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Microwave-Assisted Extraction of Polyphenol Content from Leaves of *Tristaniopsis merguensis* Griff.

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Tristaniopsis merguensis Griff. is a species of the *Myrtaceae* family and has been widely used by people of Bangka Belitung as a traditional medicine to reduce cholesterol, gastric pains, and improve cardiac performance. Extraction methods are the crucial efficacy of herbal medicine. The conventional method, like maceration, takes a long time. In this study, the leaves of *Tristaniopsis merguensis* were extracted using Microwave-Assisted Extraction (MAE) to reduce extraction time. The extraction using MARS (Microwave Accelerated Reaction System) 6 by CEM Corporation with time variation times of 5, 10, 15, 30 min with temperature of 60, 80, 100^{o}C at 1200 W. The yield using acetone extraction of *Tristaniopsis merguensis* leaves increases with time and temperature. The extraction dependent on solvent extraction, polar solvent like ethanol, and methanol were higher than semi-polar solvents like acetone and ethyl acetate. The polyphenol content of acetone extract using MAE (10 min, 80^{o}C) was found to be 234.67 mg Gallic Acid Equivalent per gram (GAE/g); it was higher than acetone extract using maceration. The phytochemical results show there are no difference in the active compound using MAE and maceration, i.e. alkaloids, tannin, and flavonoids. Yield extraction, time, and phytochemical results of MAE are more favorable than a maceration.

Keywords: Gallic Acid Equivalent (GAE), Microwave-Assisted Extraction, Polyphenol content, Phytochemical, *Tristaniopsis merguensis*

INTRODUCTION

Tristaniopsis merguensis Griff. is one of the many trees scattered in the forests of Bangka Belitung (Yarli 2011). This species is a member of the genus *Tristaniopsis* with the family *Myrtaceae*. *Tristaniopsis merguensis* Griff. is used by the community to get Pelawan honey and Pelawan fungi. Pelawan fungi contains antioxidants and essential amino acids while Pelawan honey has a bitter taste mixed with a sweet taste that is believed to be a cough medicine and antidiabetic medicine (Bellosta et al. 2003).

Phenolic compounds from natural ingredients have the best and highest biological activity, such as antibacterial, antioxidant, anticancer, and antimalarial (Carocho et al. 2014, Yap et al. 2007, Kusmardiyani et al. 2016). Extracts that have high phenolic content are often used as ingredients for herbal medicine (Dahmoune et al. 2015). Examples are ginger (*Zingiber officinale*), the crown of god (*Phaleria macrocarpa*), cumin (*Cuminum cyminum*) and others (El-Ghorab et al. 2010, Sukandar et al. 2016). The content of phenolic compounds in methanol extract of ginger (*Zingiber*

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officinale) is equal to 95.2 mg/g. Whereas in cumin (*Cuminum cyminum*), its phenolic level is equal to 35.3 mg/g. Phenolic levels in the crown of god (*Phaleria macrocarpa*) is also high at 60.5 mg/g (Hendra et al. 2011). The high phenolic content of ginger, the crown of god, and cumin indicates that plants with high phenolic levels can be used as nutritious herbal medicines (Verotta et al. 2001).

Extraction and selection of appropriate solvents is the key to the efficacy of herbal medicines (Sporring et al. 2005, Mandal et al. 2007, Li et al. 2013). Conventional extraction methods, such as maceration, take 3-7 days. Maceration itself is a cold method of extraction that is simple. But it has some disadvantages, i.e. it requires a long process within a few days and it results in an imperfect filtering process (Chigurupati et al. 2018, Hoa et al. 2002). Thus, the high demand for herbal medicines makes conventional extraction processes less efficient. Another extraction methods that are efficient in terms of time is Microwave-Assisted Extraction (MAE) (Lasano et al. 2018, Hemwimon et al. 2007, (del Mundo et al., 2018). This method combines microwave radiation with heat. The advantage of this method is that it requires shorter extraction time with higher extraction yield of active compounds (Bhuyan et al. 2015).

MAE does have better efficiency. There are several factors that influence the content of active compounds extracted using MAE (Borja et al., 2014). Heat and microwaves can indeed increase the solubility and diffusion of the active compounds (Hemwimon et al., 2007). But, if the temperature was increased too high it will not significantly influence the extraction yield (Borja et al., 2014). On the other hand, some active compounds derived from polyphenols, such as 2-methoxycinnamaldehyde, actually has lower yield when the extraction temperature was more than 80^{o}C (Kim, 2017). Too high temperature can also cause degradation of active compounds, especially phenolic compounds that are not stable to heat (thermolable) (Routray & Orsat, 2012). Therefore, it is necessary to choose the optimum conditions to produce high extraction results with low degradation of active compounds.

In addition, solvents are an important factor in MAE (Routray & Orsat, 2012). In general, the higher the dielectric constant or the more polar the solvent, the higher the capacity of the solvent to absorb microwaves, thereby causing the rate of heating and solvation of active compounds to increase. But, this does not guarantee to a higher extraction results, especially for thermolable compounds (Kaufmann & Christen, 2002). Additionally, the content of active compounds, such as polyphenols, from each plant are different (Li et al. 2013). This causes the need to search for solvents and the optimum conditions for MAE to dissolve the polyphenol compounds. Therefore, the study examined the optimum conditions to extract polyphenol of *Tristaniopsis merguensis* Grifft. leaves using the Microwave Assisted Extraction (MAE).

METHOD

Samples Preparation

The sample of this study was *Tristaniopsis merguensis* Griff. leaves, which came from Sempan Village, Pemali Subdistrict, Bangka Regency, Bangka Belitung Province. The sample was dried in the open air. After that it was ground and sifted into dry powder. The powder with a size of 40-100 mesh was extracted using the Microwave-Assisted Extraction (MAE) method and the conventional method of maceration.

Extraction

The dried powder of the *Tristaniopsis merguensis* leaves was taken by 1 gram and added 10 mL of solvent in a microwave vessel. Then the tube was inserted in a *Microwave Accelerated Reaction System* (MARS) 6 (1200 W, 2450 MHz). The solvents were varied, namely methanol, ethanol, ethyl acetate, and acetone, with time variations of 5, 10, 15, and 30 minutes and temperature variations of 40, 60, 80, 100^{o}C. Afterward, the filtrate and the residue was separated using a funnel. The filtrate obtained was concentrated with a rotary evaporator vacuum to obtain the concentrated extract. This extraction was carried out in duplicate.

Conventional extraction using maceration was done by dissolving the powder with ethanol, acetone, and methanol solvents with a ratio 1:10. Afterward, filtering was done using a büchner funnel. This maceration was carried out in duplicate

Yield (%) Extraction

The yield (%) extraction of *Tristaniopsis merguensis* Griff. leaves from MAE and maceration obtained from dry extract weight divided by sample weight and multiplied by 100 (Safdar et al. 2016).

Polyphenol Content

The polyphenol content was carried out quantitatively by the Follin-Ciocalteu method (Safdar et al. 2016). The extract was dissolved in methanol with a concentration of 10 mg/mL. This extract was then taken 0.5 ml and put in a test tube containing 2.5 mL of 10% Follin-Ciocalteu reagent and 2.5 mL 7.5% Na_2CO_3. The sample was incubated at 27^{o}C for 30 minutes. For the blanks, 0.5 mL of methanol was mixed with 2.5 mL of 1% Follin-Ciocalteu reagent and 2.5 mL of 7.5% Na_2CO_3. The change in the absorbance was measured at a wavelength of 765 nm with UV-Vis spectroscopy. The Positive control used gallic acid with variations in the concentration of gallic acid. The total phenolic extract was calculated based on the calibration standard gallic acid curve and expressed in mg Gallic Acid Equivalent (GAE) /g extract.

Phytochemical Test

Phytochemical test was carried out to find out qualitatively the active compounds in the extract of *Tristaniopsis merguensis*. This identification is carried out by several test methods, which include tannin, flavonoids, saponins, alkaloids, steroids, and terpenoids test. Tannin test using iron (III) chloride ($FeCl_3$) method, flavonoid using the Wilstater Cyanidine method, saponin using Forth, alkaloid test using Mayer and Wagner methods, and steroid using Liebermann-Buchnard method (Mahardika & Roanisca, 2018).

RESULTS AND DISCUSSION

The *Tristaniopsis* genus is found in many lowlands, usually found in forests. *Tristaniopsis merguensis* is a dicotyledonous plant with large trees. The shape of the leaf is *Obovatus* or *Oblanceolatus* with length in between 6-8 inches and width 1.25-2.25 inches (Yarli 2011). Large compound flower, solid, white with the mother flower stalk in the leaf armpit (*Axi-laris*) and hairy. The tube-shaped petals converge with the sharp lobes. *Tristaniopsis merguensis* height reaches around 20 meters to reach 80 meters. The bark is reddish and tends to peel with pinnate leaf bones (Bollesta et al. 2003).

Effect of Extraction Time

The effect of time on the extraction yield is shown in Fig. 1. The data show that increasing time has an impact on increasing extraction yield. It means that a longer extraction time makes the active components in the leaves of *Tristaniopsis merguensis* interact longer with the solvent so that the extraction yield increases. In this study, MAE at 80°C with acetone and ethanol solvents produced higher extraction yield with increasing time. The acetone and ethanol solvents at this temperature have boiled. Acetone solvents had high extraction rates at the first 5 and 10 minutes, then tended to be flat in the next minutes. This was due to the longer time that acetone was more in the vapor phase (gas) than the liquid phase, so it would make the interaction of solvent acetone with plant tissues tended to be flat. Likewise, in ethanol solvents, the level of ethanol extraction was high in the first 5 minutes and tended to be flat until the first 15 minutes, which subsequently increased in the first 30 minutes. The high extraction rate in the first 5 minutes was also caused by the active compounds on the outside of the leaf tissue of *Tristaniopsis merguensis,* which were easier to interact with the solvents.

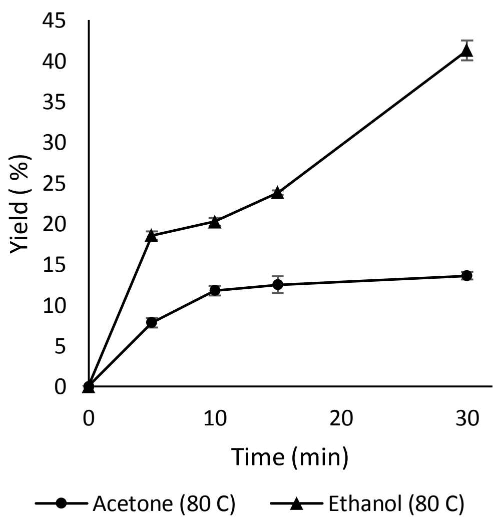

Fig. 1: Effect extraction times of MAE

In general, polar ethanol solvents had a higher extraction rate than semi-polar or non-polar solvents. Likewise, the use of ethanol solvents in MAE also had a higher extraction rate than semi-polar acetone solvents. This showed that the leaves of *Tristaniopsis merguensis* contain more polar active compounds such as flavonoids, tannins, and alkaloids.

Comparison of MAE and Maceration

Based on Fig. 2, the MAE method shows a better extraction rate than maceration with acetone solvents. Maceration at room temperature had a lower extraction rate than MAE at 60 and 80°C. The maceration extraction rate increased significantly only in the first 10 minutes, while in MAE, there was a significant increase for the first 5 minutes. MAE at 60°C had almost twice the extraction rate compared to maceration. The extraction rate was different from the two methods due to the presence of microwave irradiation that could damage the leaf tissue of *Tristaniopsis merguensis* Griff. As a result, the solvent (which in this case was acetone) could interact better with active compounds in the leaf tissue.

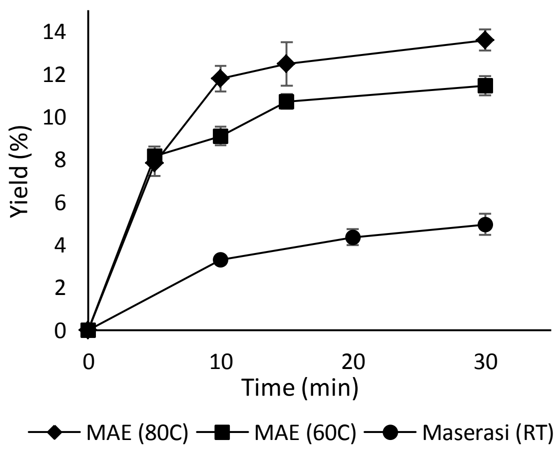

Fig. 2: Effect Extraction times of MAE and Maceration

In addition, high extraction rates were also caused by MAE temperatures. The rise in MAE temperature increased the yield of *Tristaniopsis merguensis* Griff. leaves extraction. The temperature could increase the kinetic energy of the solvent so as to increase the interaction between solvent molecules with active compounds (Hemwimon et al. 2007).

Based on SEM (Scanning Electron Microscope) analysis, the microwave irradiation treatment caused the leaf structure of *Tristaniopsis merguensis* Griff. more damage compared to maceration. Microwaves make leaf cells damaged making it easier for solvent to enter the leaf tissue (Fig. 3). This causes more solvents to interact with the active compound so that the extraction yield using MAE is better than conventional methods (maceration).

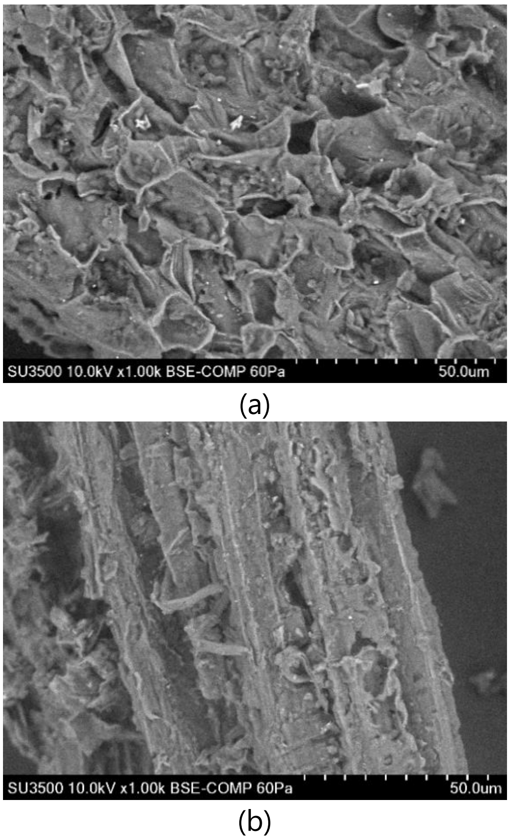

Fig. 3: Scanning electron micrograph *of Tristaniopsis merguensis* Griff. leaves: a. Maceration; b. microwave irradiation

Effect of Temperatures Extraction

Fig. 4 shows that the higher the MAE temperature results in an increased on the extraction yields. High temperatures increased the interaction of solvents with active compounds, thus increasing extraction yield. This showed that the higher the temperature would reduce the solvent viscosity, making it easier for the solvent to diffuse into the leaf tissue. In addition, high temperatures would increase the interaction between solvent molecules with active compounds so as to increased extraction efficiency.

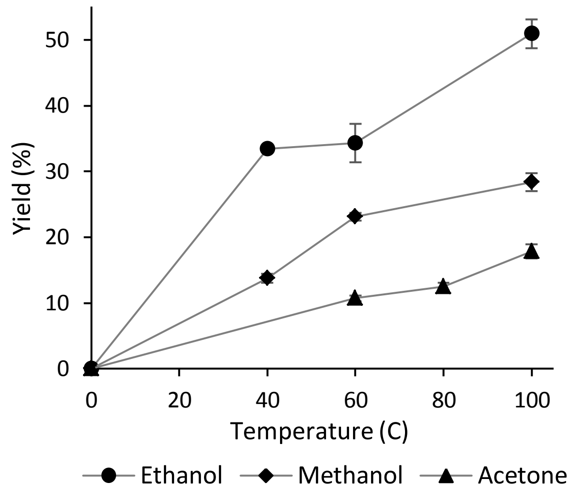

Fig. 4: Effect of temperatures MAE (Time 10 min)

The MAE, system which was equipped with a tightly closed vessel, made the solvent at high temperatures unable to get out of the extraction system. This further resulted in the highest extraction rate for acetone, methanol, and ethanol at a temperature of 100°C compared to temperatures of 40, 60, and 80°C. Acetone solvent had the lowest extraction rate compared to methanol or ethanol. This was caused by acetone, which was less polar than methanol and ethanol.

Effect of Solvent Type

The extraction rate on MAE depends on the type of solvent. Comparison of MAE and maceration at 60°C for 10 minutes is shown in Fig. 5. The highest extraction rate of MAE was ethanol. Likewise, with maceration, ethanol also had a higher extraction rate than other solvents. If the temperature is raised as shown in Fig. 4, the effect of the type of solvent was not very influential on the extraction yield. Temperature only increases the kinetic energy while the dielectric constant does not change significantly.

The nature of polar ethanol showed that the content of *Tristaniopsis merguensis* leaves was dominated by polar compounds. This was also supported by methanol solvent extraction rate data. Based on Fig. 5, polar methanol also had a high extraction rate, either MAE or maceration. Semipolar acetone and ethyl acetate had lower extraction rates. This is in accordance with research from Lovric et al., 2019, ethanol had a better extraction capability when compared with methanol in the extraction of phenolic compounds in Blackthorn Flowers (Lovrić et al., 2017). When compared to methanol-water, MAE yields of phenolic compounds such as routine and quercetin were found to be increased when the ethanol concentration increased from 30% to 50% in *Euonymus alatus* (Thunb.). In addition, ethanol solution was more suitable than acetone and methanol for extracting flavonoids in tea (Wang & Helliwell, 2001).

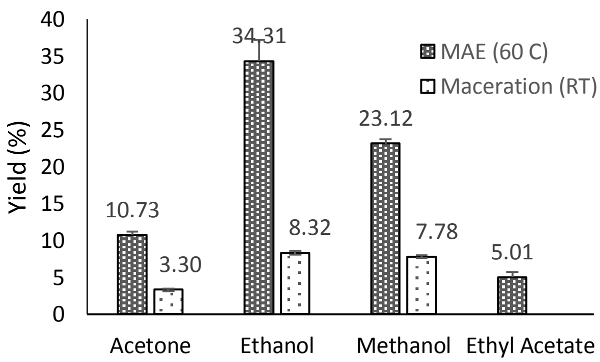

Fig. 5: Effect of solvent MAE and maceration

Although methanol also had a high extraction rate, methanol was toxic, so it was feared to have undesirable side effects when used as extraction solvents. In this case, ethanol was the best extraction solvent compared to other solvents because it was less toxic and had a high extraction rate.

Total Polyphenol Contents

Analysis of total polyphenols in this study was carried out using the Follin-Ciocalteu method. Total polyphenols were measured based on gallic acid standards. The results of measurements of the total polyphenols of *Tristaniopsis* leaves were measured by MAE and maceration methods shown in Fig. 6. The total polyphenols from MAE (60 ^{o}C, 10 min) were higher than maceration at room temperature (RT), which was carried out for 30 minutes. This showed that extraction with MAE was more effective than maceration in terms of time, where extraction rates were higher with more polyphenols. Microwave radiation and heat helped the solvent to extract more polyphenol compounds.

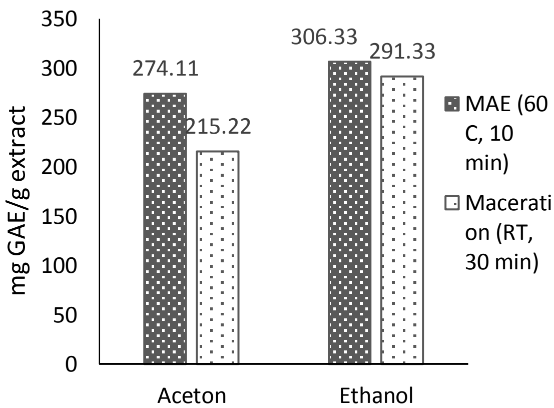

Fig. 6: Effect MAE and maceration on polyphenols content

MAE ethanol had a polyphenol content of 306.33 mg GAE / mg extract. This total polyphenol was higher than MAE acetone, which was 274.11 mg GAE / mg. The polar nature of ethanol and polyphenol compounds allowed for more intermolecular interactions. In addition, the possibility of forming hydrogen bonds between ethanol and phenol compounds added strongly to the solvent extraction rate. This was not the case with acetone; the possibility of forming hydrogen bonds between phenol compounds and acetone became less so that interactions occurred more in the polarity.

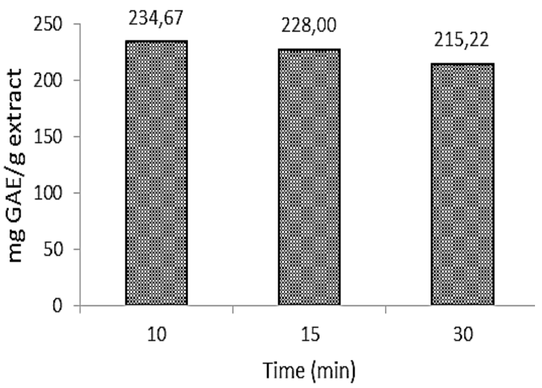

Fig. 7: Polyphenols content from acetone extract at 80 ^{o}C by microwave.

The length of MAE extraction time didn't cause an increase in polyphenol extraction. This can be seen in Fig. 7 of the influence of MAE time on the total polyphenols. The data was taken from MAE results at 80^{o}C using acetone solvent. The results showed that the addition of extraction time didn’t make the polyphenol extraction rate increase, but on the contrary, there was a downward trend. This was caused by the long-time extraction, which could damage polyphenol compounds from *Tristaniopsis merguensis* leaves. Microwave radiation and heat allowed the polyphenol compounds to degrade. Under certain conditions, it make polyphenol compounds easily form free radicals so that levels of polyphenols can decrease. MAE makes polyphenol compounds easy to form free radicals so that the yield of polyphenols can decrease. This is supported by research by Wang et al., 2008, prolonged extraction actually reduces the extraction yield of the ginsenoside compounds. The optimum time for microwave extraction of ginsenosides is 10 minutes from the time of 2, 5, 10, 15, and 30 minutes (Wang et al., 2008). As for flavonoid compounds extracted from Radix astragali, there was an increase in the results of flavonoid extraction up to 25 minutes. But, the extraction yield decreases when the time is extended (Xiao et al., 2008). Prolonged microwave heating can cause degradation of target compounds due to overheating of solutes/solvents. Similar with the case of polyphenol extraction from *Tristaniopsis merguensis*, the increased extraction time did not increase the polyphenol yield from the extraction result.

The best time to extracted *Tristaniopsis merguensis* leaf polyphenols was 10 minutes. The polyphenol content of Pelawan leaves was found that acetone extract using MAE 10 min at 80^{o}C higher than maceration 30 min that is 234.67 mg GAE / g extract.

Phytochemical Results

Phytochemical testing was conducted to determine the content of secondary metabolites in *Tristaniopsis merguensis* leaves. Phytochemical results were shown in Table 1.

Table 1. Phytochemical results of *Tristaniopsis merguensis* Griff.

Test	Method	MAE	Maceration
Alkaloid	Mayer	+	+
	Wagner	+	+
Tanin	$FeCl_3$	+	+
Flavonoid	Wilstater-Cyanidine	+	+
Saponin	Forth	-	-
Steroid	Liebermann - Buchnard	-	-

It was found no difference of active compound using MAE and maceration like alkaloid, tannin, and flavonoids. Saponin and steroid compounds were not found in *Tristaniopsis merguensis* leaves (Roanisca, et al., 2019).

CONCLUSION

Yield extraction, times, polyphenols content, and phytochemical results of MAE more favorable than a maceration. Increasing time MAE has an impact on increasing extraction yield. Extraction with MAE was more effective than maceration in terms of polyphenols content. MAE extract was higher in the polyphenols content a maceration. Microwave radiation and helped the solvent to extract more polyphenol compounds. In this case, ethanol was the best extraction solvent compared to other solvents because it was less toxic and had a high extraction rate. Phytochemical result was found no difference in the active compound using MAE and maceration like alkaloids, tannins, and flavonoids.

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