

Evaluation of Phytochemical and Antioxidant Activity of *Gomphrena celosioides* Mart. Grown in Tien Giang Province, Vietnam

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Gomphrena celosioides Mart. is well known for its medicinal values worldwide. In this study, three extracts, viz. diethyl ether extract (DEE), ethanolic extract (EE) and aqueous extract (AE), were successively obtained from the leaves and stem of *Gomphrena celosioides* to determine the polyphenol and flavonoid content in this plant. A wide variety of pharmacologically active compounds such as alkaloid, flavonoid, terpenoid, saponin, tannin and polyphenol compounds were present in *Gomphrena celosioides*. The results of quantitative determination showed that total polyphenol content of DEE, EE and AE reached 35.35 ± 1.47 , 250.17 ± 2.95 and 133.92 ± 3.17 mgGAE/g, respectively. Moreover, total flavonoid content of the DEE, EE and AE was 23.21 ± 1.87 , 50.74 ± 2.32 and 27.25 ± 1.34 mgQE/g, respectively. In comparison with DEE and AE, the ethanolic extract exhibited the highest DPPH ($IC_{50} = 13.29 \pm 0.10$ μ g/mL) and ABTS ($IC_{50} = 6.3 \pm 0.11$ μ g/mL).

Keywords: *Gomphrena celosioides* Mart, Phytochemicals, Total polyphenol, Total flavonoid, Antioxidant activity.

INTRODUCTION

In recent years, natural products derived from plants, animals, microorganisms and marine organisms play a vital role in traditional medicines [1-9]. *Gomphrena celosioides* Mart. is an herbal plant well known for its use as a common ingredient in traditional medicine. *Gomphrena celosioides* belongs to the Amaranthaceae family, which is geographically distributed in tropical and temperate regions [10-13]. The previous phytochemical investigations made on the plant have shown that they possess a wide variety of compounds like flavonoids, saponins, hydrocarbons, alcohols, steroids and terpenoids. In recent years, cultivating *Gomphrena celosioides* have been receiving a great deal of public attention due to practical applications of essential oil extracted from its leaves

and stems [14]. Moreover, the previous study shows that *G. celosioides* possesses a number of pharmacological important activities such as antimalarial, anti-inflammatory, antimicrobial, antioxidative activities, etc. [15-19]. In addition, flavonoids have been highlighted for their role as the key ingredients responsible for the activity against reactive oxygen species [20-22]. Because flavonoids and polyphenols could not be synthesized in the human body, the intake of such compounds from plants is crucial for normal function of human body. However, relatively little is explored about extraction and separation processes of polyphenols and flavonoids from *Gomphrena celosioides* plant.

Phenolic compounds play an essential role in dietary applications and have been extensively studied. Antioxidants play an essential role in the human protection body against free radical

disorders acting as radical scavengers [23-25]. Phenolics belongs to a class of chemical compounds including simple phenols and polyphenols. Polyphenols can reduce and prevent damage to the human body through the promotion of free radicals. In addition, flavonoids can provide mechanisms that may inhibit invasion and kill tumor cells. The present study was carried out to evaluate phytochemical screening, total polyphenol, flavonoids content and antioxidant activity on DPPH and ABTS of the extract of *G. celosioides* grown in Tien Giang province, Vietnam.

EXPERIMENTAL

Leaves and stems of *Gomphrena celosioides* were obtained from Tien Giang province, Vietnam in January 2019. The samples were washed and dried under shade at 40 °C to remove the water content. The dried samples were ground into fine powders before being subject to extraction. The extraction flowchart procedure is shown in Fig. 1. Briefly, powder sample (100 g) was extracted with 1 L of diethyl ether at 25 °C for 24 h and then concentrated *via* vacuum evaporation to obtain diethyl ether extract (DEE) of *Gomphrena celosioides*. The residue was further extracted with 1 L of 99.5 % ethanol and 1 L of distilled water by using the same procedures as above to produce ethanolic extract (EE) and aqueous extract (AE), respectively.

Total polyphenol content (TPC): TPC was carried out by method of Chandra *et al.* [26]. First, 0.5 mL extract was pipetted with 2.5 mL Folin-Ciocalteu reagent 10 % (v/v). After 5 min, 2 mL Na₂CO₃ 7.5% (w/v) was mixed to the sample. Next, the mixture was vigorously shaken and hatched for 30 min in the dark. Gallic acid acts as a standard. Finally, the absorbance was spectrophotometrically measured at 765 nm.

Total flavonoid content (TFC): TFC was determined by aluminum chloride colorimetric method [27]. Briefly, 0.5 mL of extract was added with 0.1 mL 10 % AlCl₃. Then, 0.1 mL 1M CH₃COOK and 4.3 mL distilled water was combined and vigorously shaken. Quercetin acts as a standard. The absorbance was spectrophotometrically measured at 415 nm.

DPPH scavenging activity: The antioxidant activity of the individual essential oil was tested using 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay with 600 µL of DPPH (O.D. 517 nm = 0.0403 ± 0.013) into 500 µL solution sample. The sample solution with a specified concentration was added to DPPH solution and allowed to stabilize at room temperature in the dark within 37 min. The optical measurement of mixture by UV/VIS-1800 Shimadzu spectrometer at 517 nm. The blank was 500 µL solution replaced with ethanol 99.7 %. Standard sample: vitamin C (0.1 g) was dissolved EtOH 99.7 % into volume flask 100 mL in dark (C = 100 µL/mL). The percent DPPH scavenging effect was calculated as follows:

$$\text{DPPH scavenging activity (\%)} = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100$$

ABTS scavenging activity: First, 10 mL of 2.6 mM K₂S₂O₈ was added into 10 mL of 7.4 mM ABTS solution in 15 h. Next, the working solution is made by combining 1 mL of stock solution and 60 mL of methanol, which take the absorbance value of 1.1 ± 0.02 at 734 nm. Then, 0.5 mL of sample was mixed with 1.5 mL of working solution for 30 min at room temperature. A UV-VIS spectrophotometer was used to measure the mixture at 734 nm. The percentage of ABTS decolorization of the sample was determined as follows:

$$\text{ABTS scavenging activity (\%)} = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100$$

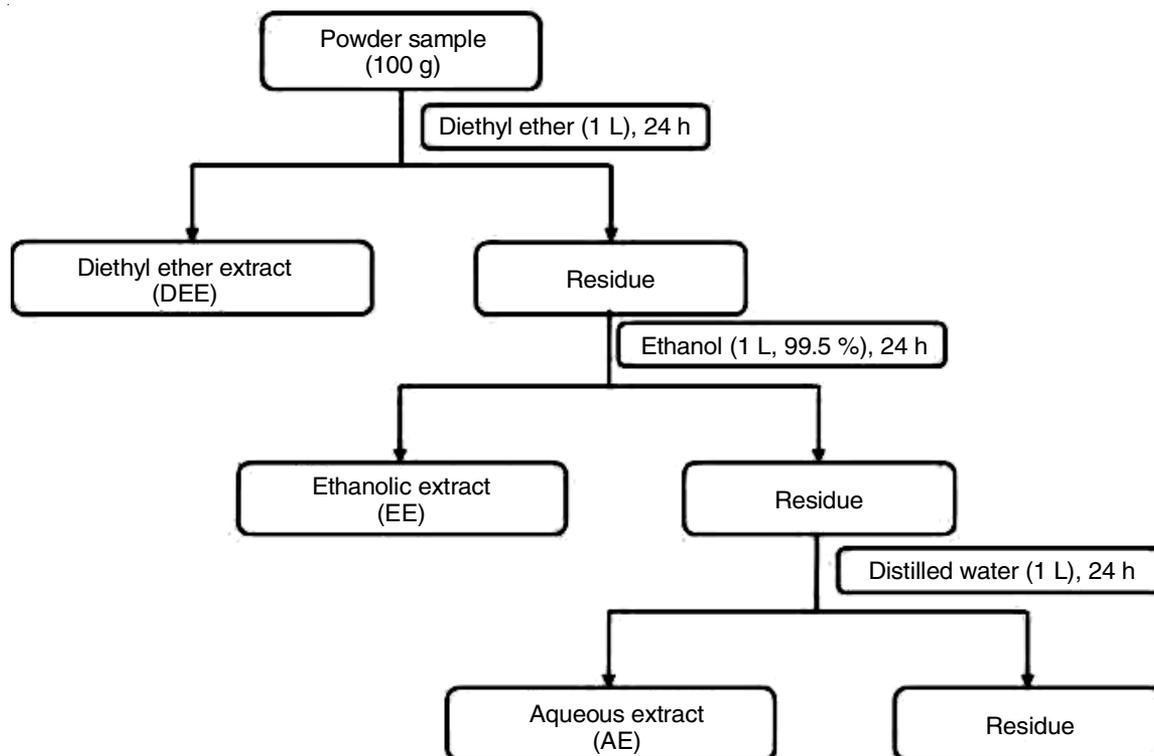


Fig. 1. Extraction scheme of the leaves and stem extracts from *G. celosioides*

Statistical analyses: All determinations were carried out in triplicate. One-way analysis of variance (ANOVA) was performed using SPSS 15 (SPSS Inc. Chicago, U.S.A) and differences between samples were compared using Tukey's test ($p < 0.05$).

RESULTS AND DISCUSSION

Phytochemical analysis: Table-1 illustrates the preliminary phytochemicals screening of extracts from *Gomphrena celosioides* leaves and stems, which were found to be composed of alkaloid, flavonoid, terpenoid, saponin, tannin and polyphenol compounds. The bioactivities of flavonoids and polyphenols have been highlighted in previous studies [28,29], where antimicrobial, anticancer, antiallergic and α -glucosidase inhibition were suggested to be the prominent properties of those compounds. In addition, other interesting bioactivities such as anti-inflammatory, antidiabetic, antioxidant activities, wound healing properties, protection of skin, health promotion and disease prevention are also reported for secondary metabolites from plants. Nowadays, isolation and structural determination phytochemicals are gaining increasing interest because such compound are not only used directly as drugs for the treatment of disease but also as modeling compounds for the discovery of new drugs having reduced toxicity and side effects to humans.

Phytochemical class	Diethyl ether extract	Ethanollic extract	Aqueous extract
Alkaloids	Present	Present	Present
Oils	Absent	Absent	Absent
Tannins	Absent	Present	Present
Flavonoids	Present	Present	Present
Terpenoids	Present	Present	Absent
Saponins	Absent	Present	Present
Polyphenols	Present	Present	Present
Courmarins	Absent	Absent	Absent

TPC and TFC in different fractions: In plants, phenolic compounds principally act as secondary metabolites, which play an essential function in antioxidant activity and stimulating the activity of these extracts [30]. Fig. 2 illustrates the level of phenolic compounds in diethyl ether extract, ethanolic extracts and aqueous extracts of *Gomphrena celosioides* leaves. The total polyphenol content in ethanolic extract is the highest (250.17 ± 2.95 mgGAE/g), followed by diethyl ether (35.35 ± 1.47 mgGAE/g) and aqueous extract (133.92 ± 3.17 mg GAE/g). These results indicated that leaves and stem of *Gomphrena celosioides* contains a large number of polyphenol compounds and the extraction solvent influenced TPC. Flavonoids is one

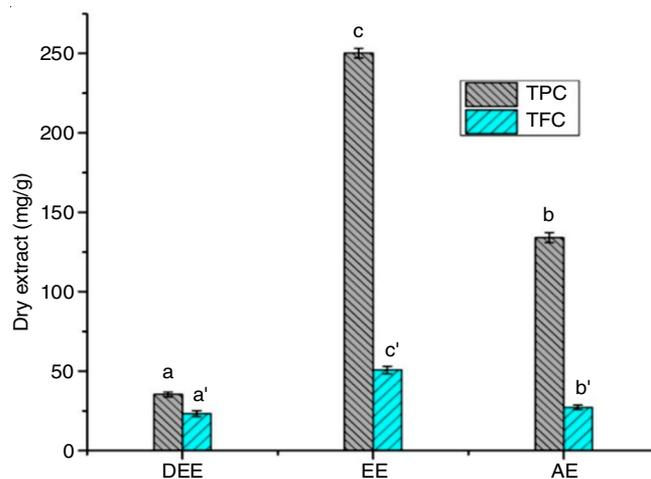


Fig. 2. Total polyphenol contents and total flavonoid contents in *G. celosioides* leaves and stem extracts

of secondary plant metabolites. These metabolites are mainly present in the plant to generate yellow pigments. Moreover, flavonoids are often consumed by humans and they play a major function in antiallergic, anticancer and anti-inflammatory activities [23]. The total flavonoid content is highest in the ethanolic extract (50.74 ± 2.32 mgQE/g), followed by diethyl ether extract (23.21 ± 1.87 mgQE/g) and aqueous extract (27.25 ± 1.34 mgQE/g).

DPPH radical cation scavenging activity: There are different techniques for estimating the antioxidant activity of both synthetic and natural compounds. The DPPH scavenging assay is broadly applied to determine the free radical scavenging of plant extracts thanks to its sensitivity, simplicity, and ease of perform. Antioxidants can remove the radical by hydrogen donation, which results in a decrease of DPPH absorbance at 517 nm. Fig. 3 illustrates the DPPH radical scavenging ability in DEE, EE and AE fractions of *G. celosioides*. Ethanolic extract showed the lowest DPPH radical scavenging measured by IC_{50} (13.29 ± 0.10 μ g/mL), while the highest IC_{50} belonged to DEE (335.88 ± 2.02 μ g/mL). The results highlight the high antioxidant activity from *G. celosioides* extracts, especially EE (Table-2).

ABTS radical cation scavenging activity: Proton radical scavenging is an essential characteristic of antioxidants. ABTS acts as a protonated radical, which has a characteristic maximum at 734 nm. ABTS plays a vital role in the antioxidant capacity of hydrogen-donating antioxidants. Color reduction shows the decrease of ABTS radical. Fig. 4 illustrates the ABTS radical scavenging ability in DEE, EE and AE fractions of the leaves and stem from *G. celosioides*. In this study, EE showed the lowest IC_{50} of ABTS radical scavenging (6.3 ± 0.11 μ g/mL), while DEE showed the highest IC_{50} (185.3 ± 3.04 μ g/mL) (Table-2).

Sample	Extraction yields (%)	TPC (mg GAE/g)	TFC (mg QE/g)	IC_{50} value (μ g/mL)	
				DPPH	ABTS
Diethyl ether extract	0.85 ± 0.06^a	35.35 ± 1.47^a	23.21 ± 1.87^a	335.88 ± 2.02^d	185.3 ± 3.04^d
Ethanolic extract	4.00 ± 0.23^b	250.17 ± 2.95^c	50.74 ± 2.32^c	13.29 ± 0.10^b	6.3 ± 0.11^b
Aqueous extract	16.10 ± 0.87^c	133.92 ± 3.17^b	27.25 ± 1.34^b	149.24 ± 2.49^c	114.3 ± 0.23^c
Ascorbic acid	—	—	—	4.80 ± 0.00^a	2.66 ± 0.01^a

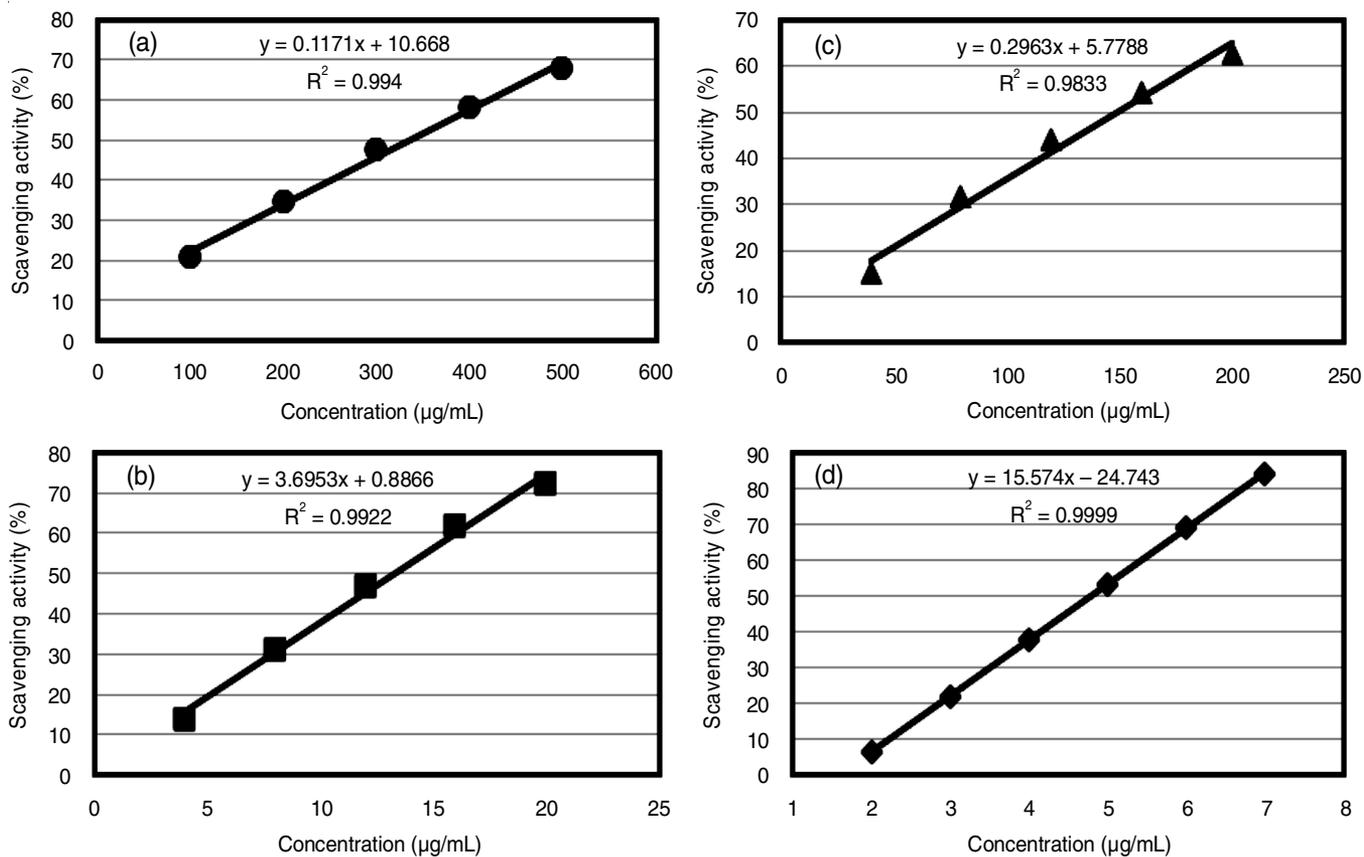


Fig. 3. DPPH radical scavenging activity of different extracts from the leaves and stem extracts of *G. celosioides*. (a) diethyl ether extract (DEE), (b) ethanolic extract (EE), (c) aqueous extract (AE) and (d) ascorbic acid (AA)

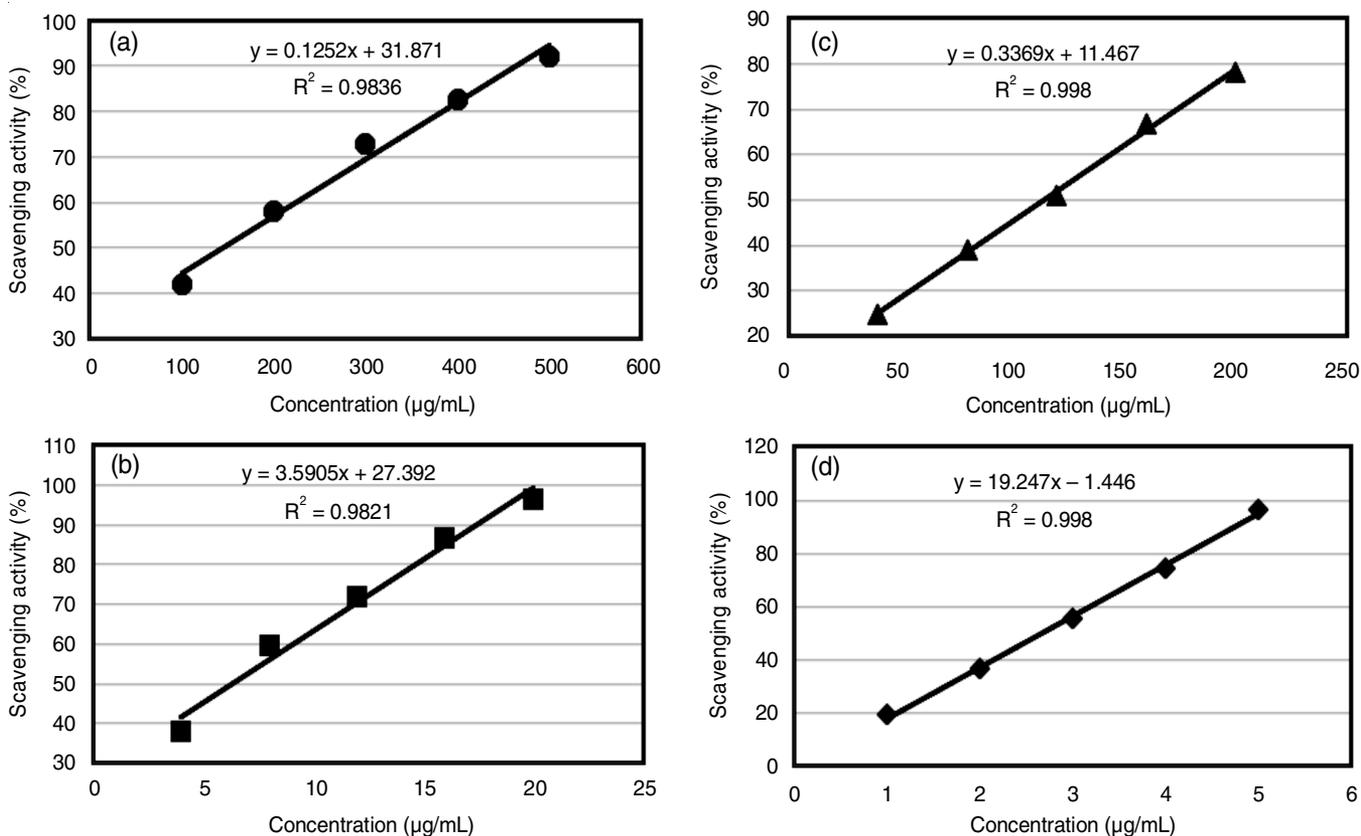


Fig. 4. ABTS radical scavenging activity of different extracts from the leaves and stem extracts of *G. celosioides*. (a) diethyl ether extract (DEE), (b) ethanolic extract (EE), (c) aqueous extract (EA) and (d) ascorbic acid (AA)

Conclusion

In this study, TPC, TFC and antioxidant activity of the leaves and stems of *Gomphrena celosioides* were investigated. Total polyphenol content (250.17 ± 2.95 mgGAE/g), Flavonoid (50.74 ± 2.32 mgQE/g), DPPH ($IC_{50} = 13.29 \pm 0.10$ μ g/mL) and ABTS ($IC_{50} = 6.3 \pm 0.11$ μ g/mL) radical scavenging activity were observed in aqueous leaf extracts of *G. celosioides*. To be specific, three compounds obtained from *G. celosioides* including diethyl ether extract (TPC achieved 35.35 ± 1.47 mgGAE/g; TFC achieved 23.21 ± 1.87 mgQE/g), ethanolic extract (TPC achieved 250.17 ± 2.95 mgGAE/g; TFC achieved 50.74 ± 2.32 mgQE/g) and the aqueous extract (TPC achieved 133.92 ± 3.177 mgGAE/g; TFC achieved 27.25 ± 1.34 mgQE/g). These results suggest that *Gomphrena celosioides* could serve as an inexpensive and abundant source of natural antioxidant for food industries.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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