Effects of Extraction Temperature on Polyphenol Compounds and Antioxidant Activity of Golden Bladderwort (*Utricularia aurea*)

Sabri Sudirman*, Ace Beahaki, Fajar Fathullah, Miftahul Janna

Department of Fisheries Product Technology, Agriculture Faculty, Sriwijaya University
Jl Palembang-Prabumulih KM. 32, Indralaya 30662, South Sumatra, Indonesia
*Corresponding author: Sabri Sudirman, Email: sabrisudirman@unsri.ac.id

Submitted: June 7, 2022; Revised: November 1, 2022, November 15, 2022;
Accepted: November 23, 2022; Published: November 30, 2023

ABSTRACT

The excess of free radicals is known to induce dysfunction in tissues and cells within the human body. This phenomenon is caused by the imbalance between antioxidants and free radical production necessitating the need for exogenous antioxidants. Golden bladderwort (*Utricularia aurea*) plant may have the potential as a source of antioxidant. Therefore, this study aimed to investigate the effect of extraction temperature on the polyphenol compounds and the antioxidant activity of the golden bladderwort plant. The dried powder from the plant was extracted using 70% ethanol at 30, 45 and 60 ºC, with each treatment repeated three times. The antioxidant activity was analyzed by using the 2,2-diphenyl-1-picrylhydrazyl method. The results showed that the yield of extraction was 1.34% (30 ºC), 4.00% (45 ºC) and 12.48% (60 ºC). The total polyphenol and flavonoid contents found at 60ºC were 50.80 mg QE/g dried sample and 60.01 mg QE/g dried sample, respectively. Furthermore, the antioxidant activity was calculated in *half-maximum inhibitory concentration* (IC_{50}), and the values obtained were 0,38 mg/mL (45 ºC), 0,35 mg/mL (30 ºC) and 0,11 mg/mL (60 ºC). These results indicated that temperature of extraction affected the level of polyphenol contents and the antioxidant activity of the golden bladderwort extract. The high extraction temperature (60 ºC) emerged as the most optimal condition for extraction with effective antioxidant activity. Therefore, the results can be used as a reference for future investigations involving the extraction of polyphenol compounds from golden bladderwort as a source of natural antioxidants.

Keywords: Antioxidants activity; extraction; golden bladderwort; polyphenol; temperature

INTRODUCTION

Oxidative stress is a condition arising from the imbalance between antioxidant potential and free radical production in the body. This phenomenon causes damage to cells and tissues, leading to metabolic disorders. Consequently, external sources of antioxidants are needed to prevent or reduce the oxidative stress condition. Antioxidants made synthetically have been utilized frequently in numerous applications, including pharmacology and the food industry but there are concerns over their potential side effects (Sasaki et al., 2002). Numerous investigations have been performed to explore alternative sources of natural antioxidants and innovative methods for extracting bioactive compounds from various biological resources. Vitamins and polyphenolics are prominent examples of bioactive compounds, extensively utilized as natural antioxidants (Sinbad, Folorunsho, Olabisi, Abimbola Ayoola, & Johnson Temitope, 2019; Zeb, 2020).

Golden bladderwort (*Utricularia aurea*) is an aquatic plant that lives floating on the surface of waters,

DOI: http://doi.org/10.22146/agritech.75223
ISSN 0216-0455 (Print), ISSN 2527-3825 (Online)

such as lakes, rivers, and waterlogged soil. Within the ecosystem, this plant is classified as a carnivore, capturing insects with its specialized traps (Kumar, Thorat, Labala, & Patra, 2018). Previous studies have highlighted the potential as a pharmaceutical agent due to the presence of bioactive substances, including polyphenols, tannins and flavonoids (Mishra & Kumar, 2020). These compounds can be obtained through the extraction process, which is affected by several parameters, such as extraction temperature, the ratio between solvent and extracted material, particle size and the number of repetitions (Chirinos, Rogez, Campos, Pedreschi, & Larondelle, 2007).

Polyphenolic compounds are easily damaged by temperature or are thermolabile (Li et al., 2017). Previous studies reported that high temperatures (>100 ºC) reduced the polyphenol content of *Thymus vulgaris* extract (Vergara-Salinas, Pérez-Jiménez, Torres, Agosin, & Pérez-Correa, 2012). Extraction with a temperature above 40 ºC also caused the degradation of certain thermolabile bioactive components in *Gordonia axillaris* extract (Li et al., 2017). The total polyphenols in *Prunus persica* fruit decreased significantly at temperatures above 50 ºC, while the total flavonoids reduced at 60 ºC (Mokrani & Madani, 2016). These findings show that temperature is an important factor in extracting polyphenolic compounds from plants. Therefore, this study aimed to investigate the extraction temperature effect on the polyphenolic compounds of golden bladderwort plant extract and its antioxidant activity.

METHODS

Materials

The main material used, namely fresh golden bladderwort plants (*U. aurea*) was obtained from swamp waters in Ogan Ilir Regency, South Sumatra, Indonesia. Other materials included ethanol (Supelco-Merck, absolute), free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH, 95.54%) purchased from HIMEDIA (Mumbai, India), quercetin (≥95%) from Sigma-Aldrich (St. Louis, MO, USA) and Folin-Ciocalteu's phenol reagent from Supelco-Merck (Darmstadt, Germany). The various types of equipment used were hot plate magnetic stirrer (IKA C-MAG HS 7, Staufen, Germany), freeze dryer (Biobase BK-FD10S, Shandong, China), evaporator (Biobase RE-301, Shandong, China), UV-Vis spectrophotometer (Genesys 150 ThermoScientific, Massachusetts, USA) and centrifuge (Oregon LC-04S centrifuge).

Preparation and Extraction Process

Fresh golden bladderwort plants were obtained from Jakabaring Swamp Waters, Palembang. The plants were then transported to the laboratory, cleaned with water, and stored at 5 ºC for further analysis. The extraction process was carried out based on methods published by previous studies (K. K. Chew et al., 2011; Sudirman, Herpandi, Safitri, Apriani, & Taqwa, 2022). The plants were oven dried at 45 ºC for 12 hours to reduce the water content, facilitate the extraction process, and maximize the amount of sample solids used. The dried samples were further pulverized to obtain the powder form, then polyphenolic compounds from these plants were extracted through the maceration method with the aid of a magnetic stirrer (120 rpm) using 70% ethanol solvent. A total of 70% ethanol solvent was used in this study because it was considered the optimal solvent to extract polyphenol compounds from water lettuce plants (Sudirman et al., 2022). The extraction temperatures used were 30, 45 and 60 ºC, each of which was carried out for three hours. After the maceration time was complete, the filtrate (liquid phase) and residue (solid phase) were separated using filter paper (Whatman Number 42). The filtrate was collected in a new collection tube, and the residue was re-extracted using a new 70% ethanol under the same conditions as the first extraction process. Total extraction was performed five times until the filtrate was colorless, indicating that the bioactive components (polyphenols) were no longer in the powder. Subsequently, the combined filtrates were evaporated at 45 °C to produce extract concentrate, which was freeze-dried to obtain a fine powdered crude extract. The extraction results were stored at cold temperatures for further analysis, and the percentage (%) yield of crude extract was calculated based on published references, as shown in Equation 1 (Sudirman et al., 2022).

$$\text{Yield (\%)} = \frac{\text{Dry extract weight (g)}}{\text{Dry sample weight (g)}} \times 100\% \quad (1)$$

Total Polyphenol Analysis

Total polyphenols were analyzed using Folin-Ciocalteu's phenol reagent based on methods published by a previous study (Chandra et al., 2014). Gallic acid was used as a standard to calculate the polyphenol content contained in the extract. A total of 0.2 mL extract (10 mg/mL in distilled water, b/v) or gallic acid was mixed with phenol reagent and reacted for 5 minutes. Afterward, 1 mL of sodium carbonate solution (8% in water, b/v) was added to the test tube, followed by the addition of distilled water to reach a volume of 3 mL. The mixture was reacted under dark conditions at room temperature for 30 minutes and then centrifuged at 1,744 × g for 30 minutes. The supernatant obtained was measured for absorbance using a UV-Vis spectrophotometer at 750

nm. Total polyphenols were expressed as mg gallic acid equivalent (GAE) per g dry sample.

Total Flavonoid Analysis

Total flavonoids were analyzed using the colorimetric aluminum chloride method based on a previous study (Chandra et al., 2014). Quercetin was used as a standard to calculate the flavonoid content contained in the extract. A total of 0.6 mL extract solution (10 mg/mL in distilled water, b/v) or quercetin was reacted with 20% aluminum chloride (1:1, v/v) at room temperature for 60 minutes. Afterward, the absorbance was immediately measured using a UV-Vis spectrophotometer at 420 nm. Total flavonoids were expressed as mg quercetin equivalent (QE) per g dry sample.

Antioxidant Activity Analysis

Antioxidant activity of the sample was measured using the DPPH method based on a previous method (Y. L. Chew, Lim, Omar, & Khoo, 2008). The extract was dissolved using distilled water in various concentrations (0 to 1000 µg/mL). A total of 1 mL extract solution was mixed with 0.2 mM DPPH solution (1:1, v/v), and the mixture was incubated in the dark at 37 ºC. Afterward, the absorbance was immediately measured using UV-Vis spectrophotometry at 517 nm. The percentage (%) of free radical inhibition was measured based on Equation 2 (Y. L. Chew et al., 2008).

$$\text{Percentage (\%) inhibition} = \frac{Abs_{blank} - Abs_{sample}}{Abs_{blank}} \times 100\% \quad (2)$$

Description: Abs_{blank} = Absorbance without extract at 517 nm; Abs_{sample} = Absorbance with added extract at 517 nm.

RESULT AND DISCUSSION

Crude Extract Yield

The results showed that the extraction at 60 ºC produced a significantly higher yield compared to other temperatures (Figure 1). The highest yield value was 12.48 ± 4.43%, and the lowest was 1.34 ± 0.45%. Higher temperatures enhanced solvent penetration of the sample resulting in increased extraction speed and efficiency (N. Y. Lee et al., 2016). The yield of bioactive components in *Ocimum gratissimum* L. extract increased with higher extraction temperatures (40-60 ºC) as reported by previous studies (Onyebuchi & Kavaz, 2020). Significantly higher yields were also observed in *Mimusops elengi* L. fruit extracted at 45 ºC and 60 ºC (Zaidiyah, Ghifari, & Abubakar, 2021).

Several factors can affect the extraction of bioactive components, including temperature (Chirinos et al., 2007). According to a previous study, temperature affected the solubility and diffusion coefficient during the extraction process (N. Y. Lee et al., 2016).

Total Polyphenol and Flavonoid Content

The extraction results at 60 ºC had significantly higher total polyphenol content compared to other temperatures as shown in Figure 2. The highest and lowest content was 50.80 ± 2.33 mg GAE/g dry sample and 35.03 ± 1.73 mg GAE/g dry sample, respectively. The polyphenol content of *Funtumia elastica* stem bark extract increased at an extraction temperature of 25-65 ºC (Frempong, Owusu Boadi, & Badu, 2021).

The highest flavonoid content namely 60.01 ± 1.37 mg QE/g dry sample, was obtained at the extraction temperature of (60 ºC) as shown in Figure 3. The lowest

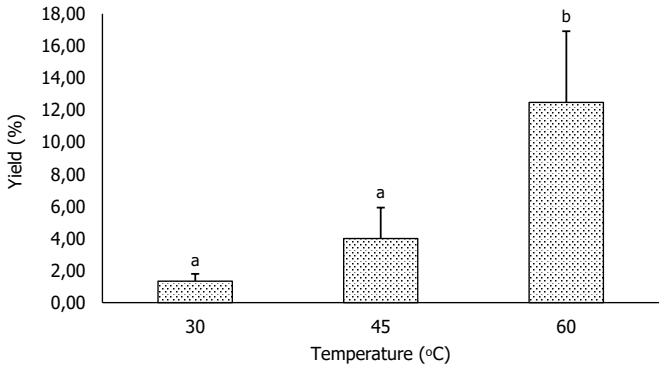

Figure 1. Yield of crude extracts from *Utricularia aurea* extracted at different temperatures. The mean ± standard deviation (n=3) is used to express the data. Different letters represent significant differences (p<0.05).

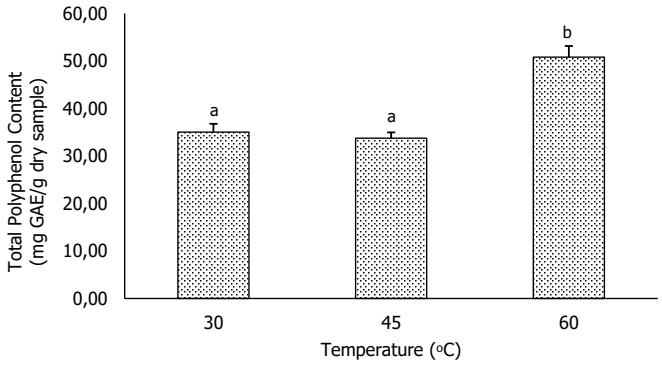

Figure 2. Total polyphenol content of *Utricularia aurea* extracted at different temperatures. The mean ± standard deviation (n=3) is used to express the data. Different letters represent significant differences (p<0.05).

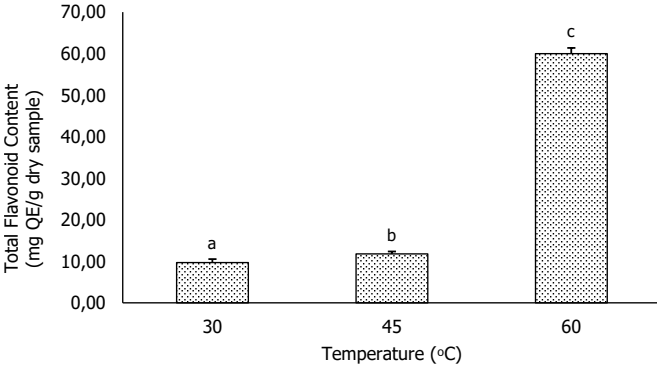

Figure 3. Total flavonoid content of *Utricularia aurea* extracted at different temperatures. The mean ± standard deviation (n=3) is used to express the data. Different letters represent significant differences (p<0.05).

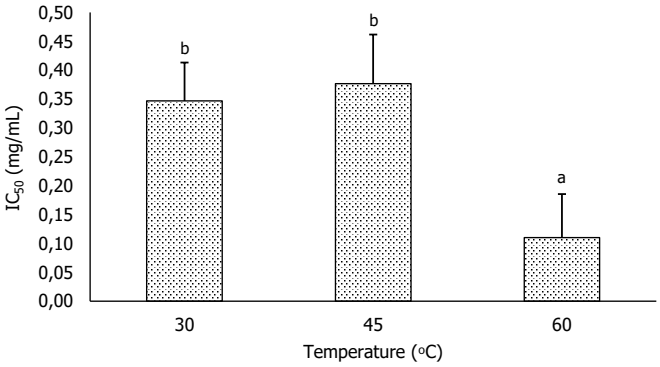

Figure 4. Antioxidant activity of *Utricularia aurea* extracted at different temperatures. The mean ± standard deviation (n=3) is used to express the data. Different letters represent significant differences (p<0.05).

flavonoid content was 9.71 ± 0.81 mg QE/g dry sample (30 ºC). Previous studies reported that the flavonoid content of *the Cannabis sativa* plant extracted at 40-60 ºC increased from 14 mg LUE/100 g to 25 mg LUE/100 g (Teh & Birch, 2014). Moreover, flavonoid levels in *Tabernaemontana heyneana* plant leaves extracted at 55-65 ºC ranged from 71.5 mg/g to 107.2 mg/g (Sathishkumar et al., 2008).

The increased temperature during the extraction process softened plant tissues, as well as weakened the protein-phenol and phenol-polysaccharide interactions, causing more polyphenols to migrate into the solvent (Shi et al., 2003). This phenomenon can also explain the observed escalation in flavonoid levels with a concurrent increase in the temperature, as flavonoid compounds frequently exist in the form of glycosides (Garcia-Salas, Morales-Soto, Segura-Carretero, & Fernández-Gutiérrez, 2010).

Antioxidant Activity

The antioxidant activity test carried out using the DPPH method showed that the extraction conducted at 60 ºC had a significantly lower half-maximum inhibitory concentration (IC_{50}) value compared to other temperatures (Figure 4). The IC_{50} value at 60 ºC was 0.11 mg/mL, while the values obtained at 30 ºC and 45 ºC had no significant difference.

The low IC_{50} value in the 60 ºC extract signifies a more effective antioxidant potential in inhibiting free radicals due to the higher total polyphenols compared to other extracts. These compounds can inhibit free radicals or oxidation reactions through the mechanism of hydrogen atoms or electron transfer (C. Y. Lee et al., 2015). The elevating the extraction temperature (45, 55 and 65 ºC) increased the total polyphenols accompanied by improved antioxidant effectiveness of the extract (Molaveisi, Beigbabaei, Akbari, Noghabi, & Mohamadi, 2019). Furthermore, the antioxidant activity reflected in the percentage inhibition increased from 25 ºC (37.97±1.84) to 50 ºC (46.97±4.57) (Alide, Wangila, & Kiprop, 2020).

CONCLUSION

In conclusion, the crude extract of golden bladderwort was found to contain polyphenolic compounds. The extraction temperature affected the bioactive compounds in the extract and its antioxidant activity. An optimal extraction temperature of 60 ºC was identified as the most effective in extracting these components from the plant. At this temperature, there was a significant increase in the polyphenol group compounds, which involved to the strong antioxidant activity and effective inhibition of free radicals. Therefore, the results can be used as a reference for future studies involving the extraction of polyphenolic compounds from golden bladderwort as a source of natural antioxidants.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest from any party.

REFERENCES

Alide, T., Wangila, P., & Kiprop, A. (2020). Effect of cooking temperature and time on total phenolic content,

total flavonoid content and total in vitro antioxidant activity of garlic. *BMC Research Notes, 13*(1). http:doi.org/10.1186/s13104-020-05404-8

Chandra, S., Khan, S., Avula, B., Lata, H., Yang, M. H., Elsohly, M. A., & Khan, I. A. (2014). Assessment of total phenolic and flavonoid content, antioxidant properties, and yield of aeroponically and conventionally grown leafy vegetables and fruit crops: a comparative study. *Evidence-Based Complementary and Alternative Medicine, 2014*, 253875. http:doi.org/10.1155/2014/253875

Chew, K. K., Ng, S. Y., Thoo, Y. Y., Khoo, M. Z., Wan Aida, W. M., & Ho, C. W. (2011). Effect of ethanol concentration, extraction time and extraction temperature on the recovery of phenolic compounds and antioxidant capacity of *Centella Asiatica* extracts. *International Food Research Journal, 18*, 571-578.

Chew, Y. L., Lim, Y. Y., Omar, M., & Khoo, K. S. (2008). Antioxidant Activity of three edible seaweeds from two areas in South East Asia. *LWT - Food Science and Technology, 41*(6), 1067-1072. http:doi.org/10.1016/j.lwt.2007.06.013

Chirinos, R., Rogez, H., Campos, D., Pedreschi, R., & Larondelle, Y. (2007). Optimization of extraction conditions of antioxidant phenolic compounds from mashua *(Tropaeolum tuberosum* Ruíz & Pavón) tubers. *Separation and Purification Technology, 55*(2), 217-225. http:doi.org/10.1016/j.seppur.2006.12.005

Frempong, F. T., Boadi, N. O., & Badu, M. (2021). Optimization of extraction conditions for polyphenols from the stem bark of *Funtumia elastica* (Funtum) utilizing response surface methodology. *AAS Open Research, 4*. http:doi.org/10.12688/aasopenres.13284.2

Garcia-Salas, P., Morales-Soto, A., Segura-Carretero, A., & Fernández-Gutiérrez, A. (2010). Phenolic-compound-extraction systems for fruit and vegetable samples. *Molecules, 15*(12), 8813-8826. http:doi.org/10.3390/molecules15128813

Kumar, S., Thorat, S. S., Labala, R. K., & Patra, J. K. (2018). Insectivorous plants of India: Sources of Bioactive Compounds to fight against antimicrobial resistance. In *Microbial Biotechnology* (pp. 305-318).

Lee, C. Y., Nanah, C. N., Held, R. A., Clark, A. R., Huynh, U. G. T., Maraskine, M. C., . . . Sharma, A. (2015). Effect of electron donating groups on polyphenol-based antioxidant dendrimers. *Biochimie, 111*, 125-134. http:doi.org/10.1016/j.biochi.2015.02.001

Lee, N. Y., Yunus, M. A. C., Idham, Z., Ruslan, M. S. H., Aziz, A. H. A., & Irwansyah, N. (2016). Extraction and identification of bioactive compounds from agarwood leaves. *IOP Conference Series: Materials Science and Engineering, 162*(1), 012028. http:doi.org/10.1088/1757-899x/162/1/012028

Li, Y., Li, S., Lin, S.-J., Zhang, J.-J., Zhao, C.-N., & Li, H.-B. (2017). Microwave-assisted extraction of natural antioxidants from the exotic *Gordonia axillaris* fruit: optimization and identification of phenolic compounds. *Molecules, 22*(9). http:doi.org/10.3390/molecules22091481

Mishra, S. & Kumar, S. (2020). Ecological mapping & pharmacological activity of *Utricularia aurea* Lour.: A carnivorous plant of Odisha. In *Medico-Biowealth of Odisha* (pp. 10 - 21). Odisha, India: Ambika Prasad Research Foundation.

Mokrani, A. & Madani, K. (2016). Effect of solvent, time and temperature on the extraction of phenolic compounds and antioxidant capacity of peach (*Prunus persica* L.) fruit. *Separation and Purification Technology, 162*, 68-76. http:doi.org/10.1016/j.seppur.2016.01.043

Molaveisi, M., Beigbabaei, A., Akbari, E., Noghabi, M. S., & Mohamadi, M. (2019). Kinetics of temperature effect on antioxidant activity, phenolic compounds and color of Iranian jujube honey. *Heliyon, 5*(1). http:doi.org/10.1016/j.heliyon.2019.e01129

Onyebuchi, C. & Kavaz, D. (2020). Effect of extraction temperature and solvent type on the bioactive potential of *Ocimum gratissimum* L. extracts. *Scientific Reports, 10*(1). http:doi.org/10.1038/s41598-020-78847-5

Sasaki, Y. F., Kawaguchi, S., Kamaya, A., Ohshita, M., Kabasawa, K., Iwama, K., . . . Tsuda, S. (2002). The comet assay with 8 mouse organs: results with 39 currently used food additives. *Mutation Research/Genetic Toxicology and Environmental Mutagenesis, 519*(1-2), 103-119. http:doi.org/10.1016/s1383-5718(02)00128-6

Sathishkumar, T., Baskar, R., Shanmugam, S., Rajasekaran, P., Sadasivam, S., & Manikandan, V. (2008). Optimization of flavonoid extraction from the leaves of *Tabernaemontana heyneana* Wall. using L_{16} Orthogonal design. *Nature and Science, 6*(3), 10-21.

Shi, J., Yu, J., Pohorly, J., Young, J. C., Bryan, M., & Wu, Y. (2003). Optimization of the extraction of polyphenols from grape seed meal by aqueous ethanol solution. *Food, Agriculture & Environment 1*(2), 42-47.

Sinbad, O. O., Folorunsho, A. A., Olabisi, O. L., Abimbola Ayoola, O., & Johnson Temitope, E. (2019). Vitamins as antioxidants. *Journal of Food Science and Nutrition Research, 02*(03). http:doi.org/10.26502/jfsnr.2642-11000021

Sudirman, S., Herpandi, Safitri, E., Apriani, E. F., & Taqwa, F. H. (2022). Total polyphenol and flavonoid contents and antioxidant activities of water lettuce (*Pistia stratiotes*) leave extracts. *Food Research, 6*(4), 205-210. http:doi.org/10.26656/fr.2017.6(4).484

Teh, S.-S., & Birch, E. J. (2014). Effect of ultrasonic treatment on the polyphenol content and antioxidant capacity of

extract from defatted hemp, flax and canola seed cakes. *Ultrasonics Sonochemistry, 21*(1), 346-353. http:doi.org/10.1016/j.ultsonch.2013.08.002

Vergara-Salinas, J. R., Pérez-Jiménez, J., Torres, J. L., Agosin, E., & Pérez-Correa, J. R. (2012). Effects of temperature and time on polyphenolic content and antioxidant activity in the pressurized hot water extraction of deodorized thyme (*Thymus vulgaris*). *Journal of Agricultural and Food Chemistry, 60*(44), 10920-10929. http:doi.org/10.1021/jf3027759

Zaidiyah, Z., Ghifari, M. G. A., & Abubakar, Y. (2021). Extraction yield, antioxidant activity and total phenolic content of *Mimusops elengi* L. fruit. *IOP Conference Series: Earth and Environmental Science, 922*(1). http:doi.org/10.1088/1755-1315/922/1/012021

Zeb, A. (2020). Concept, mechanism, and applications of phenolic antioxidants in foods. *Journal of Food Biochemistry, 44*(9). http:doi.org/10.1111/jfbc.13394