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Preparation of *Alpinia galanga* Water Extract with High Antioxidant Properties

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Abstract: *Alpinia galanga* is one of the most common herbs in Malaysia. Because of its good aroma and taste, *A. galanga* has been used widely for Asian cooking. The objective of this study is to determine the optimum parameter (temperature and period) for *A. galanga* water extract preparation with preservation of its antioxidant property. *A. galanga* was cut, dried and ground into powder. The powder was put into a tube containing water and heated at different temperatures which were 40, 60, 80, and 100°C. Each of temperature, the extract was used with different periods which were 15 min, 30 min, 1 h, 2 h and 3 h. the extract was filtered and used for DPPH, FRAP, TPC and MDA tests to determine its antioxidant value. The result show that *A. galanga* has different antioxidant property when it is prepared with different parameters. It can be concluded that the optimum parameter to prepare *A. galanga* extract with high antioxidant activity is at a the temperature of 80°C with a period of 2 h.

Keyword: Alpinia galangal;antioxidant

INTRODUCTION

Alpinia galanga (L.) Willd. (Zingiberaceae) known as Galanga, is a member of the ginger family, which originated from Southern China and Thailand (Daniel, 2006, Pillai *et al.*, 2018). The rhizome of Galanga traditionally used in Malaysia to treat fever, headache, cough, respiratory illness, stomachache, indigestion, flatulence, laxation, nausea, vomiting, diarrhea, menstrual pain, skin infection, ringworm, afterbirth and postpartum infection (Burkill, 1935, Basri *et al.*, 2017). Galanga has also exhibited numerous pharmacological activities such as anti-microbial, anti-inflammatory, aphrodisiac, eczema, bronchitis, chest pain, dyspepsia, fever, disease, tumors, diabetes and even HIV (Elyani & Risandiansyah, 2017; Verma et al., 2014; Shetty & Monisha, 2015; Ramesh, 2011). Phytochemical screening of Galanga showed the presence of terpenoids, essential oils, phenolic compounds (phenylpropanoids), and flavonoids (Alajmi et al., 2018). *A. galanga* has been reported to contain alpinin, galangin, methyl cinnamate, 1'-acetoxychavicol acetate, 1'-hydroxychavicol acetate, α-humulene, limonene, myrcene, α-pinene, β-pinene, quercetin, kaempferol, quercetin, and 3-methyl ether (Janssen et al., 1985; Nori et al, 1988; De Pooter et al., 1985). A compound isolated from Galanga, 1'S-1'-acetoxychavicol acetate (ACE), was so far the major compound reported with various biological activities (Baradwaj, 2017). An antioxidant is substances or compounds, when presence even in a low concentration, can inhibit

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or delay the oxidation of other substances. Oxidation is the chemical reaction that produces free radicals, leading to the chain reactions that may damage cells, lipids, proteins or DNA (Lyras et al, 1997). When there is an unbalance between free radicals and antioxidants, in favor of the former, a condition called oxidative stress occur. Oxidative stress may result in various health conditions including heart diseases, cancer, stroke, arthritis, Parkinson's disease, and other inflammatory or ischemic conditions (Jenner, 2003; Sayre et al., 2001; Sosa et al., 2013). Several assays have been used to determine the antioxidant activity of plant extracts including DPPH scavenging assay, total phenolics content (TPC) determination, ferric reducing antioxidant power (FRAP), and malondialdehyde (MDA) assays. However, the antioxidant properties of plant extracts can be inconsistent among different assays tested. The inconsistency can be due to the different mechanisms involved in the assay itself. For instance, FRAP assay determines the reducing capability based on ferric ion and DPPH assay determines the scavenging effect of antioxidants towards stable radicals, DPPH. There is no single method that is comprehensive enough to determine the antioxidant properties of plant extracts. Therefore, the use of more than one method is recommended (Prior et al.,2005). Different extraction temperatures and periods may affect the antioxidant properties of plant extract. There were several reports which indicate the effect of different extraction temperatures toward antioxidant properties of plant extracts and natural products (Reblova, 2012; Hossain et al., 2013; Molaveisi et al., 2019). This study was performed to determine the best extraction temperature and extraction period to produce standardized *Alpinia galanga* extract.

PROTOCOL AND METHODS

Crude extraction: First, the root of *A. galanga* was washed and cut into small pieces. Then, *A. galanga* was put into the oven for the drying process at a temperature of 55°C for two days. After that, *A. galanga* was ground into crude powdered form.

Water extraction: 1.77 g of *A. galanga* was weighed by using an electronic balance and put into glass tubes. After that, the glass tube was added with 10 ml of distilled water. Then, it was heated different temperatures (40, 60, 80, 100 °C) for different times (15 min, 30 min, 1 h, 2 h, and 3 h). The extract was filtered and put into a labeled tube.

Diphenyl picrylhydrazil (DPPH) assay: The effect of extract of extracts on DPPH radical was estimated using the method of Yen and Hsieh (1998). The positive controls were BHT (Butylated Hydroxytoluene) and Vitamin C (Ascorbic Acid). The wavelength that was used was 540nm.

Ferric Reducing Antioxidant Power (FRAP) assay: The ferric reducing ability of the plant materials was assessed using the method described by Benzie and Strain (1996). The absorbance was measured spectrophotometrically at 595nm. The final results were expressed as mol Fe (II) equivalent/g sample in the fresh sample.

TPC assay (Total Phenolic Content): The concentration of total phenolic was based on the method described by Velioglu *et*. al. (1998). The absorbance of the reaction was measured at 595nm against a blank.

MDA assay (Malondialdehyde): A modified thiobarbituric acid-reactive species (TBARS) assay was used to measure the lipid peroxide formed, using egg-yolk homogenates as lipid-rich media (Ohkawa *et al*., 1979). 250 µL of Egg homogenate and 50 µL of extract were mixed in a test tube and the volume was made up to 500 µL, by adding distilled water. Finally, 25 µL "FeSO4" (0.07 M) was added to the mixture and incubated for 30 min. Thereafter, 750 µL of 20% acetic acid (pH 3.5) and 750 µL of 0.8% TBA (w/v) (prepared in 1.1% sodium dodecyl sulfate) and 25

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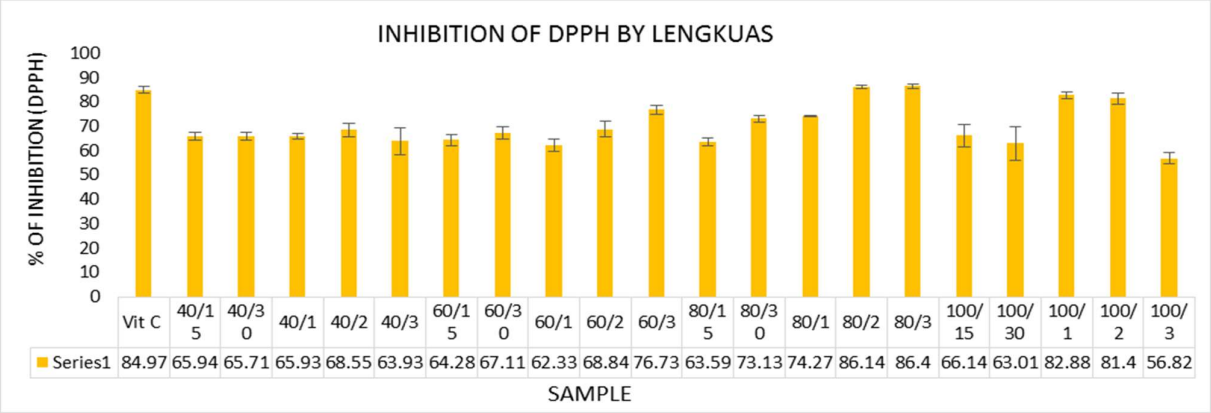
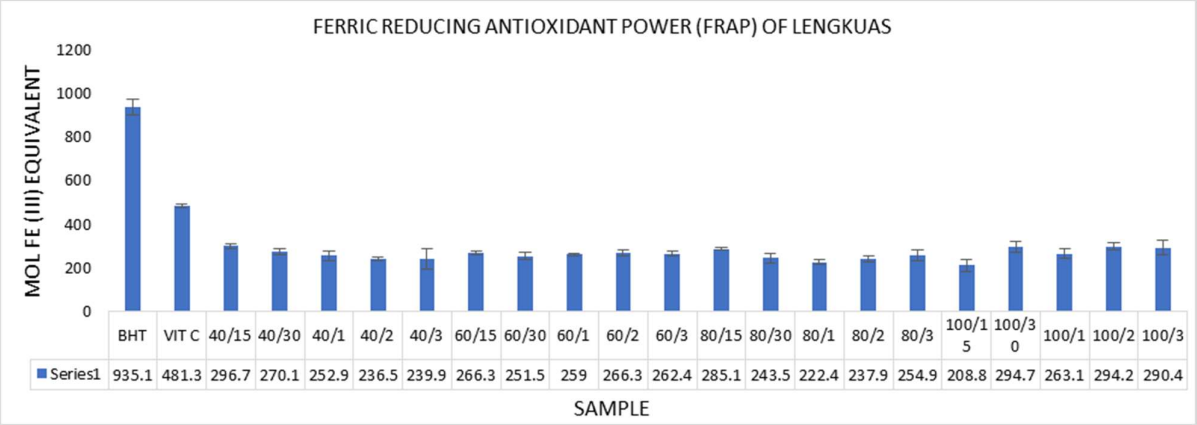
µL 20% TCA was added, vortexed, and then heated in a boiling water bath for 60 min. After cooling, 3.0 mL of 1-butanol was added to each tube and centrifuged at 3000 rpm for 10 min. The absorbance of the organic upper layer was measured against 3 mL butanol at 532 nm.

RESULTS



Graph 1: Based on graph 1, it can be seen that the temperature of 80°C with 2 hours period shows a consistent DPPH inhibition percentage compare to others.



Graph 2: Based on graph 2, it can be seen that all parameter showed lower FRAP value compared to positive control which was BHT and Vitamin C.

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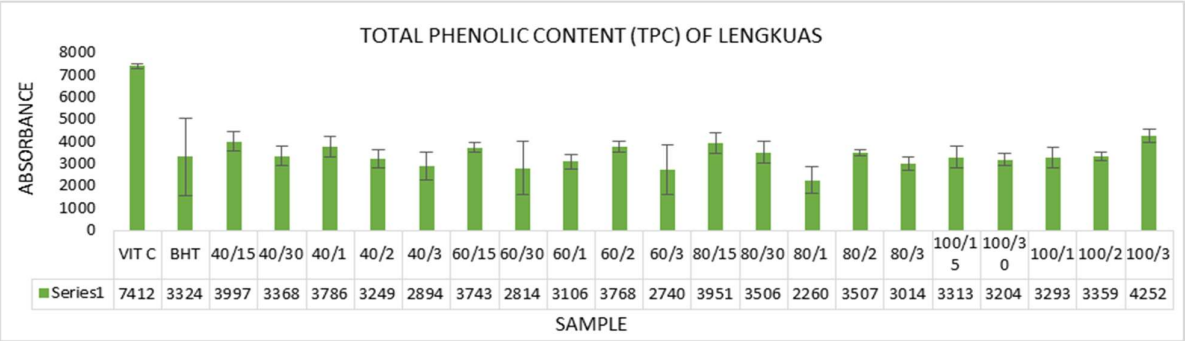
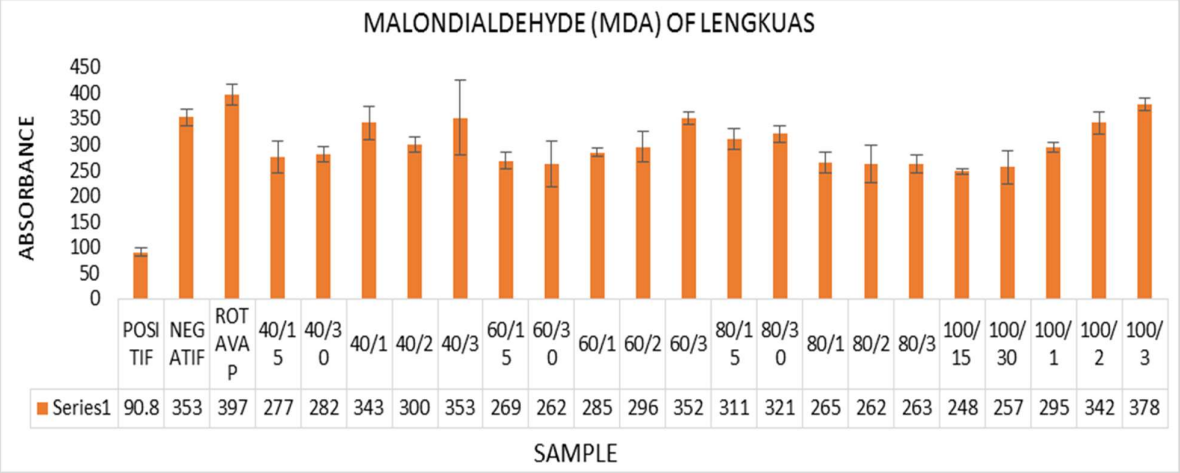


Graph 3: Based on graph 3, it can be seen that all parameter showed lower TPC value compared to positive control BHT. However, there were several parameters were not significant compared with Vitamin C.



Graph 4: Malondialdehyde (MDA), a secondary product of the oxidation of polyunsaturated fatty acids, reacts with two molecules of thiobarbituric acid (TBA), yielding a pinkish-red chromogen with an absorbance maximum at 532 nm (Janero, 1990). Reducing of MDA level showed the sample can decrease the oxidative activity. The lowest MDA level was 100°C/15h. The extract that was prepared using parameter 80°C/2h also showed lower MDA level compared to the negative control.

CONCLUSION

In the DPPH and MDA test, the most effective parameter that showed a higher value of antioxidant activity was 80°C at 2 hours. Besides that, the extract can reduce MDA concentration compared to the negative control.

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DECLARATION OF CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

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