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Preparation of *Cleome gynandra* Linn. (Maman) water extract with high antioxidant properties

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Abstract *Cleome gynandra Linn.* (Maman) is a common herb eaten and used as a medicinal plant by many people around the world. The main objective of this study was to determine the antioxidant activity in various parameters which are temperature (40°C, 60°C, 80°C, and 100°C) and time (30 min, 1hr, 2hrs, and 3hrs) for the preparation of *C. gynandra* water extract. *Cleome gynandra* powder was placed in a tube containing water and heated at different temperatures of 40°C, 60°C, 80°C, and 100°C. For each temperature, the extract was incubated at different intervals of 30 min, 1 h, 2 hrs, and 3 hrs. In this study, four tests such as DPPH, FRAP, TPC, and MDA assay are performed. The results showed that different parameters had different antioxidant activities. Based on the results, the parameter of 80°C for 30 min showed the highest antioxidant activity.

Keywords*: Cleome gynandra Linn*, antioxidant activity, DPPH, FRAP, TPC, MDA assay

Abstrak: *Cleome gynandra Linn*. (Maman) adalah herba yang selalu dimakan dan digunakan sebagai tumbuhan ubatan oleh ramai orang di seluruh dunia. Objektif utama kajian ini adalah untuk menentukan aktiviti antioksida pada pelbagai parameter iaitu suhu (40°C, 60°C, 80°C dan 100°C) dan masa (30 min, 1jam, 2jam dan 3jam) bagi penyediaan ekstrak air *C. gynandra*. Serbuk *Cleome gynandra* dimasukkan ke dalam tabung yang berisi air dan dipanaskan pada suhu yang berbeza iaitu 40°C, 60°C, 80°C, dan 100°C. Bagi setiap suhu, ekstrak dipanaskan pada selang masa yang berbeza 30 minit, 1 jam, 2 jam dan 3 jam. Dalam kajian ini, empat ujian iaitu ujian DPPH, FRAP, TPC dan MDA dilakukan. Keputusan menunjukkan bahawa parameter (suhu dan masa) yang berbeza mempunyai aktiviti antioksidan yang berbeza. Berdasarkan keputusan ujian, parameter pada 80°C selama 30 minit menunjukkan aktiviti antioksidan yang paling tinggi.

Katakunci*: Cleome gynandra Linn*, aktiviti antioksida, DPPH, FRAP, TPC, ujian MDA

INTRODUCTION

Cleome gynandra (*C. gynandra*) is a green leafy vegetable (Figure A, B, and C) known as Maman in Malay (Awang *et al*., 2020). It belongs to the family Cleomaceae (formerly Capparaceae). According to Awang *et al*., (2020), Malay people used *C. gynandra* to treat itchiness. *C. gynandra* was also used to treat many diseases such as protozoal and worm infections, irritable bowel syndrome, and epilepsy (Adhikari and Paul, 2018; Chandradevan *et al*., 2020). Other medicinal uses of *C. gynandra* include the treatment of migraines, stomachache, earache, sepsis, diphtheria, vomiting, postpartum pregnancy, and snakebite (Imanirampa and Alele, 2016). People around the world use *C. gynandra* as a medicinal plant because of its numerous pharmacological properties (Adhikari and Paul, 2018). Current studies show that it has free radical scavenging, antidiabetic, immunomodulatory, anti-inflammatory, and anti-cancerous activities (Adhikari and Paul, 2018).

Besides that, many studies have reported that *C. gynandra* was rich in secondary metabolites such as tannins, saponins, alkaloids, steroids, glycosides, flavonoids, and phenolic (Sowunmi and Afolayan, 2015; Moyo *et al*., 2018). Previous research has identified many secondary metabolites such as β-amyrin, sitosterol, β-amyrin-3-O-β-Glu-copyranoside, and stigmasterol by using NMR and GC-MS (Chandradevan *et al*., 2020; Tiveron *et al*., 2012). The secondary metabolites extracted from *C. gynandra* known as the phenolic compound protect against pathogens and radiation (Pandey *et al*., 2009). Moreover, they are directly related to antioxidant activity. An antioxidant is a substance that can reduce oxidative stress (Tiveron *et al*., 2012; Saeed *et al*., 2012; Sabir *et al*., 2017).Unbalanced reactive oxygen production that occurs in the body is called oxidative stress (Pizzino *et al*., 2017). This condition is constantly occurring in the human body (Chang and Kim, 2018).

Figure 1: *Cleome gynandra*

ASIAN JOURNAL OF PHARMACOGNOSY
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Oxidative stress is a harmful process that can damage certain cell structures, such as membranes, lipids, proteins, lipoproteins, and deoxyribonucleic acid (Kelly, 2003). For example, excess hydroxylic radicals and peroxynitrites can cause lipid peroxidation, damaging cell membranes, and lipoproteins. This in turn will lead to the formation of malondialdehyde (MDA) and conjugated dietary compounds, which are known as cytotoxic and mutagenic. Antioxidants from plants can reduce the hydroxylic radicals and peroxynitrites (Nimse and Pal, 2015). The ability of plant-derived antioxidants to scavenge free radicals can reduce the damage of cells and maintain a more active physiological state (Xiaoji *et al.*, 2020). However, the temperature, extraction time, and solvent used in the extraction process affect the antioxidant concentration and activities in the extract (Spigno *et al.*, 2007).

Determination of the antioxidant activity of plant extracts can be performed through many techniques such as 2,2-diphenyl-1-picrylhydrazyl free radical scavenging assay (DPPH), Total phenol content (TPC), malondialdehyde assays (MDA), and ferric antioxidant-reducing power (FRAP) (Chandradevan *et al.*, 2020; Tiveron *et al.*, 2012).

Therefore, this study was performed to determine the optimum temperature and duration used to prepare the *C. gynandra* extract.

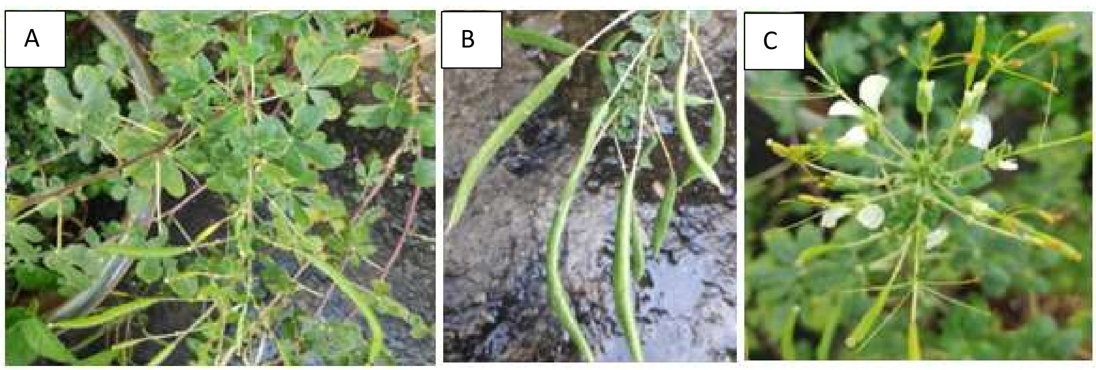

Figure (A): *C. gynandra*, Habit: Herbs or shrubs. Leaf: Alternate, simple or digitately compound; stipules when present minutes or spiny. **Figure (B):** fruit of *C. gynandra***,** Fruit: Oblong or linear capsule, seeds without endosperm and small. **Figure (C):** flower of *C. gynandra*. Flower: Regular or slightly irregular. Sepals and petals are often four. Stamens are four, six, or many. The ovary is typical with one-loculate and gynophore may be long or short.

MATERIAL AND METHODS

Extraction of C. gynandrai: Five milligrams of *C. gynandra* were weighed using an electronic balance and placed in falcon tubes. One milliliter of distilled water was added. The tubes were placed in a water bath and heated at different temperatures (40ºC, 60ºC, 80ºC, and 100ºC) and at different times for each temperature (15 min, 30 min, 1h, 2hrs, and 3hrs). The extract was then centrifuged for 15 min at 2000 rpm. The suspension was kept in a freezer at -20ºC until used.

2,2-Diphenyl-1-picrylhydrazyl (DPPH) Scavenging Assay: DPPH scavenging assay was performed according to Tiveron *et al.*, (2012) with modifications. A DPPH reagent was prepared by mixing 1.77g of DPPH powder in 10 mL methanol. Previously prepared *C. gynandra* extract were taken from the freezer and prepared for DPPH testing. Fifty microliters of *C. gynandra* extract were then mixed with 100 μL of DPPH reagent into a 96-well microplate. The microplate was

ASIAN JOURNAL OF PHARMACOGNOSY
Asian Journal of Pharmacognosy 6(1): 5-13
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incubated for 30 min in the dark. The absorbance of the solution was measured at a 540nm wavelength. Ascorbic acid with a concentration of 5 mg/mL was used as the positive control. The experiments were performed three times.

Ferric Reducing Antioxidant Power (FRAP) assay: FRAP assessment followed the procedure described by Benzie and Strain (1996). The FRAP reagent contained 2.5 mM of 10 mM TPTZ solution in 40 mM HCl, 2.5 mL of 20 mM $FeCl_3 \cdot 6H_2O$, and 25 mM of 300 mM acetate buffer (pH = 3.6). A standard curve was prepared using a concentration of 0.1–2 mmol / l $FeSO_4 \cdot 7H_2O$. The reaction mixture was stirred at 37°C for 30 min and the absorbance was measured at 593 nm using the spectrophotometer. For the *C. gynandra* sample, 30 µL of the prepared extract was placed in a 96-well plate. Then 200 µL of FRAP reagent was added to the microplate and mixed using a microplate mixer for a few minutes. Absorbance was measured spectrophotometrically. The positive control used in this study was Vitamin C (Vit C). The experiments were performed three times.

Total Phenolic Content (TPC) Assay: The TPC of each extract was determined by the Folin-Ciocalteu method following Singleton and Rossi (1965) with some modifications. In short, 20 µL of the water extract was mixed with 10 µL of Folin-Ciocalteu reagent in each microplate well-containing sample and positive control. The mixture was kept for 5 min at 37°C. Then, 40 µL of 7.6% sodium carbonate was added. The microplate was then placed for 2 hours in a dark condition. Subsequently, 765 nm was used to measure the absorbance against the blank.

Malondialdehyde (MDA) Assay: The modified thiobarbituric acid reactive species (TBARS) test was used to measure the lipid peroxide formed, using egg yolk homogeneity as the lipid-rich medium (Upadhyay *et al.*, 2014). Two hundred and fifty microliters of 10% egg homogeneity were placed into a test tube. Then, 50 µL of *C. gynandra* extract was then mixed in the test tube. Distilled water was added to the test tube to make 500 µL. Finally, 25 µL "$FeSO_4$" (0.07 M) was added to the above mixture and incubated for 30 min, for lipid peroxidation induction. 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 was heated in a boiling water bath for 60 minutes and cooled under tap water. Each test tube was then added with 3.0 ml of 1-butane and centrifuged at 3000 rpm for 10 min. The top layer was measured at 532 nm.

RESULTS AND DISCUSSION

The DPPH method is a simple, fast, stable, and sensitive method for determining the antioxidant activity of a particular compound or plant extract (de Torre *et al.*, 2019). DPPH assay was performed to determine the optimum temperature and extraction time for the preparation of *C. gynandra* extract. The scavenging activity of the extracts is a method to determine the antioxidant properties of plants (Rahman *et al.*, 2015). Antioxidant properties from a plant extract contribute to the scavenging activity for free radicals (Irshad *et al.*, 2012). In addition, electron transfer activity or hydrogen donation potential was shown in the DPPH test (Rahman *et al.*, 2015). In this test, DPPH free radicals received hydrogen and were reduced by antioxidants (Kedare and Singh, 2011). In Figure 2, Vit C showed the highest DPPH percentage. This indicated that Vit C showed the maximum DPPH scavenging activity. Besides that, the temperature at 60°C showed the consistent highest DPPH scavenging activity compared to the other temperatures. This indicated that the extract heated at a temperature of 60°C showed a higher hydrogen donation

ASIAN JOURNAL OF PHARMACOGNOSY
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potential. This condition occurred due to the high ability to scavenge free radicals compared to other temperatures (Johari and Khong, 2019).

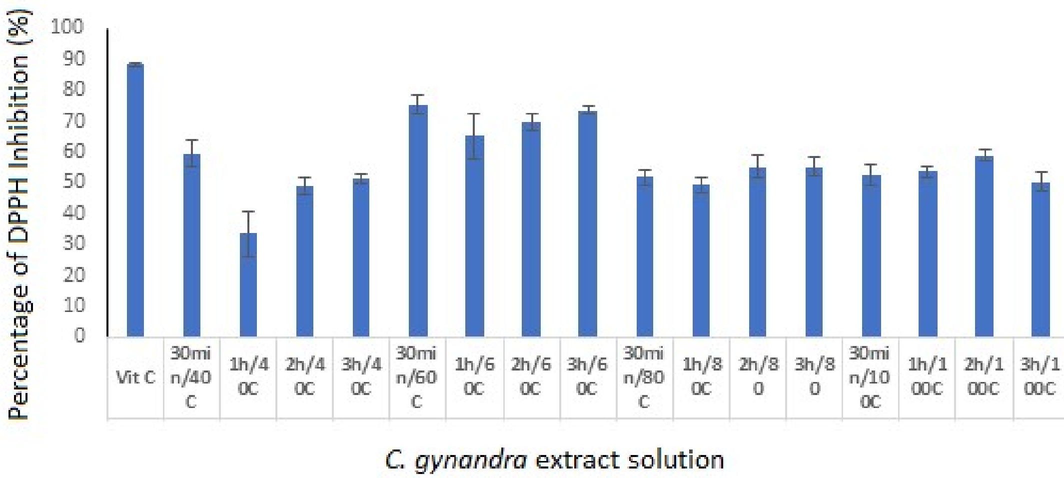

Figure 2: DPPH free radical scavenging activity of *C. gynandra* extract with different temperatures and times.T

The extract heated at temperatures of 80ºC and 100ºC showed a lower DPPH inhibition percentage compare to Vit C. Moreover, Figure 1 showed a consistent highest percentage of DPPH inhibition at an extraction time of 30 min compared to the other times. These results suggested that *C. gynandra* extraction at temperature of 60ºC for 30 min could produce the highest activity of DPPH radical scavenging activity. Based on a previous study, the redox potential of phenolic compounds played a key role in determining the antioxidant potential (Irshad *et al.*, 2012). The components present in the extract contributed to the neutralization of free radicals (Payne *et al.*, 2013; Rahman *et al.*, 2015). In Figure 2, temperature influenced the concentration of electron-donating antioxidants reflected in FRAP assay values.

The reducing power value of *C. gynandra* extract increased as the temperature increased. An extract prepared at temperatures of 80ºC and 100ºC had greater reducing power compared to the extract prepared at temperatures of 40ºC and 60ºC. There was no significant difference in the reducing power of an extract prepared at a temperature of 100ºC with different extraction times. Moreover, Figure 3 showed that all extracts had a lower FRAP value compared to Vit C. The extract prepared using a temperature of 100ºC for 30 min showed the highest value of FRAP value compared to the other extracts.

ASIAN JOURNAL OF PHARMACOGNOSY
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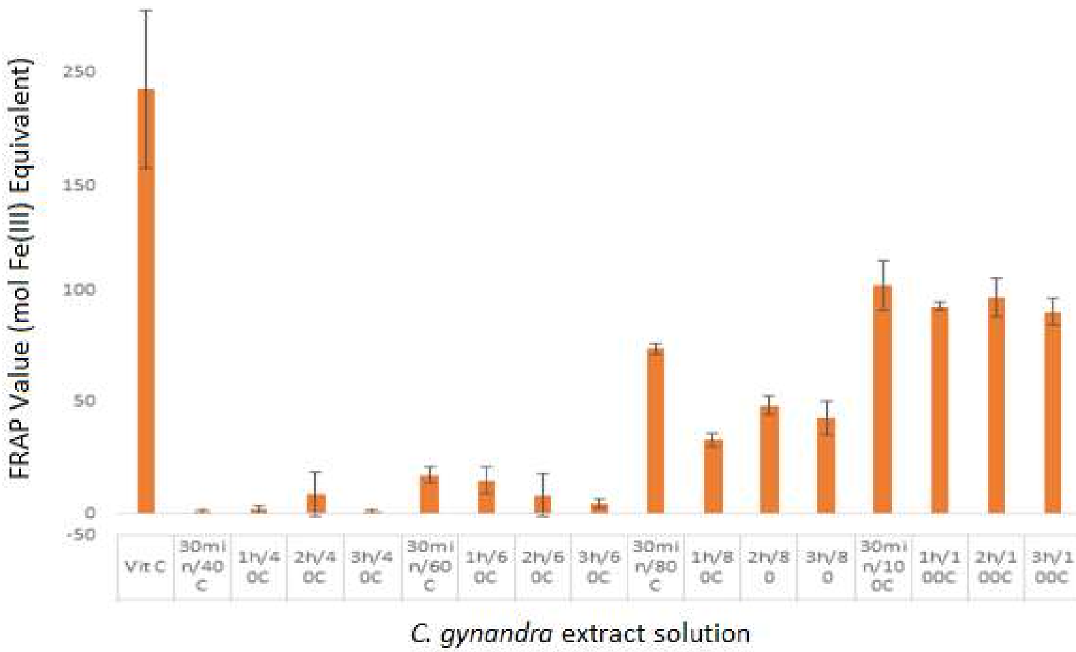

Figure 3: FRAP value of *C. gynandra* extract with different temperatures and times

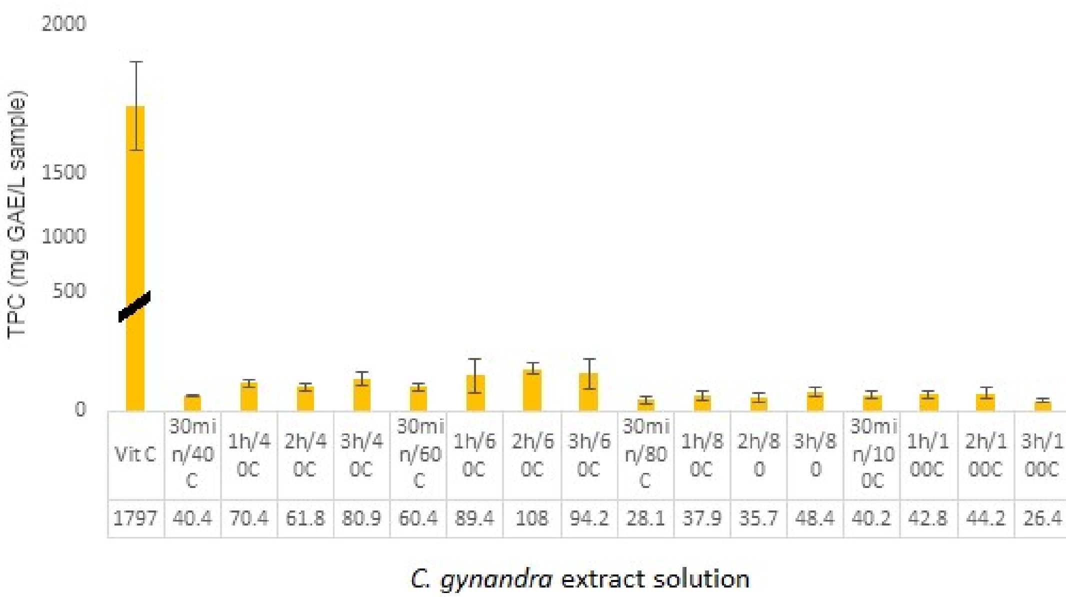

Figure 4: TPC level of *C. gynandra* extract with different temperatures and times.

A large amount of the phenolic compound in an extract could contribute to the higher antioxidant activity (Phuyal *et al*., 2020). The antioxidant activity also depends on the amount and types of phenolic groups that existed in the sample (Parikh *et al*., 2018; Phuyal *et al*., 2020).

ASIAN JOURNAL OF PHARMACOGNOSY
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According to Figure 4, the temperature of 40°C and 60°C showed higher TPC values as compared to the temperature of 80°C and 100°C. It was shown that the temperature of 60°C showed a higher TPC value compared to the other temperatures. Besides that, the lower value of the TPC showed in the extract that was prepared with the temperature of 80°C and 100°C. Moreover, the TPC graph in Figure 3 had a similar pattern to the DPPH graph in Figure 1. The relationship between phenolic content and radical scavenging in the DPPH test was reported earlier by Aryal *et al*., (2019) and Kumar *et al*., (2014). The antioxidant activity of phenolic compounds was associated with the hydroxyl groups linked to the aromatic ring capable of donating hydrogen atoms to neutralize electrons and free radicals (Wan Yahaya *et al*., 2019). Phenolic compounds that existed in the samples had antioxidant properties that allowed them to scavenge the DPPH radical. Moreover, many studies showed that low temperature in the extraction process contributed to good results in antioxidant activity because the phenolic compound structure was maintained and not affected by lower temperature (Johari *et al*., 2019). However, Figure 2 showed that the FRAP value had increased with the temperature increase. This indicated the type of phenolic compound also played a key role in antioxidant activities. The previous study had reported that various polyphenolic compounds exhibited antioxidant activities were due to the reactivity of the phenolic moiety (Parikh *et al*., 2018).

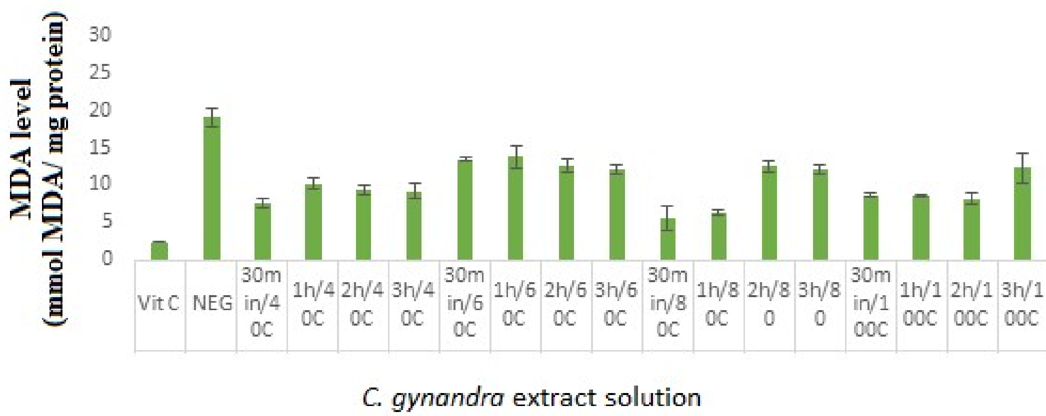

Figure 5: MDA level after treatment of *C. gynandra* extract with different temperature and time

Malondialdehyde (MDA) is a secondary oxidation product of polyunsaturated fatty acids that reacts with two molecules of thiobarbituric acid (TBA) and yields a pinkish-red crust with maximum absorption at 532 nm (Wan Yahaya *et al*., 2019; Onoja *et al*., 2014). In Figure 4, the level of MDA in the group treated with *C. gynandra* extract had decreased compared with the negative control. This condition suggested that treatment of the sample could reduce oxidative activities. In Figure 5, the MDA level graph of each extract treatment showed a similar pattern to the FRAP graph. Temperatures of 80°C and 100°C showed a decrease in MDA level compared to the temperatures of 40°C and 60°C. This suggested that a higher temperature was more effective for the preparation of *C. gynandra* extract. Besides that, treatment of Vit C showed the lowest concentration of MDA which indicated its antioxidant properties and effectiveness to significantly reduce the level of MDA compared to the other treatment. Moreover, the temperature at 80°C was more effective in reducing the level of MDA compared to 100°C. At the temperature of 80°C, the extraction time of 30 min and 1 hour showed the lowest concentration of MDA level. The extracts with higher antioxidant activity might lower the lipid peroxidation process (Zeb & Ullah., 2016).

Therefore, it was concluded that the optimum temperature and time for *C. gynandra* extraction was at 80°C for 30 min. However, it is suggested for further evaluation of antioxidant activities by using other methods such as superoxide (SOD) inhibition assessment or ABTS-free radical assay in the future study.

CONCLUSION

Based on frap and TPC assay, it has been concluded that the temperature of 80°C and the extraction time of 30 min are the best parameters for the preparation of *C. gynandra* extract to produce an extract with high antioxidant activity.

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DECLARATION OF CONFLICT OF INTEREST

No conflict of interest to declare.

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