



Preparation of *Legazpia polygonoides* Water Extract with Different Parameters (Temperatures and Extraction Times): Antioxidant and Anti-lipid peroxidation

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Abstract: *Legazpia polygonoides* is an herb belonging to the Linderniaceae family. This plant is traditionally used to treat eye infections and improve eyesight. However, scientific experiments using *L. polygonoides* are still scarce. This study aimed to determine the level of antioxidants and their ability to prevent lipid peroxidation. The dried *L. polygonoides* powder was placed in a tube of water and heated to different temperatures of 40°C, 60°C, 80°C and 100°C. For each temperature, the extracts were incubated at different times, e.g. 30 minutes, 1 hour, 2 hours, and 3 hours. The results of the DPPH test showed that the water extract of *L. polygonoides* prepared with the parameter 60°C / 3 hours had the highest inhibition of DPPH compared to the other parameters. Furthermore, the FRAP test showed that the aqueous extract of *L. polygonoides* prepared with the parameter 60°C/3 h had the highest FRAP value compared to the other parameters. For the TPC test, the preparation of aqueous extract of *L. polygonoides* with the parameter 100°C / 30 min had the highest phenol content compared to the other parameters. For the MDA test, the test results showed that the extracts produced using the 100°C/3 h parameter had lower levels of malondialdehyde compared to the other parameters. This study shows that *L. polygonoides* has the potential to be used as a source of antioxidants. It can be used to prevent lipid peroxidation.

Keywords: *L. polygonoides*, temperature, duration time, antioxidant, toxicity

INTRODUCTION

Free radicals such as reactive oxygen species (ROS) and reactive nitrogen species (RNS) are produced as byproducts of aerobic metabolism and cause harm to the

human body (Nakai *et al.*, 2021). Excessive ROS in cells causes oxidative damage to DNA, proteins, and lipids (Juan *et al.*, 2021). Besides that, free radicals also can cause diseases such as cancer, atherosclerosis, and stroke (Čipak, 2020). However, the human body has several mechanisms to survive oxidative stress by producing endogenous antioxidants enzyme such as superoxide dismutase, catalase and glutathione peroxidase (Ighodaro and Akinloye, 2018) or either receiving the antioxidant from food, dietary supplements and herbs (Pham-Huy *et al.*, 2008, Xu *et al.*, 2017).

In the previous time, human uses the herb to cure diseases (Petrovska, 2012). According to Rahman *et al.*, (2021), herbs contain many phenolic compounds that can help to reduce oxidative stress. Besides that, herbal medicine can inhibit enzymes related to the development of human diseases in the early step (Rahman *et al.*, 2021). However, there are many issues in the production of herbal and its preparation that need to be handled to get the herbal product of high quality. The inconsistency of method and parameter used in the herbal preparation causes the inconsistency of its efficacy. In addition, the phenolic compound may be demolished when heated at a higher temperature for a long time during herbal preparation (Xu *et al.*, 2007). Therefore, the study on the parameter must be performed to ensure the herbal extract contains high and effective compound. One of the herbs that were used for centuries was *Legazpia polygonoides*.

Legazpia polygonoides (*L. polygonoides*) belong to family of Linderniaceae (Schoch *et al.*, 2020). It is also called *Torenia polygonoides* Benth or *Lindernia crustacea* var. *godefroyi* (Bonati) T. Yamazaki. It can be found in Malaysia and ASEAN countries. Local name of this herb is Malayan Eyebright. In Malay language, this herb is called as Kerak merah, Rumpit sisik naga or Terutup batu. It is a trailing herb with white flowers (Henderson, 1959). The leaves of *L. polygonoides* is oppositely arranged, triangular with rounded edges, and serrated along the margins (Henderson, 1959). Traditionally, the leaves are used in the treatment of eye infections and improve eyesight.



A) Leaves



B) Plant,



C) Leave stems



MATERIALS AND METHODS

Collection of L. polygonoides and Preparation of water extract: This herb was collected at the Forest Research Institute Malaysia (FRIM). The sample was submitted to Natural Product Division for authentication. The plant was identified by a certified botanist and kept at Kepong Herbarium (KEP). The SBID code was SBID 015/22. Then, the herb was washed and dried using an oven at 55°C for 48 hours. After that, the dried herb was ground into powder. Then, 5 mg of *L. polygonoides* powder are weighed using electronic scales and placed in a falcon tube. Five milliliters of distilled water has been added to the falcon tube. The tube was heated to different temperatures of 40°C, 60°C, 80°C, and 100°C at different times for each temperature which were 15 min, 30 min, 1h, 2 hours, and 3 hours. Then the extract was centrifuged for 15 minutes at a speed of 2000 rpm. The suspension was stored in the refrigerator at -20°C until it is used.

2,2-diphenyl-1-picrylhydrazyl (DPPH) Scavenging Assay: The DPPH test was performed using the method shown by Tiveron *et al.*, (2012) with a slight modification. DPPH reagents are provided by adding 1.77g of DPPH powder to the falcon tube. Then, 10 mL of methanol was added into the tube to prepare the DPPH reagent. Then, 50 µL of previously prepared *L. polygonoides* extract was mixed with 100 µL of DPPH reagents in a 96-well microplate. The microplate was incubated in a dark place for 30 minutes. The absorbance of the solution was measured at a wavelength of 540nm. This experiment was conducted in triplicates.

Ferric Reducing Antioxidant Power (FRAP) Assay: The measurement of FRAP in *L. polygonoides* extract was according to the procedure described by Benzie and Strain (1996). The FRAP reagent contained 2.5 mM 10 mM TPTZ solution in 40 mM HCl, 2.5 mL 20 mM FeCl₃ • 6H₂O and 25 mM 300 mM acetate buffer (pH = 3.6). A standard curve was prepared using a concentration of 0.1 to 2 mmol / l FeSO₄ • 7H₂O. 30 µL of *L. polygonoides* extract was added to each 96-well plate. Then 200 µL of FRAP reagent was placed on the same microplate. The reaction mixture was stirred at 37°C for 30 min and the absorbance was measured at 593 nm using a spectrophotometer. This experiment was repeated three times.

Total Phenolic Content (TPC): The TPC of *L. polygonoides* extract was measured using the Folin-Ciocalteu method as reported by Singleton and Rossi (1965) with minor modifications. Briefly, 20 µL of *L. polygonoides* extract was mixed with 10 µL of Folin-Ciocalteu reagent in each well. The mixture is stored for 5 minutes at 37° C. Then, 40 µL of 7.6% sodium carbonate was added to the microplate. The microplates were incubated for 2 hours in the dark. Absorption measurements were then performed at wave 765 nm. The experiment was performed up to three times.

Thiobarbituric Acid Reactive Species (TBARS) test: The modified TBARS test was used to measure the level of lipid peroxide formed using egg yolk as the lipid medium to be oxidized (Upadhyay *et al.*, 2014). Two hundred and fifty microliters of 10% egg yolk were placed in a test tube. Subsequently, 50 µL of *L. polygonoides* extract was then

mixed in the test tube. Distilled water was added to the test tube to make it 500 μL . Finally, 25 μL of ferrous sulfate (FeSO_4) (0.07 M) was added to the mixture and incubated for 30 minutes to ensure that the lipid peroxidation process occurred.

Subsequently, 750 μL of 0.8% TBA, 750 μL of 20% acetic acid and 25 μL of 20% TCA were added to the test tube. The test tube is boiled in a water bath for 1 hour and cooled under running water. To each test tube was added 3.0 ml of 1-butane, separated and centrifuged at 3000 rpm for 10 min. The top layer was taken and measured at a wavelength of 532 nm. The experiment was performed three times.

Statistical analysis: Each experiment performed on the sample was three times. All the results presented in the graph of each study result are $\text{min} \pm$ standard deviation. Statistical analysis was performed with SPSS 21. Statistical significance was observed using one-way ANOVA ($p < 0.05$).

RESULTS

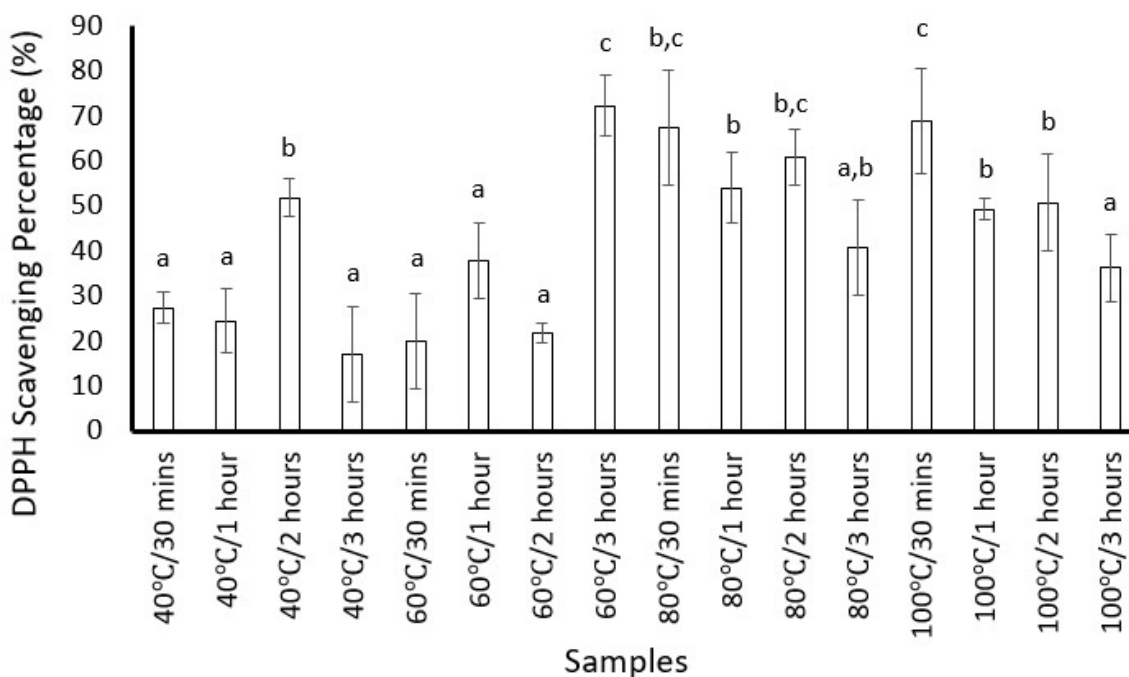


Figure 1: DPPH free radical scavenging activity of *L. polygonoides* extract with different temperatures and times. Values are $\text{mean} \pm$ standard deviation ($n = 8$). Different superscript letters indicate a significant difference at $p < 0.05$.

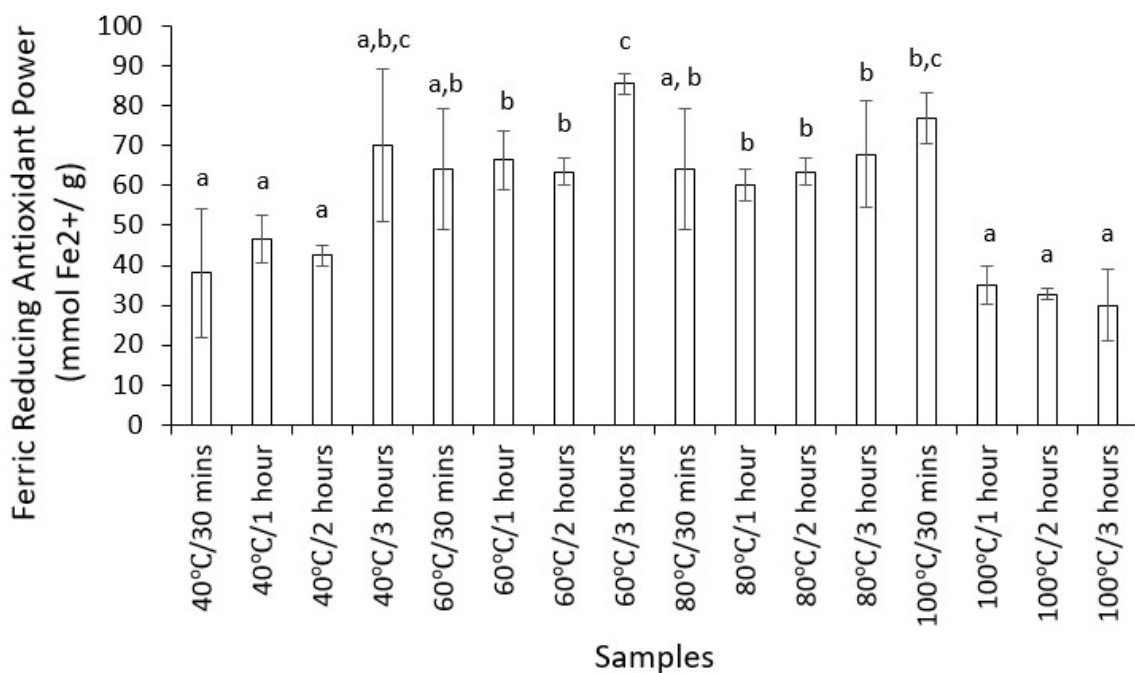


Figure 2: FRAP value of *L. polygonoides* extract with different temperatures and times. Values are mean \pm standard deviation (n = 8). Different superscript letters indicate a significant difference at $p < 0.05$.

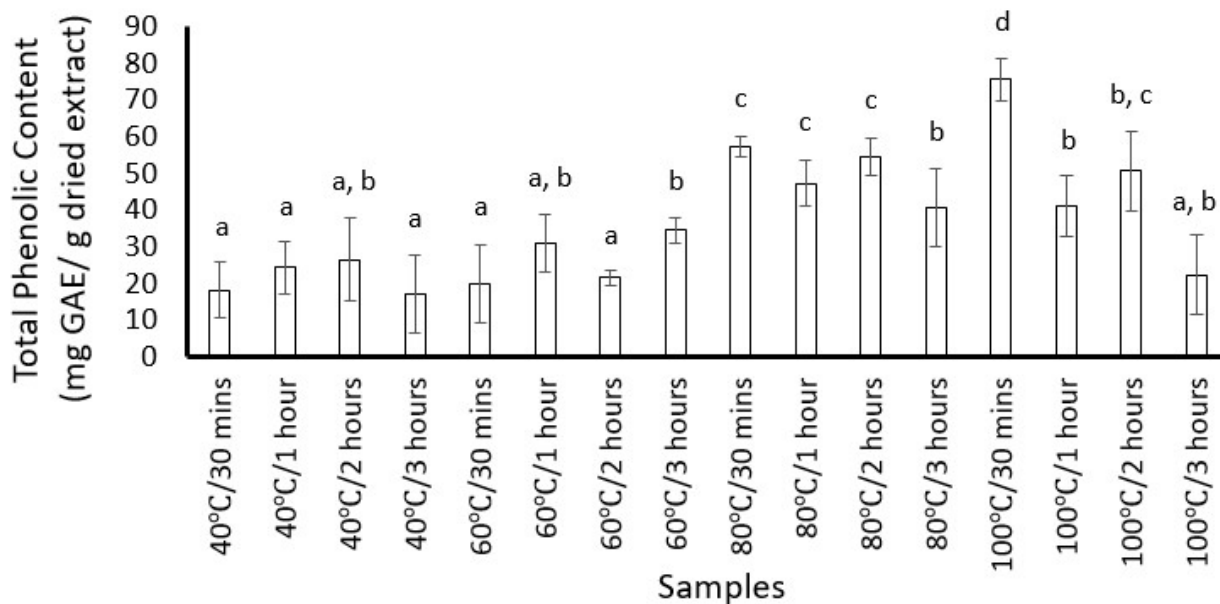


Figure 3: TPC level of *L. polygonoides* extract with different temperatures and times. Values are mean \pm standard deviation (n = 8). Different superscript letters indicate a significant difference at $p < 0.05$.

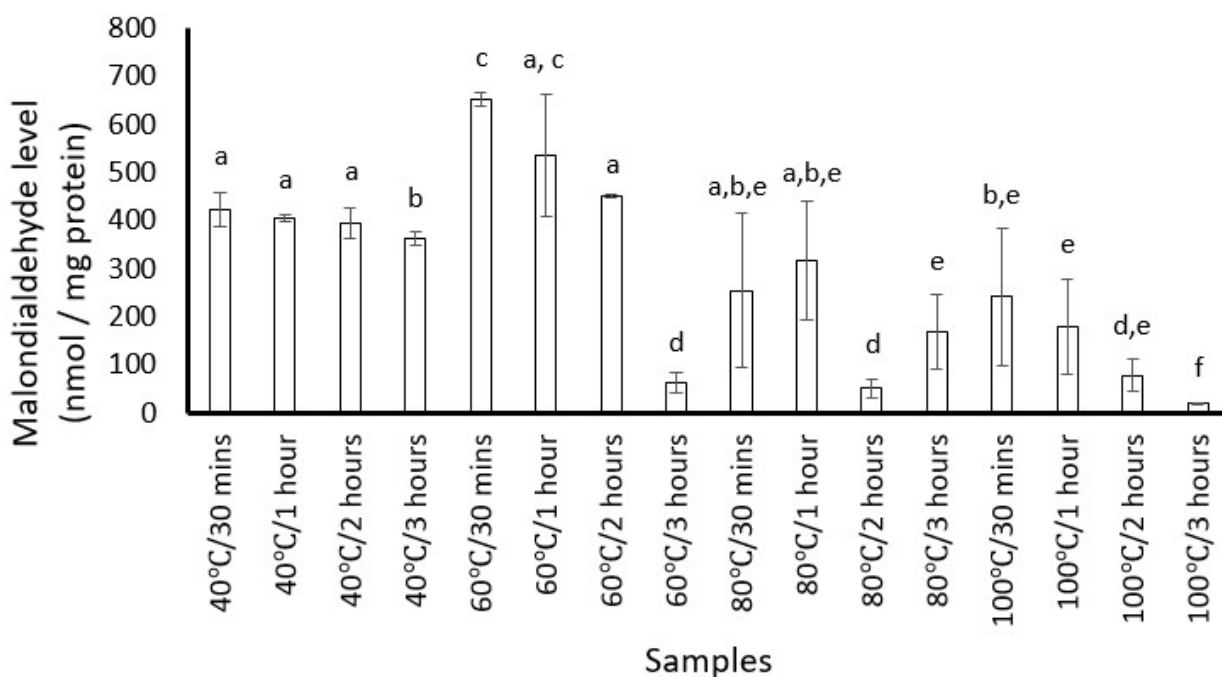


Figure 4: MDA level after treatment of *L. polygonoides* extract with different temperatures and times. Values are mean \pm standard deviation (n = 8). Different superscript letters indicate a significant difference at $p < 0.05$.

DISCUSSION

Linderniaceae have been used for a century as a medicinal plant (Gunatilaka *et al.*, 2015). *Lindernia* species are broadly distributed in moist tropical lands mostly in Asia and African continents (Umakrithika, 2021). This plant family gives many benefits to treating diseases and pain. However, studies on this family of herbs in laboratory work for the preparation of extracts are almost zero or very rarely reported, probably due to the difficulty in sampling. Besides that, the preparation of extract needs to be optimized (Chaves *et al.*, 2020). Based on the potential of herbs and the importance of applications for human use, researchers have extensively studied specific techniques and conditions for the optimization of herbal extraction for analytical, preparatory, or industrial purposes (Zhang *et al.*, 2018). Besides that, the unstable nature of bioactive compounds from herbs requires an individual approach to extraction and optimization during its preparation process (Tusek *et al.*, 2018). The temperature used during extraction may reduce the quality of the extract because high temperatures cause oxidation and degradation of the active compound (Che Sulaiman *et al.*, 2017).

There are many parameters that need to be controlled during the extraction of herbs (Rocha *et al.*, 2011). Based on Fig 1, it was shown that the use of different temperatures and extraction times produced different activities of DPPH inhibition. It was clearly shown that temperatures of 40°C and 60°C produced an extract with lower DPPH inhibition properties compared to temperatures of 80°C and 100°C. Onyebuchi and Kavaz (2020) reported that the extraction temperature has a significant effect on



the phenolic content yield and antioxidant capacity of the herbal extract. Furthermore, extraction time is also a very important parameter by which it can affect the content level of the active substance and the quality of the extract (Claassen *et al.*, 2021). Use of 60°C showed an increase in DPPH inhibition only if the herb was extracted for 3 hours compared to other extraction times of 30 minutes, 1 hour and 2 hours did not increase DPPH inhibition by more than 50%. However, the use temperatures of 80°C and 100°C should not use a long extraction period as it will cause the DPPH inhibition to decrease. It might be due to the use of high temperatures over a long period of time that will damage the active compound in the extract as reported by Kim (2017).

In addition, Fig 2 showed that the temperature and time of extraction can also affect the content of the extract to act as an active ingredient that will be involved in the reduction of the ferric ion (Fe^{3+}) ligand complex to the blue ferrous complex (Fe^{2+}).

This result was parallel with the previous study as reported by Yim *et al.*, (2013). The use of a certain temperature will cause a certain active compound to exist in the extract and in this case, it is the active compound that increases the FRAP value. The use of temperature 40°C with short time extraction showed a lower FRAP value. This indicates that the temperature and extraction time is insufficient to extract the active compound to give a higher value of FRAP. The use of higher temperatures to prepare the extract such as 60°C, 80°C, and 100°C increased the FRAP value. The parameter of 60°C/ 3 hours showed the highest FRAP value compared to the other parameters. Besides that, the parameter of 100°C/ 30 min displayed a second higher FRAP value. However, the results of the FRAP value are not compatible with the TPC value. This indicates that a higher FRAP value did not relate to a higher TPC value as shown in Fig 3.

Moreover, there are various compounds in plant extracts that provide antioxidant effects (Lourenço *et al.*, 2019). There are many soluble compounds in water extract especially ionic compounds which are water-soluble and will have an antioxidant effect (Catena *et al.*, 2020). They will give different effects in the antioxidant test, namely DPPH and FRAP. TPC tests were performed to detect phenolic compounds in the extracts prepared in this study. There are many past studies proving that phenolic compounds give good readings in antioxidant tests. Fig 3 showed that higher temperatures such as 80°C and 100°C produced an extract with a high TPC value compared to a lower temperature which is 40°C and 60°C. The parameter of 100°C/ 30 min has the highest TPC value. Besides that, a temperature of 80°C with a shorter extraction time displayed a higher TPC value compared to a longer extraction time. Besides that, the results of the TPC value are compatible with the MDA level. A higher TPC value causes a lower MDA level as shown in Fig 4.

Furthermore, malondialdehyde (MDA) is a biomarker of oxidative stress-induced lipid peroxidation (Weiss *et al.*, 2014). A compound that can reduce MDA levels is considered a good antioxidant (Saputra *et al.*, 2020). In Fig 4, it was shown that the higher temperature used for extraction produced an extract with high antioxidant activity and effectively reduced MDA level. The lowest MDA level can be detected at the parameter between 60°C/ 30 min to 100°C/ 3 hours. All extract that was produced which used those particular parameters can decrease the MDA level significantly. It indicated that phenolic compound from the extract which prepared at temperature of 60°C, 80°C and 100°C can reduce the peroxidation of egg yolk. Besides that, extraction time must be selected to get an extract which can reduce the MDA level to the minimum level.



From the results of the tests performed in this study, it was suggested that the preparation of *L. polygonoides* water extract can be done based on its usefulness. To obtain an aqueous extract of *L. polygonoides* with the highest inhibitory activity of DPPH and with the highest FRAP value, it must be prepared with a parameter of 60°C / 3 hours. Meanwhile, preparation of the aqueous extract of *L. polygonoides* with the parameter 100°C / 30 min produced the aqueous extract with the highest value of TPC. Finally, preparation of the aqueous extract of *L. polygonoides* using the parameters 60°C / 3 hours, 80°C / 2 hours, and 100°C / 3 hours produced an extract that could lower MDA levels in oxidized egg yolks. MDA results showed that preparation of aqueous extract of *L. polygonoides* at 60°C / 3 hours produced an aqueous extract with stable levels of antioxidant and anti-lipid peroxidation activity.

CONCLUSION

Preparation of aqueous extract of *L. polygonoides* at 60°C / 3 hours produced an aqueous extract with stable levels of antioxidant and anti-lipid peroxidation activity.

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DECLARATION OF CONFLICT OF INTEREST

None.

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