Effect of extraction time on extract temperature and TPC

Solvent Ratio (v/v)	time (min)	T ekstract (°C)	TPC (mg GAE /g ekstract)	Average TPC (mg GAE/g ekstract)
50:50	5	33	1768,2353	1434,9019
60:40			1650,5882	
70:30			885,8823	
50:50	10	36	2662,3529	2219,2157
60:40			1768,2353	
70:30			2227,0588	
50:50	20	46	4862,3529	3979,9999
60:40			3850,5882	
70:30			3227,0588	
50:50	40	60	4238,8235	2807,4509
60:40			3156,4706	
70:30			1027,0588	

Table 2. shows that the optimum time of extraction is in the 20th dimenite, this can be proven by the higher TPC produced with the average TPC value of 3979.9999 mg GAE / g extract compared with the extraction time of 5 minutes, 10 minutes and 40 minutes. The results of this study were supported by (Handayani et al., 2016) who reported that the phenolic content of soursop leaf in the 15th minute reached 14216.50 ppm and in the 20th minute reached 4527.62 ppm, it showed that TPC results only slightly changed Because the suspected solution has entered the saturation point so it can not produce more extracts.

Effect of Solvent on Total Phenolic Content of Ketapang Leaf

The results of the total phenolic content test on ketapang leaves showed that the use of ethanol-water mixture solvent with ratio 50:50, 60:40 and 70:30 was very influential. This can be seen from the result of the total phenolic content of different leaves of ketapang with the use of different solvent ratio. The results obtained showed that the highest total phenolic content was obtained by using 50:50 ethanol-water solvent which can be seen in Fig. 3.

In principle a material will readily dissolve in the same solvent polarity (Ujic et al., 2016). Phenolic compounds are generally polar in that they are more soluble in polar solvents (Margaretta et al., 2011). In this study the highest phenolic compound content was found in solvent ratio ethanol:water 50:50, followed by solvent ratio ethanol:water 60:40 and the lowest was obtained at solvent ratio ethanol: water 70:30. This shows that as the water composition in the solvent increases, the more polar phenolic compounds in the ketapang leaves can diffuse into the solvent.

Phenolic compounds generally are polar will dissolve in polar solvents such as water which is polar solvent and solvent is ethanol group has polar and non-polar. The hydroxyl group (-OH) is a highly polar group because of the high degree of electronegativity of oxygen. On the other hand, ethanol also has a non-polar carbon (C2H5-) so it can dissolve non-polar compounds (Azis et al., 2014). This means that the highest concentration of phenolic compound in the solvent ratio ethanol 96% -air 50:50 corresponds to the polarity level of the solvent, the higher the polarity of the solution the more dissolved the phenolic compound. The soluble phenolic compound is influenced by solvent polarity. This is supported by the research conducted (Rivai et al., 2013) on the total phenolic content test on soursop leaves reported that the use of solvent rthanol 96% -air with a ratio of 1: 1 resulted in the highest total phenolic compound content of 9.071 mg / g, With the use of solvent ethanol 96% -air with a ratio of 1: 0 and 2: 1 ie 6.569 mg / g and 8.159 mg / g.

The results of this study showed that the optimum time for ultrasonic extraction of phenolic compounds from ketapang leaves was 20 minutes using ethanol 96% -air 50:50 solvent.

4. CONCLUSION

Research on total phenolic content test of the extract of leaves of Ketapang (Terminalia catappa) assisted

ultrasonic waves that have to be got some conclusions as follows:

a. The longer the time of ultrasonic assisted extraction can increase the temperature of the extract, so the extraction at the 40 minute extract temperature reaches 60 °C and there is a decrease in the total phenolic content of the operating conditions.
b. Use of solvent 96% ethanol: water with a ratio of 50:50 yields a greater total phenolic content than the 60:40 and 70: 30 ratio.
c. The content of total phenolic ketapang leaf extract obtained at the time of ultrasonic extraction for 20 minutes, with a 96% ethanol: water 50:50 in the amount of 4862.3529 mg GAE/g extract

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