Process of microwave assisted extraction (MAE) for *Rhodomyrtus tomentosa* fruit and its bioactive compounds

M Ridlo, S Kumalaningsih and D Pranowo

Department of Agro-industrial Technology, Faculty of Agricultural Technology, Universitas Brawijaya, Malang, Indonesia

E-mail: dodykpranowo@ub.ac.id

Abstract. Karamunting (*Rhodomyrtus tomentosa* (Ait.) Hassk) is one of the plants contains high bioactive compounds. The fruit parts have been used widely as traditional herbs in Malaysia, Vietnam, Thailand and China. In Indonesia, this plant grows as wild plants in shrubs and forests of Sumatra, Kalimantan and Sulawesi Islands. The degradation of bioactive compounds from karamunting fruit can be minimised by the extraction process. The purpose of research was to obtain the best operational condition in the extraction of the bioactive compounds from karamunting fruit. Microwave Assisted Extraction (MAE) was used in this study. MAE is a modern extraction method that utilises microwaves to increase the effectiveness and efficiency of cell breakdown. The molecular movement of microwaves produces friction and heat energy in the material so that the cell wall and tissue material will be damaged, and the solute can finally come out. The extraction process consists of 2 factor variables, namely extraction time (seconds) and solvent volume (mL). The parameters of the analysis to determine the best conditions of the extraction process were total flavonoid content (TFC), total anthocyanin content (TAC) and extract yield. The results showed that the highest yield of extract was 1.68% (v/w), the highest total flavonoid was 2563.79 mg QE/g BK, and total anthocyanin was 651.26 mg/g.

1. Introduction

An adequate way of handling fresh fruit may cause a degradation in the bioactive compounds. Several studies have reported that the extraction of plant parts is one of the potential routes to preserve and obtain the bioactive compounds [1-3]. Currently, the bioactive compounds in karamunting fruit are extracted conventionally using the maceration method with extensive time and high solvent requirements. Relatively new extractions have been carried out in several studies, include the microwave assisted extraction (MAE) method [1-5].

The MAE method is widely proven and more effective for extracting bioactive compounds from plants that are prone to high temperatures [4]. As a modern extraction method, MAE is known with a higher extraction rate, lower solvent consumed and faster extraction time than the conventional method extraction. MAE is an electromagnetic wave with a frequency threshold between 300 MHz and 300 GHz. MAE with solvent combinations have been applied to extract polar or non-polar flavonoid compounds [6].

To date, there has been limited studies on the use MAE method to optimise the time and the volume of solvents in the extraction process of karamunting fruit. Indeed, different microwave systems may differ in the extraction performance, as the microwave power and the extraction time can...
only be achieved on certain ranges. This is because the volume of solvents affects the absorption of microwave energy during the extraction process [5].

Therefore, this study was aimed to obtain the best operational condition in the extraction of the bioactive compounds from karamunting fruit. The bioactive compounds produced from the extraction process using the MAE method is potentially to be applied as one of strategies in developing plant-based bioactive agro-industry.

2. Materials and Method

2.1. Sample collection and preparation

Karamunting fruits was freshly collected from Tarakan City, North Kalimantan, Indonesia. Ripe karamunting fruits were selected as sample which identified with purple color, and the fruits were in the morning. During the ripening process, karamunting fruits changed the color from green to red or purple, indicated by the phenol content. Several studies have reported that environmental conditions can affect the phenol content in fruit [7-9].

Then, the selected karamunting fruits were packed with styrofoam box in cold conditions and the box was wrapped with plastic to minimize air penetration, thus minimizing the respiration process. Such handling treatment was aimed to minimize the degradation of bioactive compounds during the delivery process. The packaged sample was then sent to Malang City by cargo services. After arriving in Malang City, West Java, karamunting fruits were immediately brought to the laboratory and stored in the refrigerator until the extraction experiment was carried out. Other chemicals used in this study include ethanol solvent 96% (pa), methanol, buffer solution pH 1 and pH 4, potassium chloride (KCl), Na acetate, HCl (pa), NaOH 45% (pa), alcohol 95% (pa), quercetion, NaNO₂ and AlCl₃.

2.2. Microwave assisted extraction

Karamunting fruit (10 g) was weighed and placed in a 500-mL Erlenmeyer bottle. Then, mixed with ethanol solvent according to the ratio of feed: solvent (v/w) (i.e. 10: 1, 15: 1 and 20: 1). The Erlenmeyer bottle was tightly closed and placed in the microwave. The extraction of karamunting fruits was carried out at 400 watts of power with extraction time of 60, 120 and 180 s. The extract results were bright yellow color. The extract was then evaporated using a rotary vacuum evaporator at 55°C for 5 minutes. The use of a fixed temperature is due to the degradation and instability of anthocyanins starting at 80°C [10, 11]. The filtrate was separated from the solid by means of a centrifuge of 5000 rpm for 10 minutes at room temperature.

2.3. Calculation of absorbed power and energy

The efficiency of microwave heating systems in various extraction conditions can be identified based on absorbed power density (APD). APD is defined as the heating power absorbed per unit volume of solvent (W/mL). Extraction solvent (V in mL) is heated in a microwave system with a predetermined time (tH in s). The value of APD is calculated based on the following equation:

\[
APD = \frac{Q}{V \cdot t_H}
\]

Heat (Q) in formula (1) is obtained from the difference in temperature of the solvent based on the calorimetry method [12]. The calculation formula for heat absorbed is determined according to the following two conditions:

Condition a: warm up the solvent before boiling

\[
Q = m_L \cdot C_p \cdot \Delta T
\]

Condition b: heating the solvent after boiling
\[ Q = m_L C_p \Delta T + m_v \Delta H_{vap} \]  \hspace{1cm} (3)

Information: solvent mass (mL), solvent heat capacity (Cp), solvent heat evaporation (Hvap).

\[ Q = m_L C_p \Delta T + m_v \Delta H_{vap} \]  \hspace{1cm} (4)

Where \( t \) is extraction time.

APD is associated with heating efficiency in extraction systems, while absorbed energy density (AED) is related to achieving equilibrium MAE with energy in solvents as indicators [5].

2.4. Experimental design
The research design used was a central composite design (CCD), with two factors, include extraction time (X1) for 60 seconds, 120 seconds and 180 seconds, and solvent: material (X2) ratios of 10:1, 15:1 and 20:1. The parameters analysed were extraction yield (%), total flavonoids (mg/g) and total anthocyanin (mg/g).

3. Results and Discussion
The solvent used was ethanol as it is not carcinogenic and has high absorption of bioactive compounds. Microwave extraction of karamunting fruit at 400 watts and extraction time according to the design of the research unit. The extract obtained was bright yellow, then evaporated with a rotary vacuum evaporator at 55°C for 5 minutes, due to the instability of anthocyanin compounds starting at 80°C [12]. The filtrate is separated from the solid by means of a 5000-rpm speed centrifuge for 10 minutes at room temperature. Figure 1 shows that microwave devices used was an open system to obtain a safe extraction process and an efficient extraction of prone bioactive compounds to high temperatures. This system operates a room atmospheric conditions and only a portion of Erlenmeyer glass bottles is directly exposed to microwave radiation. Radiation rays was emitted by the magnetron to all corners in the microwave cavity. The upper part of the vessel is connected to the reflux unit for melting the solvent vapor.

Figure 1. Design on the microwave system

The principle of microwave energy heating is based on the direct effect of microwave radiation on material molecules. Microwaves are used for disrupting cell in plants, thus bioactive compounds can be absorbed by solvents. Biomolecules are efficiently released due to an increase in pressure on the
cell tissue, followed by the breakdown of cellular matrices and increased porosity, causing an increase in mass transfer from inside to outside the cell [13].

The transformation of electromagnetic energy in heat energy is carried out with two mechanisms include ionic conduction and dipole rotation. Both mechanisms take place simultaneously. Ion friction is caused by the migration of electrophoretic ions in electromagnetic fields. The resistance of the solution to the flow of ions and collisions between molecules produce heat energy. Dissolved ion migration increases the solvent penetration into the material, causing the dissolution of the target compounds. Meanwhile, the dipole rotation consists of the movement of polar molecules which are affected by dipoles and by the direction of the electric field [14].

3.1. Determination of absorbed power and energy
Microwave energy can be controlled during the extraction process and often considered as a variable for experimental design and optimisation. Increased microwave power has been observed to have impact on increasing the cell structure’s damage. This was mainly due to the rapid pressure formed in the bio-cellular matrix and the increase in temperature according to the dielectric constant of the solvent [15, 16]. Microwave energy absorbed by each sample is shown in Table 1.

| Sample ID | Extraction time (s) | Solvent volume (mL) | Initial temperature (°C) | Final temperature (°C) | APD (W/mL) | AED (J/mL) |
|-----------|---------------------|---------------------|--------------------------|------------------------|------------|------------|
| 1         | 120                 | 220.7               | 26.9                     | 71                     | 1.08       | 134.96     |
| 2         | 120                 | 150                 | 26.8                     | 67                     | 0.86       | 103.21     |
| 3         | 60                  | 100                 | 27                       | 63                     | 1.62       | 97.36      |
| 4         | 120                 | 150                 | 27.1                     | 67                     | 0.86       | 102.81     |
| 5         | 35.16               | 150                 | 27                       | 45                     | 2.38       | 83.60      |
| 6         | 60                  | 200                 | 27                       | 50                     | 1.32       | 78.94      |
| 7         | 204.84              | 150                 | 26.8                     | 115                    | 0.82       | 168.42     |
| 8         | 120                 | 150                 | 27                       | 67                     | 0.85       | 102.53     |
| 9         | 180                 | 200                 | 26.9                     | 101                    | 0.61       | 109.15     |
| 10        | 180                 | 100                 | 26.9                     | 87                     | 0.92       | 165.16     |
| 11        | 120                 | 150                 | 27                       | 67                     | 0.86       | 102.74     |
| 12        | 120                 | 150                 | 26.8                     | 67                     | 0.86       | 103.37     |
| 13        | 120                 | 70.93               | 27                       | 64                     | 0.67       | 80.77      |

Table 1 shows that the biggest power (APD) absorbed was by the 5th sample (2.38 W/mL), with the largest difference in temperature of 13°C. The highest energy (AED) absorbed was from the 7th sample (168.42 J/mL), with a difference in temperature of 88.213°C. AED correlates strongly with the extraction time and weakly correlates with solvent volume. Calculation of APD is known to be closely related to the type of solvent, microwave system, ratio of solvent-ingredients, and microwave power to microwave energy absorption [5, 11]. In this study, the low extraction time and solvent to material ratio had an impact on the high value of APD. High APD values are obtained from higher heating rates with shorter extraction times for equilibrium results. APD can also be used to determine the best extraction results and optimum extraction times without loss of control over heating conditions and extraction scales [5].

3.2. Effect of energy on the extraction yield
The definition of yield extract is the weight ratio of the extract produced with the weight of the raw material used, in percentage unit. The yield value is used as the criteria for the success of the production process, as a basis for efficiency in cost and production processes. Comparison of solids to solutions is one of the important factors in the extraction process with microwave radiation. Bioactive compounds and larger yields are obtained from larger volumes of solvents [16, 17].
Microwave irradiation time influences the amount of the extraction yield produced. In Table 2, the extract yield value increases as the extraction time increases. Extraction time functions as a controller of the amount of microwave radiation that the system presents to the material. Increased extraction time affects the rupture of larger cell structures. The high volume of solvents also affects the high amount of extract produced.

**Table 2. Characteristics of karamunting fruits extract**

| Sample ID | Extraction time (s) | Solvent volume (mL) | Extraction yield (%) | Anthocyanin (mg/g) | Flavonoids (mg/g) |
|-----------|---------------------|---------------------|----------------------|--------------------|-------------------|
| 1         | 120                 | 220.07              | 1.26                 | 3.43               | 11.77             |
| 2         | 120                 | 150                 | 0.97                 | 4.01               | 13.23             |
| 3         | 60                  | 100                 | 0.70                 | 3.56               | 12.64             |
| 4         | 120                 | 150                 | 1.03                 | 3.73               | 15.14             |
| 5         | 35.16               | 150                 | 0.77                 | 2.89               | 10.47             |
| 6         | 60                  | 200                 | 0.95                 | 3.01               | 10.90             |
| 7         | 204.84              | 150                 | 1.68                 | 4.34               | 20.12             |
| 8         | 120                 | 150                 | 0.91                 | 3.67               | 14.55             |
| 9         | 180                 | 200                 | 1.42                 | 4.09               | 18.38             |
| 10        | 180                 | 100                 | 1.25                 | 3.83               | 16.34             |
| 11        | 120                 | 150                 | 0.96                 | 3.93               | 13.40             |
| 12        | 120                 | 150                 | 1.01                 | 3.79               | 14.88             |
| 13        | 120                 | 70.93               | 0.87                 | 2.91               | 10.26             |

Figure 2 shows that the extraction yield produced was directly proportional to the amount of AED. The highest extract yield in the 7th sample (1.68% (w/v)), resulting from AED of 168.42 J/mL. Whereas, the lowest extraction yield was from the 3rd sample (0.70%), resulted from AED of 97.36 J/mL. This finding confirmed that the extraction time influences the yield of the extraction process. Similarly, Azadmard-Damirchi et al. [2] reported that using microwave radiation in plant oils can result higher extraction yield and increased mass transfer coefficients due to a disruption of more cell membranes. In this study, the karamunting fruit extract is expected to have higher quantity and high quality of the expected bioactive compounds.

**Figure 2. Relationship of AED (J/mL) to extraction yield (%)**

### 3.3. Effect of energy on total anthocyanins

Analysis of secondary metabolites in karamunting fruit extracts was carried out on the total anthocyanins contained. Anthocyanin is a secondary metabolite of the family of flavonoids, largely
found in fruits and vegetables [18]. Anthocyanins are composed of an aglycone (anthocyanidin) which is esterified with one or more sugar groups (glycons). Most anthocyanins are found in six forms of anthocyanidin, namely pelargonidin, cyanidin, peonidine, delphinidine, pentunidine and malvidin [19].

Table 2 shows that the highest antiseptic content was found in the 8th sample (4.34 mg/g), resulted from the treatment with ingredients: solvent ratio of 1:15 (w/v) and extraction time of 120 s. While, the smallest anthocyanin content was found in the 5th sample (2.89 mg/g), resulted from ingredients: solvent ratio of 1:15 (w/v) for 35.16 s. Microwave heating in excessive solvents influences the uptake of solvents and causes ineffective extraction of the bioactive compounds due to limited absorbed power [20].

In Figure 3, the total value of the anthocyanin produced is directly proportional to the amount of AED absorbed, with a fluctuate trend. The highest total anthocyanin was found in the 7th sample (4.34 mg/g) resulting from AED of 168.42 J/mL. While, the lowest total anthocyanin value was from the 5th sample (2.89 mg/g) resulting from AED of 78.20 W/mL. This study found that increasing ingredients: solvent ratio and extraction time increases the total anthocyanin compounds produced.

![Figure 3. Relationship of AED (J/mL) to total anthocyanins (mg/g)](image)

Microwave energy exposure is expected to detach the anthocyanin compounds in plant cells. Solvent ratio: material have no significant effect on the microwave energy and heating time in cell breakdown mechanisms [3]. Longer irradiation time influences the high process recovery period and the extraction efficiency of anthocyanin compounds from the cell matrix. However, too high exposure to microwave energy resulted in a major decomposition of the pigments and reducing the color of the compounds [21].

3.3.1. Effect of energy on total flavonoids

The total analysis of flavonoids in karamunting fruit was determined by the aluminium chloride method and quercetin was used as a comparison. MAE has proven to be an excellent method for polar extraction as well as nonpolar flavonoids. With the application of water and ethanol solvents, pure polar flavonoid extract has been produced by varying the extraction time [10, 22, 23].

This study demonstrated that an increase in the microwave power influenced the total flavonoids produced. This was possibly due to an increase in the damage of the cellular matrix structure causing by a high pressure and increased temperature from microwave dielectric heating [6, 24].

Table 2 shows that the highest flavonoid content was from the 7th sample (20.12 mg/g) using ingredients: solvent 1:15 (w/v) and extraction time of 204.84 s. While, the lowest flavonoid content was found in the 13th sample (10.26 mg/g) using ingredients: solvent ratio of 1:7.09 (w/v) and extraction time of 120 s. Larger solvent volume are generally used for dissolving bioactive compounds and, in convention extraction method, such approach may result in higher extraction yield [9, 24].

This study indicates that ingredient: solvent has little effect on the flavonoids content of the karamunting fruit extract, as shown in Figure 3. This was possibly due to a low influence of the ratio
of ingredients: solvent to microwave power and extraction time [3]. The relation between microwave power and extraction time interacts can be optimised with ratio of solvents: ingredient [25].

![Figure 4. Relationship of energy AED (J/mL) to total flavonoids (mg/g)](image)

In Figure 4, the total flavonoids produced is directly proportional to the amount of AED. The highest total flavonoid was found in the 7th sample (20.12 mg/g) with AED of 168.42 J/mL. While, the lowest total flavonoid was in the 13th sample (0.26 mg/g) with AED of 99.13 W/mL. An increase in flavonoid compounds is in accordance with increasing in AED during the MAE process. The high electromagnetic energy absorbed by the system increases the efficiency of the extraction process [15]. In this case, AED functions as an indicator of the amount of microwave energy needed for heating solvents for breakdown of plant cells, thus optimisation of the MAE process can be achieved [4]. AED by solvents is used for temperature changes (sensible heat) and changes in substances (latent heat in evaporation) [26].

4. Conclusions
The finding in this study confirmed that MAE method can be used as one of the alternative strategies to increase the efficacy of bioactive compound extraction. The study shows that, using MAE, with combination of ingredients: solvent ratio of 1:15 (w/v) and extraction time of 204.8 s, has resulted AED of 168.42 J/mL. Such combination produced the highest extraction yield of 1.68%, the total anthocyanin of 4.34 mg/g, and total flavonoids of 20.12 mg/g, respectively. Yet, further study is required on increasing the efficacy of fractionation to obtain pure karamunting fruit extract, as well as to improve the quality of the extracted bioactive compounds.

References
[1] Hendrawan Y, Pramesi N D, Rachmawati M, Susilo B, Wibisono Y, Dewi S R, Izza N 2018 Application of microwave assisted extraction in extracting Torbangun leaves (Coleus ambonicus, L.) and its effects on polyphenol and flavonoids content Adv. Food Sci. Sustain. Agric. Agroind. Eng. 1 2 8–16.
[2] Azadmard-Damirchi S, Alirezalu K, Achachlouei B F 2011 Microwave pretreatment of seeds to extract high quality vegetable oil World Acad. Sci. Eng. Technol. 57 72–75.
[3] Chan C H, Yusoff R, Ngoh G C, Kung F W L 2011 Microwave-assisted extractions of active ingredients from plants J. Chromatogr. A 1218 37 6213–6225.
[4] Chan C H, Yusoff R, Ngoh G C 2013 Modeling and prediction of extraction profile for microwave-assisted extraction based on absorbed microwave energy Food Chem. 140 1–2 147–153.
[5] Chan C H, Yusoff R, Ngoh G C 2017 An energy-based approach to scale up microwave-assisted extraction of plant bioactives. In Ingredients Extraction by Physicochemical Methods in Food Elsevier pp. 561–597.

[6] Routray W, Orsat V 2012 Microwave-assisted extraction of flavonoids: a review Food Bioprocess Technol. 5 2 409–424.

[7] Guo J, Han W, Wang M 2008 Ultraviolet and environmental stresses involved in the induction and regulation of anthocyanin biosynthesis: a review African J. Biotechnol. 7 25 4965–4972.

[8] Shin Y, Ryu J A, Liu R H, Nock J F, Watkins C B 2008 Harvest maturity, storage temperature and relative humidity affect fruit quality, antioxidant contents and activity, and inhibition of cell proliferation of strawberry fruit Postharvest Biol. Technol. 49 2 201–209.

[9] Wang L, Li D, Bao C, You J, Wang Z, Shi Y, Zhang H 2008 Ultrasonic extraction and separation of anthraquinones from Rheum palmatum L Ultrason. Sonochem. 15 5 738–746.

[10] Chebrolu K K, Jayaprakasha G K, Jifon J, Patil B S 2011 Optimization of flavanones extraction by modulating differential solvent densities and centrifuge temperatures Talanta 85 1 353–362.

[11] Bergman T L, Incropera F P, DeWitt D P, Lavine A S 2011 Fundamentals of heat and mass transfer John Wiley & Sons.

[12] Adawiyah D R 2009. Effect of natural dyes of anthocyanin kopigmentation from rosela (Hibiscus sabdarifita L.) with rosmarinic acid on color stability in soft drink models Undergraduate Thesis Universitas Brawijaya Malang. [In Indonesian]

[13] Yeoh S, Shi J, Langrish T A G 2008 Comparisons between different techniques for water-based extraction of pectin from orange peels Desalination 218 1–3 229–237.

[14] Delazar A, Nahar L, Hamedeyazdan S, Sarker S D 2012 Microwave-assisted extraction in natural products isolation In Natural products isolation Springer pp. 89–115.

[15] Routray W, Orsat V 2014 MAE of phenolic compounds from blueberry leaves and comparison with other extraction methods Ind. Crops Product. 58 36–45.

[16] Xiao W, Han L, Shi B 2008 Microwave-assisted extraction of flavonoids from Radix astragali Sep. Purific. Technol. 62 3 614–618.

[17] Zhang H F, Yang X H, Wang Y 2011 Microwave assisted extraction of secondary metabolites from plants: current status and future directions Trends Food Sci. Technol. 22 12 672–688.

[18] Yang Z, Zhai W 2010 Optimization of microwave-assisted extraction of anthocyanins from purple corn (Zea mays L.) cob and identification with HPLC–MS Innov. Food Sci. Emerg. Technol. 11 3 470–476.

[19] Mateus N, de Freitas V 2008 Anthocyanins as food colorants In Anthocyanins Springer pp. 284–304.

[20] Mandal V, Mandal S C 2010 Design and performance evaluation of a microwave based low carbon yielding extraction technique for naturally occurring bioactive triterpenoid: oleanolic acid Biochem. Eng. J. 50 1–2 63–70.

[21] Farzaneh V, Carvalho I S 2017 Modelling of microwave assisted extraction (MAE) of anthocyanins (TMA) J. Appl. Res. Med. Aromatic Plant. 6 92–100.

[22] Rostagno M A, Palma M, Barroso C G 2007 Microwave assisted extraction of soy isoflavones Anal. Chim. Acta 588 2 274–282.

[23] Terigar B G, Balasubramanian S, Boldor D, Xu Z, Lima M, Sabliov C M 2010 Continuous microwave-assisted isoflavone extraction system: design and performance evaluation Bioresour. Technol. 101 7 2466–2471.

[24] Zhang H F, Yang X H, Zhao L D, Wang Y 2009 Ultrasonic-assisted extraction of epimedin C from fresh leaves of Epimedium and extraction mechanism Innov. Food Sci. Emerg. Technol. 10 1 54–60.

[25] Chen Y, Gu X, Huang S, Li J, Wang X, Tang J 2010 Optimization of ultrasonic/microwave assisted extraction (UMAE) of polysaccharides from Inonotus obliquus and evaluation of its anti-tumor activities Int. J. Biol. Macromol. 46 4 429–435.
[26] Krishnan R Y, Chandran M N, Vadivel V, Rajan K S 2016 Insights on the influence of microwave irradiation on the extraction of flavonoids from *Terminalia chebula* Sep. Purif. Technol. 170 224–233.