BIOACTIVE COMPOUNDS FROM PURPLE ROSELLE CALYX (*HIBISCUS SABDARIFFA* L.) EXTRACT USING MULTISTAGE COUNTERCURRENT METHOD

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ABSTRACT

Multistage Countercurrent Extraction (MCE) is a new extraction technique used to extract bioactive compounds (anthocyanin, quercetin, antioxidants) from purple roselle calyxes (*Hibiscus sabdariffa* L.). This study of purple roselle calyxes extract with three-stage MCE was carried out at a comparison of roselle calyxes and distillation water solvent 1:10, extraction temperatures of 50°C, 60°C, 70°C and extraction time of 15, 30, 45 minutes. Purple roselle calyxes using the MCE method contained the highest anthocyanin content of 2815.43 mg/L, quercetin content 59.25 mg/L, and antioxidant capacity 197.6 ppm. The results showed that the content of bioactive compounds increased by increasing the extraction temperature and extraction time. MCE is an efficient technique for extracting bioactive compounds from roselle calyxes. Roselle calyxes that are rich in antioxidants have the potential as a good food colorant and natural antioxidants.

Keywords: antioxidants, anthocyanins, *Hibiscus Sabdariffa*, multistage counter current, quercetin.

INTRODUCTION

Roselle (*Hibiscus sabdariffa* L.) is a plant from the Malvaceae family that is used as an herbal drink, natural coloring in food and beverages. This plant has health benefits to prevent and avoid diseases of digestive stimulation, inflammation, microbial infection, hypolipidemia, mutagenic and carcinogenic effects (Da-Costa-Rocha et al., 2014; Jabeur et al., 2017). This is due to the activity of bioactive compounds, especially flavonoids and anthocyanins, which are obtained in the extract of roselle calyxes (Cid-Ortega & Guerrero-Beltrán, 2015). Bioactive compounds from roselle include phenolics, flavonoids, anthocyanins, antioxidant capacity and antibacterial activity (Borrás-Linares et al., 2015). Anthocyanin pigments of roselle calyxes are 4 types of color namely purple dark red roselle, light red roselle, bright red roselle and deep red roselle (Obadina & Oyewole, 2007). Roselle calyx has a high anthocyanin content 1,5% (dry weight) (Tsai et al., 2002). The highest anthocyanin content is found in purple roselle so purple roselle has the potential as a good source of natural food colorant and a source of antioxidants that can free radical scavenging (Anel et al., 2016). Anthocyanin compounds are very sensitive to heat so that the necessary extraction technology appropriate to maintain the quality of the anthocyanin compound. Extraction process with high temperature resulted in brown roselle extract (browning). The main problem in the extraction process is the degradation of bioactive compounds. Therefore, effective and efficient extraction technology is needed that produces good quality product quality. In addition, the need for natural colorant and antioxidant products that are benefit health (Triyastuti et al., 2017; Djaeni et al., 2015).

Several conventional and unconventional extraction techniques have been reported to extract bioactive compounds in herbal plants (Azmir et al., 2013). The right extraction method is an extraction method that can extract bioactive compounds, be inexpensive and environmentally friendly extraction process, and result consistent extraction (Sarker & Nahar, 2012). Therefore it needs right extraction method to produce optimum extraction results of bioactive compounds. The Multistage Counter Current Extraction (MCE) method produces the highest extract. In addition, MCE has considerable time, energy and solvent efficiency compared to other extraction methods such as single pot extraction (SPE), microwave-assisted extraction (MAE), ultrasound assisted extraction (USE), Soxhlet extraction (SHE) and room

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temperature extraction (RTE) (Azmir et al., 2013). The maceration and soxhlet extraction processes are less efficient, require a long time and the use of high temperatures in the extraction process results in degradation in anthocyanins (Jordheim, 2007). MCE has been used successfully for the extraction of several bioactive components from plant materials (Cid-Ortega & Guerrero-Beltrán, 2015). Yu et al. (2012) produce a high content of bioactive compounds (flavonoids, total phenols, and antioxidants) from *Ginkgo Biloba* L. Leaves using multistage countercurrent extraction. In addition, the MCE method has various advantages over heat-reflux extraction. MCE is a method of combining extractions that circulate dynamically and extraction technology with continuous currents (Wang et al., 2004).

The theoretical basis for the MCE method is the exchange of extracts between different extraction stages by maintaining a stable concentration gradient between solvents and herbal ingredients (Zhang et al., 2015). MCE technology is the method of choice to increase extract concentration and provide reproducible analytical results compared to using a single stage (Gokmen et al., 2009). The main objective of this study was to obtain phytochemicals with anthocyanin, quercetin and antioxidant levels from purple roselle calyx extract using multistage countercurrent extraction method.

MATERIALS AND METHODS

Multi-stage Countercurrent Extraction (MCE)

The dried purple roselle was obtained from Selopanggung Village, Semen District, Kediri Regency, East Java. Reduces dry purple roselle measuring 60 mesh. MCE extraction method has the advantage of providing effective separation (Wang et al., 2004).

High extraction efficiency is influenced by differences in the concentration of bioactive compounds between samples and extracts of solvents during the MCE process (Gokmen et al., 2009). Yu et al. (2012) conducted research on extracting *Ginkgo Biloba* leaves using the Multi-stage Countercurrent Extraction method to obtain antioxidant content. The variables used include

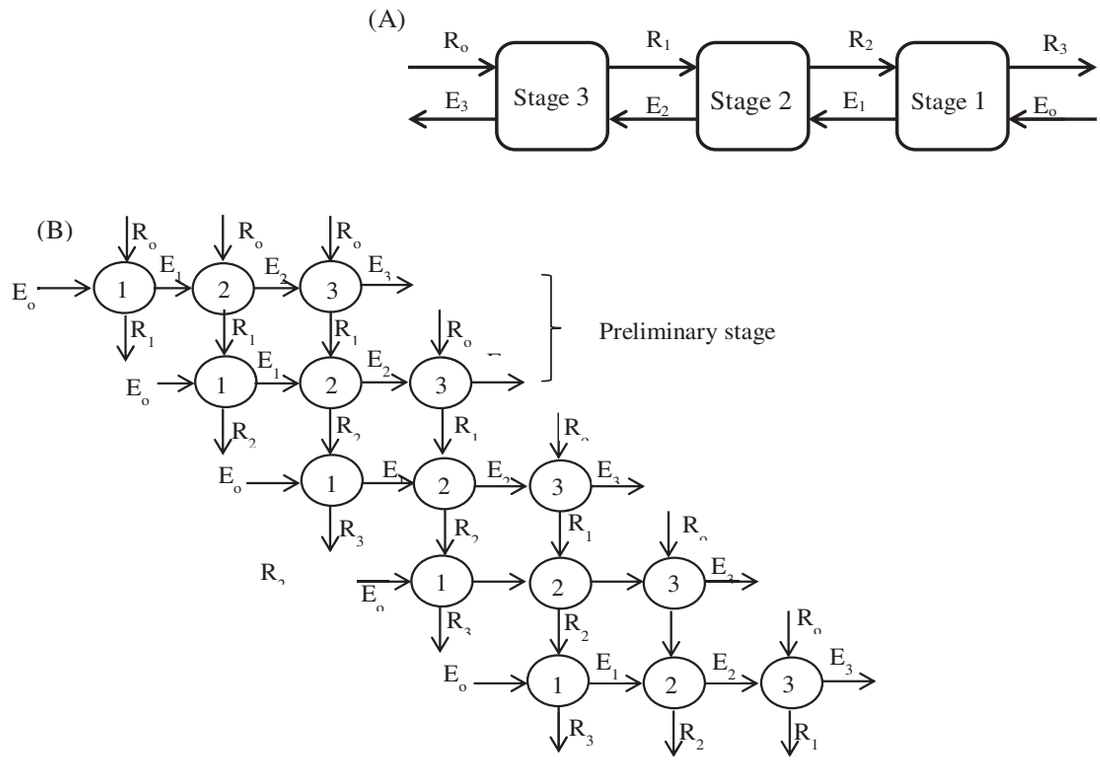


Figure 1. Simulation of Countercurrent Extraction of Roselle Calyxes. (A) General Scheme; (B) Flow Diagram of Three-stage Countercurrent Extraction Process. (Ro = Roselle calyxes raw material; Eo = fresh solvent; R = residue from stage n; E = Extraction from stage n).

the ratio of ethanol and *Ginkgo Biloba* leaves of 8–16 mL/g, extraction time of 30–60 minutes, extraction temperature of 60–80°C. Meanwhile, in the study of Qiu et al. (2018) it shows that at a temperature of 65°C produces high anthocyanin content and antioxidant content. In this research, three-stage MCE was carried out in a 500 mL glass beaker with a comparison of roselle calyxes and distillation water solvent 1:10 at a temperature of 50–60°C and extraction time of 15–45 minutes. Countercurrent multistage technique continuously using a batch simulation staged three counterflow. Step operation as shown in the schematic diagram of Figure 1. To fulfill the mass flow in Figure 1A the preliminary stage is carried out, then proceed to the main stage. The main step in Figure 1B is in accordance with Figure 1A. The main stage is carried out in 3 stages so that the process takes place in a steady state. Separating extracts and raffinates from each stage using a centrifuge.

Determination of Quercetin

Prepare a standard quercetin curve by dissolving 0.1 gram standard quercetin in the 1000 ml volumetric flask using a water distillate solvent. Dilute the standard quercetin solution at a concentration of 20–80 ppm (Pejic et al., 2004). This solution was measured using a UV-VIS Shimadzu 1800 spectrophotometer at a wavelength of 373. Quercetin contents can be calculated using the linear regression equation Y=0.0055* x + 0.1193.

Determination of Anthocyanin contents

PH difference method is used to determine the contents of anthocyanin. Making a buffer solution at pH 1 using potassium chloride solution and water solvent while at pH 4.5 using sodium acetate and water solvent by adding HCl. Dilute to homogeneous roselle calyx extract with 50 mL pH 1 buffer and do the same for pH 4.5. Measuring the absorbance of Roselle calyx extract solution in pH 1 buffer and pH 4.5 with a wavelength of 520 nm and 700 nm using a UV-Vis spectrophotometer. The following equation total contents of anthocyanin (Lee et al., 2005):

Total anthocyanin contents(mg/L)

$$= \frac{A \times MW \times DF \times 10^3}{\varepsilon \times l}$$

where:

$$A=(A_{520}-A_{700})\ pH\ 1.0 - (A_{520}-A_{700})\ pH\ 4.5$$

Determination of Antioxidant Capacity

Free radical-scavenging capacity was measured using the DPPH test method. A 20 µL purple roselle calyx extract was mixed using 1ml DPPH 1mM, then added 5 ml of water distillate. The samples were incubated in a dark room for 30 minutes at room temperature and measured the absorbance of the sample solution at a wavelength of 516 nm using a Shimadzu 1800 UV-VIS spectrophotometer. Percent antioxidant capacity was calculated using the following equation:

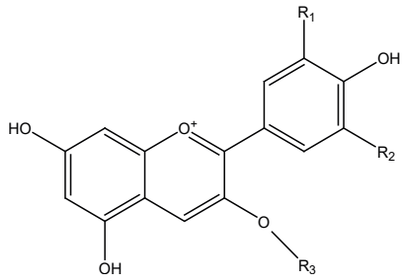
$$\text{Antioxidant capacity } (\%) = \frac{C-S}{C} \times 100\%$$

Where C is the absorbance of the control (methanol) and S is the absorbance of the sample. Making a graph of percent inhibition on concentration, then the line equation is used to get the IC_{50} value. Lower IC_{50} values indicate greater antioxidants (Einbond et al., 2004).

RESULTS AND DISCUSSION

Effect of time and temperature on anthocyanin contents

Anthocyanins are phenolic groups which are found in a wide variety of flowers, fruits, and vegetables. Anthocyanin colorant application in the field of food becomes more popular and growing rapidly. The synthetic colorant is not allowed to be used in food, so anthocyanin is an important ingredient as a source of natural food coloring. Anthocyanin have the good color stability to produce orange, red, purple and blue pigments. Natural color pigments have the potential as



Cyanidin-3-sambubioside (R1=OH; R2=H; R3= Sambubioside)
Delphinidin-3- sambubioside (R1=OH; R2=OH; R3= Sambubioside)
Cyanidin-3-glucoside (R1=OH; R2=H; R3= Glucose)
Delphinidin-3-glucoside (R1=OH; R2=OH; R3= Glucose)

Figure 2. Anthocyanin Structure

a natural colorant source to replace GB violet synthetic coloring additives in the food industry (Giusti & Wrolstad, 2003; Barhe & Tchouya, 2016; Horbowicz et al., 2008). The structure of anthocyanin is shown in Figure 2 (Jordheim, 2007).

Roselle calyxes contain 3 types of anthocyanins including delphinidin-3- glucoside, cyanidin-3-glucoside, delphinidin-3-sambubioside dan cyanidin-3-sambubioside. Roselle has 4 different genotypes including dark purple, pink roselle, bright red roselle and deep red roselle (Borrás-Linares et al., 2015; Obadina & Oyewole, 2007). Cyanidin-3-glucoside compounds are purple pigments that are dominant in anthocyanin content (Hosseinian et al., 2008; Chen et al., 2013). Factors that influence differences in composition and contents of anthocyanins such as the environment (eg quantity of UV-B rays), genetics, plants, and extraction/analysis methods (Abdel-Aal & Hucl, 1999; Bustos et al., 2012; Knievel et al., 2009).

The MCE extraction process is carried out using the extraction time at 15, 30, 45 minutes. Figure 3A shows that there is an increase in anthocyanin contents from 15 to 45 minutes. Purple roselle anthocyanin pigments at 45 minutes extraction were dark purple in black compared to 15 minutes. The more intense the red color, the higher the anthocyanin content (Triyastuti et al., 2017). According to Mardiah et al. (2015), the anthocyanin content of fresh purple rosella is higher (487.18 ppm) compared to red roselle (255.83 ppm). Extraction time is a factor that influences the efficiency and selectivity of MCE. It is in accordance with the research of Zhang et al.

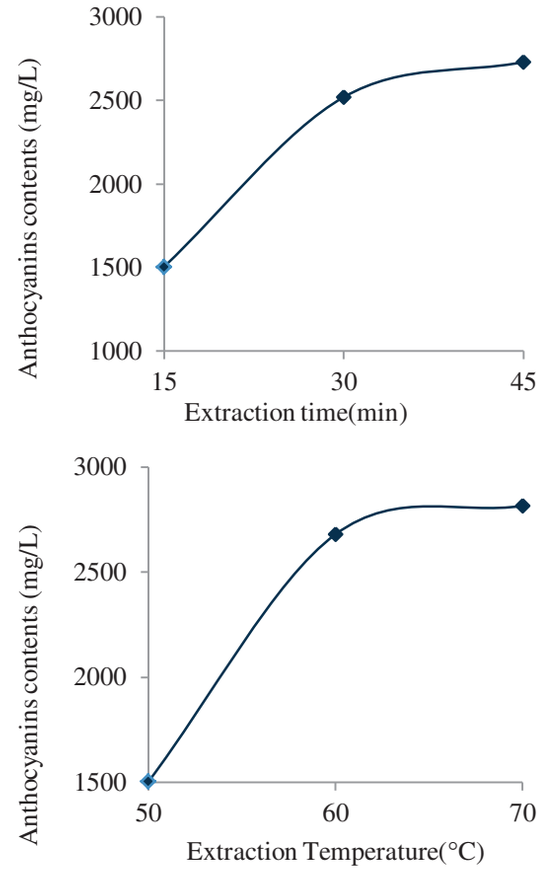


Figure 3. Effect of Different Extraction Parameters on Bioactive Compounds. (A) Extraction Time at Anthocyanin Contents; (B) Extraction Temperature at Anthocyanin Contents.

(2015) that the MCE method has the advantage of efficient extraction time and energy to produce.

Extraction temperature parameters for anthocyanin contents were carried out at low temperatures below 80°C, in figure 3B showed an increase in anthocyanin contents at 50°C (1505.07 mg/L), 60°C (2680.17 mg/L) and 70°C (2815.43 mg/L). The highest anthocyanin content at 70°C compared to 50°C and 60°C. A study by Horbowicz et al. (2008) showed that the stability of anthocyanin is influenced by pH, temperature, light, phenolic content, enzymes, metal ions, sugar, ascorbic acid, and oxygen. Temperature is an important factor that is exponential to produce good color pigments and avoid anthocyanin degradation. The use of low temperatures in the processing and storage of food can increase the stability of anthocyanin (Vargas & Lopez, 2003). According to Corrales et al. (2008) that the increase

in temperature causes pigment damage to brown due to anthocyanin degradation. Anthocyanin degradation at heating temperatures of 80°C (Yue & Xu, 2008; Qiu et al., 2018).

Effect of Time and Temperature on Quercetin Contents

Quercetin (3,5,7-trihydroxy-2-(3,4-dihydroxyphenyl)-4Hchromen-4-one) in figure 4, quercetin is a group of flavonoids that are useful as a cancer treatment (Bischoff, 2008; Siegel et al., 2016). Quercetin potential is good for health so it is used as a nutraceutical ingredient in the food and pharmaceutical industries. Quercetin is a content that is sensitive to heat and easily has chemical changes during processing and storage.

Figure 5A showed that the content of quercetin on the extraction time is 15 minutes (35.46 mg/L), 30 minutes (41.3 mg/L), 45 minutes (41.89 mg/L) at a temperature of 50°C. The difference in extraction time has a significant increase in quercetin contents but extending excessive extraction time can cause damage to flavonoid glycosides (Calabrò et al., 2004; Liu & Zhu, 2007). Comparing images 3A and 5A, the levels of anthocyanin and quercetin contents are influenced by the same factors. The results showed that in the same extraction conditions it produced the high content of anthocyanin and quercetin.

The stability of quercetin is influenced by pH, temperature, metal ions, and also other compounds such as glutathione (GSH) (Boots et al., 2005; Dehghan & Khoshkam, 2012; Moon et al., 2008 ; Price et al., 1997). Figure 5B showed that the effect of quercetin contents was at a temperature difference of 50°C (35.46 mg/L), 60°C (41.75 mg/L) and 70°C (59.25 mg/L). The temperature at 70°C has increased compared to 50°C dan 60°C.

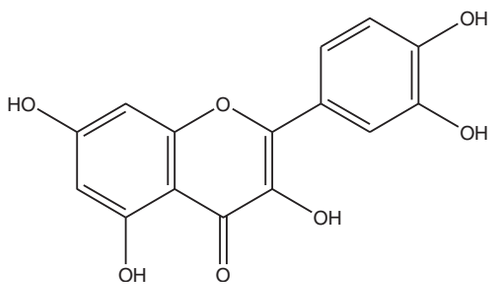


Figure 4. Quercetin Structure

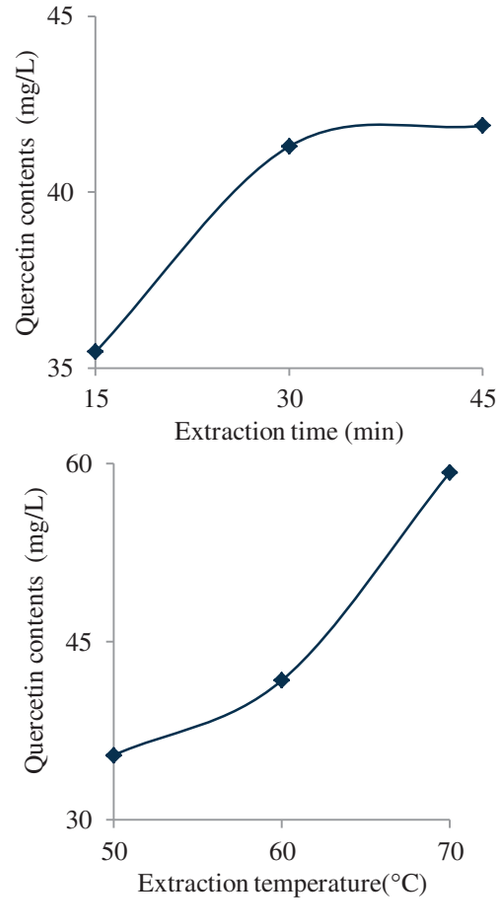


Figure 5. Effect of Different Extraction Parameters on Bioactive Compounds. (A) Extraction Time at Quercetin Contents; (B) Extraction Temperature at Quercetin Contents

Increasing temperature results in a significant increase in quercetin contents. According to Liao et al. (2016), the higher the temperature the yield decreases, this phenomenon showed that the increase in temperature does not give a good effect for ultrasound-assisted extraction. Therefore, extraction temperature is a very sensitive process parameter for extracting quercetin.

Effect of Time and Temperature on Antioxidant Capacity

The antioxidant activity of a plant has two important benefits. First, antioxidants can prevent or delay the oxidation of major biomolecules in cells by free radicals scavengers so as to reduce the risk of cancer by increasing the activity of detoxification enzymes. Second, antioxidants are useful in preserving food by preventing food from occurring oxidized so that it can increase the

shelf life of food (Isik et al., 2015). Antioxidants include nutrients such as vitamins C, E, beta-carotene, and non-nutrients such as phenolic compounds (phenolic acids, lignans) (Oztaskin et al., 2015; Andlauer & Fürst, 1999). It is known that roselle calyxes are very rich in vitamin C, anthocyanins, polyphenols, and water-soluble antioxidants. Roselle calyxes are a good source of natural antioxidants and have high antioxidant activity from raspberries and blueberries (Hussein et al., 2010; Carvajal-Zarrabal et al., 2005). The mechanism of free radical scavengers in antioxidant activity includes (i) release of hydrogen atoms from hydroxyl groups (fast kinetic from phenolic derivatives and certain acidic compounds); (ii) release of electrons (slow kinetic from glycolysis and anthocyanin derivatives) (Nanjo et al., 1996). The main mechanism in phenolic compounds is to free radicals scavengers with the capture of H atoms in DPPH (1,1-diphenyl-2-picrylhydrazyl) to produce a stable DPPHH molecule (Molyneux, 2004; Sanchez-Moreno et al., 1998).

Purple roselle, which has already known inIn Indonesia, is also known as dark-red roselle in Egypt. According to Hussein et al. (2010), purple roselle calyxes have the highest water-soluble antioxidant capacity compared to red and green roselle. Figure 6 showed that the effect of antioxidant capacity on the extraction time difference was 15 minutes (186.97 ppm), 30 minutes (192.96 ppm), 45 minutes (197.6 ppm) with temperature of 50°C. The highest antioxidant capacity at 45 minutes compared to 15 and 30 minutes. These results are consistent with the study Yu et al. (2012) investigating antioxidant extraction from *Ginkgo Biloba* L. leaves, which showed that antioxidant yields increased with a significant increase in extraction time in the range of 15–60 minutes. Zhang et al. (2015) research showed that the use of multistage countercurrent technology is an economical and efficient technology because it saves extraction time, energy and costs for producing resveratrol from peanuts.

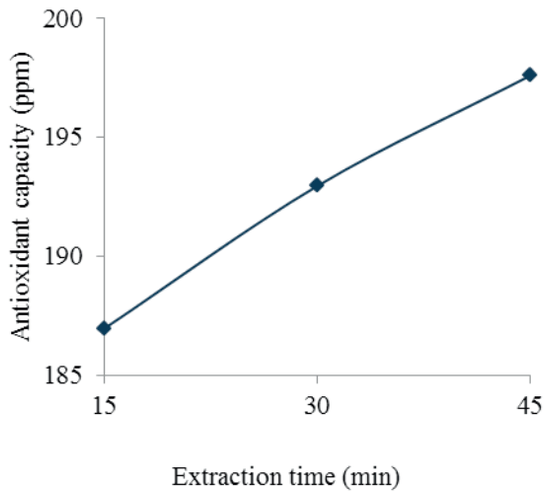


Figure 6. Effect of Extraction Time on Antioxidant Capacity

The bioactive compounds from the purple roselle calyxes in this study include contents of anthocyanin, quercetin, and antioxidants, the third content of these compounds has a correlation. According to Kita et al. (2013), in red potatoes and sweet potatoes contain anthocyanins and total polyphenols which correlate with antioxidant capacity. In addition, according to Conklin. (2009), quercetin as an anticancer is basically associated with strong antioxidant capacity. Therefore, the results reported in accordance with the study of Wong et al. (2006), Turkmen et al. (2007), Barhe & Tchouya. (2016) and Djeridane et al. (2006) that phenol content and antioxidant activity have a significant accurate correlation ($R^2 > 95\%$).

CONCLUSION

Effective and efficient extraction techniques have a significant influence on operating costs and quality of product quality. MCE (Multistage Countercurrent) is an extraction technique that can maintain a stable concentration gradient between solvents, fast extraction time, efficient and effective energy consumption. Purple roselle calyxes as a functional food ingredient rich in anthocyanins, quercetin, and antioxidants. Purple roselle calyxes using the MCE method contained the highest anthocyanin content of 2815.43 mg/L, quercetin content 59.25 mg/L, and antioxidant capacity 197.6 ppm. Thus, the extract of purple rosella calyxes using the MCE method has high anthocyanin, quercetin, and antioxidant content which has the potential as a natural colorant and antioxidant activities that are beneficial to health.

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REFERENCES

Abdel-Aal, E. S. M., & Hucl, P. (1999). A rapid method for quantifying total anthocyanins in blue aleurone and purple pericarp wheats. *Cereal Chemistry*, *76*(3), 350–354. doi:10.1094/CCHEM.1999.76.3.350

Andlauer, W., & Fürst, P. (1999). Does cereal reduce the risk of cancer? *Cereal Foods World*, *44*(2), 76–78.

Anel, T. C., Thokchom, R., Subapriya, M. S., Thokchom, J., & Singh, S. S. (2016). Hibiscus sabdariffa -A natural micro nutrient source. *Int. J. Adv. Res. Biol. Sci. International Journal of Advanced Research in Biological Sciences*, *3*(4), 243–248. Retrieved from http://www.ijarbs.com/pdfcopy/apr2016/ijarbs33.pdf

Azmir, J., Zaidul, I. S. M., Rahman, M. M., Sharif, K. M., Mohamed, A., Sahena, F., Jahurul, M. H. A., Ghafoor, K., Norulaini, N. A. N., & Omar, A. K. M. (2013). Techniques for extraction of bioactive compounds from plant materials : A review. *Journal of Food Engineering*, *117*(4), 426–436. doi:10.1016/j.jfoodeng.2013.01.014

Barhe, T. A., & Tchouya, G. R. F. (2016). Comparative study of the anti-oxidant activity of the total polyphenols extracted from Hibiscus Sabdariffa L., Glycine max L. Merr., yellow tea and red wine through reaction with DPPH free radicals. *Arabian Journal of Chemistry*, *9*(1), 1–8. doi:10.1016/j.arabjc.2014.11.048

Bischoff, S. C. (2008). Quercetin: Potentials in the prevention and therapy of disease. *Current Opinion in Clinical Nutrition and Metabolic Care*, *11*(6), 733–740. doi:10.1097/MCO.0b013e32831394b8

Boots, A. W., Balk, J. M., Bast, A., & Haenen, G. R. M. M. (2005). The reversibility of the glutathionyl-quercetin adduct spreads oxidized quercetin-induced toxicity. *Biochemical and Biophysical Research Communications*, *338*(2), 923–929. doi:10.1016/j.bbrc.2005.10.031

Borrás-Linares, I., Fernández-Arroyo, S., Arráez-Roman, D., Palmeros-Suárez, P. A., Del Val-Díaz, R., Andrade-Gonzáles, I., Fernández-Gutiérrez, A., Gómez-Leyva, J. F., & Segura-Carretero, A. (2015). Characterization of phenolic compounds, anthocyanidin, antioxidant and antimicrobial activity of 25 varieties of Mexican Roselle (Hibiscus sabdariffa). *Industrial Crops and Products*, *69*, 385?394-394. doi:10.1016/j.indcrop.2015.02.053

Bustos, D. V., Riegel, R., & Calderini, D. F. (2012). Anthocyanin content of grains in purple wheat is affected by grain position, assimilate availability and agronomic management. *Journal of Cereal Science*, *55*(3), 257–264. doi:10.1016/j.jcs.2011.12.001

Calabrò, M. L., Galtieri, V., Cutroneo, P., Tommasini, S., Ficarra, P., & Ficarra, R. (2004). Study of the extraction procedure by experimental design and validation of a LC method for determination of flavonoids in Citrus bergamia juice. *Journal of Pharmaceutical and Biomedical Analysis*, *35*(2), 349–363. doi:10.1016/S0731-7085(03)00585-5

Carvajal-Zarrabal, O., Waliszewski, S. M., Barradas-Dermitz, D. M., Orta-Flores, Z., Hayward-Jones, P. M., Nolasco-Hipólito, C., Angulo-Guerrero, O., Sánchez-Ricaño, R., Infanzón, R. M., & Trujillo, P. R. L. (2005). The consumption of Hibiscus sabdariffa dried calyx ethanolic extract reduced lipid profile in rats. *Plant Foods for Human Nutrition*, *60*(4), 153–159. doi:10.1007/s11130-005-9023-x

Chen, W., Müller, D., Richling, E., & Wink, M. (2013). Anthocyanin-rich purple wheat prolongs the life span of Caenorhabditis elegans probably by activating the DAF-16/FOXO transcription factor. *Journal of Agricultural and Food Chemistry*, *61*(12), 3047–3053. doi:10.1021/jf3054643

Cid-Ortega, S., & Guerrero-Beltrán, J. A. (2015). Roselle calyces (Hibiscus sabdariffa), an alternative to the food and beverages industries: a review. *Journal of Food Science and Technology*, *52*(11), 6859–6869. doi:10.1007/s13197-015-1800-9

Conklin, K. A. (2009). *Dietary Antioxidants During Cancer Chemotherapy : Impact on Chemotherapeutic Effectiveness and Development of Side Effects Dietary Antioxidants During Cancer Chemotherapy : Impact on Chemotherapeutic Effectiveness and Development of Side Effects*. *37*(1), 1–18. doi:10.1207/S15327914NC3701

Corrales, M., Lindauer, R., Butz, P., & Tauscher, B. (2008). Effect of heat/pressure on cyanidin-3-glucoside ethanol model solutions. *Journal*

of Physics: Conference Series, *121*(PART 14). doi:10.1088/1742-6596/121/14/142003

Da-Costa-Rocha, I., Bonnlaender, B., Sievers, H., Pischel, I., & Heinrich, M. (2014). Hibiscus sabdariffa L. - A phytochemical and pharmacological review. *Food Chemistry*, *165*, 424–443. doi:10.1016/j.foodchem.2014.05.002

Dehghan, G., & Khoshkam, Z. (2012). Tin(II)-quercetin complex: Synthesis, spectral characterisation and antioxidant activity. *Food Chemistry*, *131*(2), 422–426. doi:10.1016/j.foodchem.2011.08.074

Djaeni, M., Triyastuti, M. S., Asiah, N., Annisa, A. N., & Novita, D. A. (2015). The effect of air temperature on the sappan wood extract drying. *AIP Conference Proceedings*, *1699*. doi:10.1063/1.4938360

Djeridane, A., Yousfi, M., Nadjemi, B., Boutassouna, D., Stocker, P., & Vidal, N. (2006). Antioxidant activity of some algerian medicinal plants extracts containing phenolic compounds. *Food Chemistry*, *97*(4), 654–660. doi:10.1016/j.foodchem.2005.04.028

Einbond, L. S., Reynertson, K. A., Luo, X. D., Basile, M. J., & Kennelly, E. J. (2004). Anthocyanin antioxidants from edible fruits. *Food Chemistry*, *84*(1), 23–28. doi:10.1016/S0308-8146(03)00162-6

Giusti, M. M., & Wrolstad, R. E. (2003). Acylated anthocyanins from edible sources and their applications in food systems. *Biochemical Engineering Journal*, *14*(3), 217–225. doi:10.1016/S1369-703X(02)00221-8

Gokmen, V., Morales, F. J., Ataç, B., Serpen, A., & Lorenzo, G. A. (2009). Multiple-stage extraction strategy for the determination of acrylamide in foods. *Journal of Food Composition and Analysis*, *22*(2), 142–147. doi:10.1016/j.jfca.2008.09.007

Horbowicz, M., Grzesiuk, A., Debski, H., & Kosson, R. (2008). Anthocyanins of Fruits and Vegetables - Their Occurrence, Analysis and Role in Human. *Vegetable Crops Research Bulletin*, *68*, 5–22. doi:10.2478/v10032-008-0001-8

Hosseinian, F. S., Li, W., & Beta, T. (2008). Measurement of anthocyanins and other phytochemicals in purple wheat. *Food Chemistry*, *109*(4), 916–924. doi:10.1016/j.foodchem.2007.12.083

Hussein, R. M., Shahein, Y. E., Hakim, A. E. El, & Awad, H. M. (2010). Biochemical and molecular characterization of three colored types of roselle (Hibiscus sabdariffa L.). *Journal of American Science Org . Americanscience*, *11*(6), 726–733. https://www.kau.edu.sa/Files/857/Researches/58170_28293.pdf%0Ahttp://www.jofamericanscience.org/journals/am-sci/am0611/105_3886am0611_726_733.pdf

Isik, M., Korkmaz, M., Bursal, E., Gulcin, I., Koksal, E., & Tohma, H. (2015). Determination of Antioxidant Properties of Gypsophila bitlisensis Bark. *International Journal of Pharmacology*, *11*(4), 366–371.

Jabeur, I., Pereira, E., Barros, L., Calhelha, R. C., Soković, M., Oliveira, M. B. P. P., & Ferreira, I. C. F. R. (2017). Hibiscus sabdariffa L. as a source of nutrients, bioactive compounds and colouring agents. *Food Research International*, *100*, 717–723. doi:10.1016/j.foodres.2017.07.073

Jordheim, M. (2007). *Isolation, identification an properties of pyranoanthocyanins and anthocyanin forms*. 98.

Kita, A., Bakowska-Barczak, A., Hamouz, K., Kułakowska, K., & Lisińska, G. (2013). The effect of frying on anthocyanin stability and antioxidant activity of crisps from red- and purple-fleshed potatoes (Solanum tuberosum L.). *Journal of Food Composition and Analysis*, *32*(2), 169–175. doi:10.1016/j.jfca.2013.09.006

Knievel, D. C., Abdel-Aal, E. S. M., Rabalski, I., Nakamura, T., & Hucl, P. (2009). Grain color development and the inheritance of high anthocyanin blue aleurone and purple pericarp in spring wheat (Triticum aestivum L.). *Journal of Cereal Science*, *50*(1), 113–120. doi:10.1016/j.jcs.2009.03.007

Lee, J., Durst, R. W., & Wrolstad, R. E. (2005). Determination of total monomeric anthocyanin pigment content of fruit juices, beverages, natural colorants, and wines by the pH differential method: Collaborative study. *Journal of AOAC International*, *88*(5), 1269–1278. doi:10.5555/jaoi.2005.88.5.1269

Liao, J., Qu, B., & Zheng, N. (2016). Effects of Process Parameters on the Extraction of Quercetin and Rutin from the Stalks of Euonymus Alatus (Thumb.) Sieb and Predictive Model Based on Least Squares Support Vector Machine Optimized by an Improved Fruit Fly Optimization Algorithm. *Applied Sciences*, *6*(11), 340. doi:10.3390/app6110340

Liu, B., & Zhu, Y. (2007). Extraction of flavonoids from flavonoid-rich parts in tartary buckwheat

and identification of the main flavonoids. *Journal of Food Engineering*, *78*(2), 584–587. doi:10.1016/j.jfoodeng.2005.11.001

Mardiah, Zakaria, F. R., Prangdimurti, E., & Damanik, R. (2015). CHANGES IN CHEMICAL CONTENT OF RED AND PURPLE ROSELLE (Hibiscus sabdariffa L.) EXTRACTDRIED INCABINET DRYER AND FLUIDIZED BED DRYER. *Jurnal Teknologi Industri Pangan*, *25*(1), 1–7.

Molyneux, P. (2004). The use of the stable free radical diphenylpicryl- hydrazyl (DPPH) for estimating antioxidant activity. *Songklanakarin Journal of Science and Technology*, *26*(November 2003), 211–219. doi:10.1287/isre.6.2.144

Moon, Y. J., Wanga, L., DiCenzob, R., & Morris, M. E. (2008). Quercetin Pharmacokinetics in Humans Young. *Biopharmaceutics & Drug Disposition*, *29*(4), 205–217. doi:10.1002/bdd

Nanjo, F., Goto, K., Seto, R., Suzuki, M., Sakai, M., & Hara, Y. (1996). Scavenging effects of tea catechins and their derivatives on 1,1- diphenyl-2-picrylhydrazyl radical. *Free Radical Biology and Medicine*, *21*(6), 895–902. doi:10.1016/0891-5849(96)00237-7

Obadina, A. O., & Oyewole, O. B. (2007). Assessment of the antimicrobial potential of roselle juice (ZOBO) from different varieties of roselle calyx. *Journal of Food Processing and Preservation*, *31*(5), 607–617. doi:10.1111/j.1745-4549.2007.00151.x

Oztaskin, N., Cetinkaya, Y., Taslimi, P., Goksu, S., & Gulcin, I. (2015). Antioxidant and acetylcholinesterase inhibition properties of novel bromophenol derivatives. In *Bioorganic Chemistry* (Vol. 60). doi:10.1016/j.bioorg.2015.04.006

Pejic, N., Kuntic, V., Vujic, Z., & Micic, S. (2004). Direct spectrophotometric determination of quercetin in the presence of ascorbic acid. *Farmaco*, *59*(1), 21–24. doi:10.1016/j.farmac.2003.07.013

Price, K. R., Bacon, J. R., & Rhodes, M. J. C. (1997). Effect of Storage and Domestic Processing on the Content and Composition of Flavonol Glucosides in Onion (*Allium cepa*). *Journal of Agricultural and Food Chemistry*, *45*(3), 938–942. doi:10.1021/jf9605916

Qiu, G., Wang, D., Song, X., Deng, Y., & Zhao, Y. (2018). Degradation kinetics and antioxidant capacity of anthocyanins in air-impingement jet dried purple potato slices. *Food Research International*, *105*, 121–128. doi:10.1016/j.foodres.2017.10.050

Sanchez-Moreno, C., Larrauri, J. a., & Saura-calixto, F. (1998). A procedure to measure the antiradical efficienc y of polyphenols. *Journal of the Science of Food and Agriculture*, *270*(199802), 270–276. doi:10.1002/(SICI)1097-0010(199802)76:2<270::AID-JSFA945>3.0.CO;2-9

Sarker, S. D., & Nahar, L. (2012). An introduction to natural products isolation. *Methods in Molecular Biology*, *864*, 1–25. doi:10.1007/978-1-61779-624-1_1

Siegel, R. L., Miller, K. D., & Jemal, A. (2016). Cancer statistics. *CA Cancer J Clin*, *66*(1), 7–30. doi:10.3322/caac.21332.

Triyastuti, M. S., Kumoro, A. C., & Djaeni, M. (2017). Physical properties evaluation of roselle extract-egg white mixture under various drying temperatures. *AIP Conference Proceedings*, *1823*. doi:10.1063/1.4978116

Tsai, P. J., McIntosh, J., Pearce, P., Camden, B., & Jordan, B. R. (2002). Anthocyanin and antioxidant capacity in Roselle (Hibiscus sabdariffa L.) extract. *Food Research International*, *35*(4), 351–356. doi:10.1016/S0963-9969(01)00129-6

Turkmen, N., Velioglu, Y. S., Sari, F., & Polat, G. (2007). Effect of extraction conditions on measured total polyphenol contents and antioxidant and antibacterial activities of black tea. *Molecules*, *12*(3), 484–496. doi:10.3390/12030484

Vargas, F. D., & Lopez, O. P. (2003). Natural Colorants For Food and Nutraceutical for Uses. In *CRC Press LLC*. doi:10.1016/S0924-2244(03)00076-1

Wang, Q. E., Ma, S., Fu, B., Lee, F. S. C., & Wang, X. (2004). Development of multi-stage countercurrent extraction technology for the extraction of glycyrrhizic acid (GA) from licorice (Glycyrrhiza uralensis Fisch). *Biochemical Engineering Journal*, *21*(3), 285–292. doi:10.1016/j.bej.2004.06.002

Wong, C. C., Li, H. Bin, Cheng, K. W., & Chen, F. (2006). A systematic survey of antioxidant activity of 30 Chinese medicinal plants using the ferric reducing antioxidant power assay. *Food Chemistry*, *97*(4), 705–711. doi:10.1016/j.foodchem.2005.05.049

Yu, C. H., Chen, J., Xiong, Y. K., Li, X. X., Dai, X. Y., & Shi, C. C. (2012). Optimization of multi-stage countercurrent extraction of antioxidants from Ginkgo biloba L. leaves. *Food and Bioproducts Processing*, *90*(2), 95–101. doi:10.1016/j.fbp.2011.05.003

Yue, X., & Xu, Z. (2008). Changes of anthocyanins, anthocyanidins, and antioxidant activity in bilberry extract during dry heating. *Journal of Food Science*, *73*(6), 494–499. doi:10.1111/j.1750-3841.2008.00845.x

Zhang, Q., Bian, Y., Shi, Y., Zheng, S., Gu, X., Zhang, D., Zhu, X., Wang, X., Jiang, D., & Xiong, Q. (2015). An economical and efficient technology for the extraction of resveratrol from peanut (Arachis hypogaea) sprouts by multi-stage countercurrent extraction. *Food Chemistry*, *179*, 15–25. doi:10.1016/j.foodchem.2015.01.1