การประชุมเสนอผลงานวิจัยระดับบัณฑิตศึกษา มหาวิทยาลัยสุโขทัยธรรมาธิราช ครั้งที่ 2
The 2nd STOU Graduate Research Conference

คุณสมบัติต้านออกซิเดชั่นของสารสกัดเอทานอลจากจักรนารายณ์ (*Gynura Divaricata* (L.) DC.

Antioxidant Properties of the Ethanolic Extract from *Gynura Divaricata* (L.) DC.

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บทคัดย่อ

จักรนารายณ์ (*Gynura divaricata*, Family Asteraceae) เป็นพืชสมุนไพรชนิดหนึ่งที่นิยมใช้ในประเทศไทย เพื่อบรรเทาอาการผิดปกติหรือโรคต่าง ๆ เช่น โรคเบาหวาน โรคความดันโลหิตสูง โรคหัวใจ โรคภูมิแพ้ โรคหอบหืด โรคมะเร็ง โรคอ้วน โรคหลอดเลือดหัวใจ และอาการผิดปกติทางช่องท้อง เป็นต้น ปัจจุบันเชื่อว่า oxidative stress มีบทบาทสำคัญต่อการเกิดโรคและอาการผิดปกติต่าง ๆ ของร่างกาย ดังนั้นการศึกษาในครั้งนี้จึงมีวัตถุประสงค์เพื่อ (1) ศึกษาคุณสมบัติต้านออกซิเดชั่นของสารสกัดเอทานอลจากจักรนารายณ์ ซึ่งประกอบด้วยการขจัดอนุมูล $ABTS^{+\bullet}$ อนุมูลซุปเปอร์ออกไซด์แอนไอออน และอนุมูลไฮดรอกซิล และการป้องกันการเกิดภาวะลิปิดเปอร์ออกซิเดชั่น (2) หาปริมาณรวมของสารฟีโนลิคในสารสกัดเอทานอลจากจักรนารายณ์

การศึกษาทำโดยนำใบสดของจักรนารายณ์มาล้างให้สะอาดและบดให้ละเอียด แล้วสกัดด้วยการหมักกับ 95% เอทานอลเป็นเวลา 2 วันที่อุณหภูมิห้อง จากนั้นกรอง ระเหยเอาเอทานอลออก และทำให้แห้ง จะได้เป็นสารสกัดเอทานอลจากจักรนารายณ์ (GDE) การศึกษาคุณสมบัติต้านออกซิเดชั่นของ GDE โดยการขจัดอนุมูล $ABTS^{+\bullet}$ อนุมูลซุปเปอร์ออกไซด์แอนไอออน อนุมูลไฮดรอกซิล และการป้องกันการเกิดภาวะลิปิดเปอร์ออกซิเดชั่นของ GDE จะใช้วิธีของ Re *et al.* (1999) Nishimiki *et al*. (1972) Halliwell *et al*. (1987) และ Masao *et al.* (1993) ตามลำดับ รวมทั้งการหาปริมาณรวมของสารฟีโนลิคใน GDE ใช้วิธีของ Hammerschmidt และ Pratt (1978)

ผลการศึกษาพบว่า การสกัดใบจักรนารายณ์ได้ GDE ได้ปริมาณ 2.34% และมีคุณสมบัติต้านออกซิเดชั่น โดยมีค่า EC_{50} ของความสามารถในการขจัดอนุมูล $ABTS^{+\bullet}$ อนุมูลซุปเปอร์ออกไซด์แอนไอออน อนุมูลไฮดรอกซิล และการป้องกันการเกิดภาวะลิปิดเปอร์ออกซิเดชั่น เท่ากับ 0.476 ± 0.017, 0.539 ± 0.006, 0.014 ± 0.001 และ 16.134 ± 0.461 มิลลิกรัม/มิลลิลิตร ตามลำดับ ความสามารถในการต้านออกซิเดชั่นของ GDE คำนวณได้จากการเปรียบเทียบกับสารมาตรฐาน (Trolox, gallic acid) ในค่าของ TEAC และ GAE และแสดงผลเป็นกรัมของ GDE ต่อมิลลิกรัมของสารมาตรฐาน ค่า TEAC และ GAE ของ GDE ในการขจัดอนุมูล $ABTS^{+\bullet}$ อนุมูลซุปเปอร์ออกไซด์แอนไอออน และอนุมูลไฮดรอกซิล เท่ากับ 764.74 ± 8.24, 0.0021 ± 0.00005 และ 998.25 ± 28.37 ตามลำดับ จากผลการทดลองแสดงให้เห็นว่า GDE มีคุณสมบัติเป็นสารต้านออกซิเดชั่น นอกจากนี้ยังพบว่า GDE มีสารฟีโนลิคเป็นองค์ประกอบ โดยมีค่า GAE เท่ากับ 0.017 ± 0.0003 ซึ่งเป็นที่ทราบกันดีว่าสารประกอบฟีโนลิคเป็นสารต้านออกซิเดชั่นที่สำคัญชนิดหนึ่ง ดังนั้นการศึกษานี้จึงแสดงให้เห็นว่า สารสกัดจากจักรนารายณ์อาจเป็นสารต้านออกซิเดชั่นที่ได้จากธรรมชาติชนิดหนึ่ง ซึ่งสามารถนำมาพัฒนาเป็นยาสมุนไพร หรืออาหารเสริม ก่อให้เกิดประโยชน์ต่อการดูแลรักษาสุขภาพได้ต่อไปในอนาคต

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Abstract

Gynura divaricata (Jakr-Na-Rai, Family Asteraceae) is popularly used in Thailand for alleviating various diseases and disorders such as diabetes, high blood pressure, heart disease, allergies, asthma, cancer, obesity, atherosclerosis and stomach disorders. Oxidative stress is now been suggested to play roles in many diseases and disorders. The present study was carried out with the following objectives: (1) to investigate antioxidant properties of the ethanolic extract from *G. divaricata* which including radicals scavenging activity ($ABTS^{+\bullet}$, superoxide anion, hydroxyl) and anti-lipid peroxidation activity; (2) to determine the total phenolic contents of the ethanolic extract from *G. divaricata*.

Fresh leaves of *G. divaricata* were washed, sliced thinly, ground with 95% ethanol, macerated for 2 days at room temperature and then filtered, concentrated, and dried to yield the ethanolic extract from *G. divaricata* (GDE). The GDE was assayed for radicals scavenging activity which included $ABTS^{+\bullet}$, superoxide anion, and hydroxyl radicals scavenging activity using methods described by Re *et al.* (1999), Nishimiki *et al*. (1972), and Halliwell *et al*. (1987), respectively. Anti-lipid peroxidation activity of the GDE was evaluated according to the method of Masao *et al.* (1993). Additionally, the phenolic content in the GDE was determined by following the method of Hammerschmidt and Pratt (1978).

The yield of the GDE was 2.34%. The GDE showed $ABTS^{+\bullet}$, superoxide anion, and hydroxyl radicals scavenging activity with EC_{50} values of 0.476 ± 0.017, 0.539 ± 0.006, and 0.014 ± 0.001 mg/mL, respectively. The EC_{50} of anti-lipid peroxidation activity was found to be 16.134 ± 0.461 mg/mL. The activities were calculated in terms equivalent to the standards (Trolox, gallic acid) used and expresses as g of GDE per mg of standard. The $ABTS^{+\bullet}$, superoxide anion, hydroxyl radicals scavenging activity showed TEAC value of 764.74 ± 8.24, GAE value of 0.0021 ± 0.00005, and TEAC value of 998.25 ± 28.37, respectively. The results obtained suggest that the GDE possesses antioxidant properties. The GDE showed the presence of phenolic contents with the GAE value of 0.017 ± 0.0003. It is likely that the phenolic compounds play roles in its antioxidant activities. Thus, the present study indicates that *G. divaricata* is possibly be the potential sources for natural antioxidants to be further developed which will be beneficial as medicinal plant products, food supplements or nutraceuticals in healthcare system.

Keywords: Antioxidant properties, Gynura Divaricata, Radicals scavenging activity, Phenolic contents

INTRODUCTION

Reactive free radicals (ROS) such as hydroxyl radical ($^{\bullet}OH$), superoxide anion radical ($O_2^{\bullet-}$), and hydrogen peroxide (H_2O_2), are unstable molecules and generated in living organisms through numerous metabolic pathways. The oxidative stress caused by ROS can contribute to various diseases such as aging, arthritis, cancer, and heart diseases. (Meerson *et al.*, 1982; Busciglio and Yankner, 1995; Abe and Berk, 1998). Antioxidants may protect cells from the damage caused by oxidative stress by stabilizing the free radicals.

A Chinese medicinal plant: *Gynura divaricata* (L.) DC. (Family Asteraceae) is used in Chinese medicine for the treatment of many diseases such as bronchitis, pulmonary tuberculosis, pertussis, sore eye, toothache, rheumatic arthralgia, peptic ulcer, hypertension, diabetes and cancer in folk medicine (Chen *et al.*, 2009). The plant is known as Jakr-Na-Rai (Figure 1) and popularly used in Thailand. Leaves and fresh stems are used for treating diabetes, high blood pressure, heart disease, allergies, asthma, cancer, obesity, atherosclerosis, stomach disorders and kidney stones (Promrungraeng, 2007). The chemical constituents of *G. divaricata* leaves include flavonoids, phenolics, cerebrosides, polysaccharide, alkaloids, terpenoids, and sterols (Chen *et al.*, 2003; Roeder *et al.*, 1996). It has been reported that *G. divaricata* exhibited a free radical-scavenging capacity by using 1,1,-diphenyl-2-picryl-hydrazil (DPPH) assay and phosphomolybdenum method, and that phenolics and flavonoids are the major antioxidant components (Wan *et al.*, 2011).

Figure 1 *Gynura divaricata* (L.) DC., Family Asteraceae

PURPOSES OF THE STUDY

The present study was carried out to evaluate the ethanolic extract from *G. divaricata* for antioxidant properties by assaying for free radicals (2,2'-azino-bis 3-ethylbenzthiazoline-6-sulfonic acid: $ABTS^{+\bullet}$, superoxide anion, and hydroxyl) scavenging activity, and anti-lipid peroxidation activity. Additionally, the ethanolic extract from *G. divaricata* was determined for phenolic contents.

MATERIALS AND METHODS

Materials

1. 2,2'-azino-bis 3-ethylbenzothiazoline-6-sulfonic acid (ABTS) (Sigma-Aldrich, Steinheim, Germany)
2. Potassium persulfate ($K_2S_2O_8$) (Sigma-Aldrich, Steinheim, Germany)
3. 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) (Sigma-Aldrich, Steinheim, Germany)

4. Nitro blue tetrazolium (NBT) (Sigma-Aldrich, Steinheim, Germany)
5. *β*-nicotinamide adenine dinucleotide (NADH) (Sigma-Aldrich, Steinheim, Germany)
6. Phenazine methosulphate (PMS) (Sigma-Aldrich, Steinheim, Germany)
7. Gallic acid (Sigma-Aldrich, Steinheim, Germany)
8. 2-deoxy-D-ribose (Sigma-Aldrich, Steinheim, Germany)
9. Hydrogen peroxide (H_2O_2) (Merck, Darmstadt, Germany)
10. Ferric chloride ($FeCl_3$) (Sigma-Aldrich, Steinheim, Germany)
11. Nitrilotriacetic acid (NTA) (Sigma-Aldrich, Steinheim, Germany)
12. 2-thiobarbituric acid (TBA) (Fluka, Switzerland)
13. Trichlorotic acid (TCA) (Sigma-Aldrich, Steinheim, Germany)
14. Bovine serum albumin (BSA) (Invitrogen™, Germany)
15. Ferrous chloride tetrahydrate (Sigma-Aldrich, Steinheim, Germany)
16. Ascorbic acid (Sigma-Aldrich, Steinheim, Germany)
17. Acetic acid (Sigma-Aldrich, Steinheim, Germany)
18. *n*-butanol (Lab Scan Asia CO., Bangkok, Thailand)

Preparation of the ethanolic extract from *Gynura divaricata*

The fresh leaves of *G. divaricata* obtained from Chiang Mai province, were prepared as an ethanolic extract and the steps involved in the extraction procedure is shown as diagram in Figure 2. The yield of the ethanolic extract from *G. divaricata* (GDE) was 2.34%. The GDE was dissolved in deionized water before used.

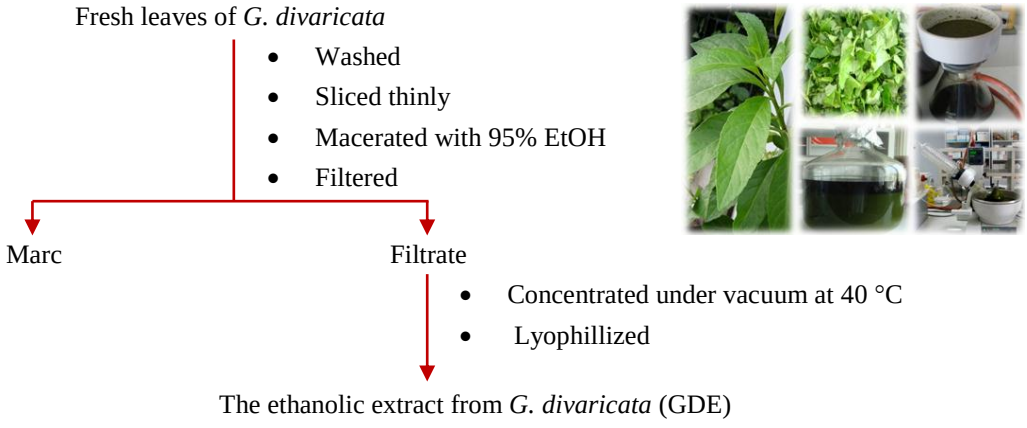

Figure 2 Steps involved in the preparation of the ethanolic extract from *G. divaricata* (GDE)

Evaluation of the GDE for antioxidant properties

1. $ABTS^{+\bullet}$ (2,2'-azino-bis 3-ethylbenzthiazoline-6-sulfonic acid) radical scavenging activity

The method described by Re *et al.* (1999) with some modifications was used. The $ABTS^{+\bullet}$ reagent was prepared by mixing 5 mL of 7 mM ABTS with 88 µL of 140 mM $K_2S_2O_8$. After the mixture was kept in the dark at room temperature for 16 h to allow the completion of radical generation, it was diluted with deionized

water so that its absorbance was adjusted to 0.70 ± 0.05 at 734 nm. To determine the scavenging activity, 1 mL ABTS reagent was mixed with 10 µL of the GDE and the absorbance was measured at 734 nm 6 min after the initial mixing. Trolox, a water soluble derivative of vitamin E, was employed as a positive control.

2. Superoxide anion ($O_2^{-\bullet}$) scavenging activity

Measurement of superoxide anion scavenging activity of the GDE was performed by following the method described by Nishimiki *et al*. (1972). Briefly, the nitroblue tetrazolium (NBT), nicotinamide adenine dinucleotide (NADH) and phenazine methosulphate (PMS) solution at concentrations of 156 µM, 468 µM and 60 µM, respectively were prepared in 0.1 M phosphate buffer at (pH 7.4). The 1 mL of NBT solution, 1 mL of NADH solution, and 0.1 mL of the GDE were mixed. The reaction was started by adding 0.1 mL of PMS solution to the mixture. After 5 min of incubation at room temperature, the absorbance was measured at 560 nm. Gallic acid was used as a positive control.

3. Hydroxyl radical ($OH^{\bullet}$) scavenging activity

Scavenging of hydroxyl radicals ($^{\cdot}OH$) was determined by the method of Halliwell *et al*. (1987). The solutions of 2.8 mM deoxyribose, 2.8 mM H_2O_2, 25 µM $FeCl_3$, 100 µM nitrilotriacetic acid (NTA), 1% (w/v) thiobarbituric acid (TBA) and 2.8% (w/v) trichlorotic acid (TCA) were prepared in 0.1 M phosphate buffer at (pH 7.4). The reaction mixtures contained, in a final volume of 1.2 mL, the following reagents: 0.22 mL of the GDE, 0.2 mL of deoxyribose solution, 0.38 mL of H_2O_2 solution, 0.2 mL of $FeCl_3$ solution, and 0.2 mL of NTA solution. The mixtures were incubated in water bath at 37 °C for 60 min. Degradation of deoxyribose sugar induced by $^{\cdot}OH$ was determined by the addition of 1 mL of TBA solution and 1 mL of TCA solution and heated at 100 °C for 20 min. The pink chromogen formed was determined by measuring its absorbance at 532 nm. Trolox was used as a positive control.

4. Inhibition of lipid peroxidation

The lipid peroxide formation was measured by the method of Masao *et al.* (1993). The rats weighing 200-250 g were sacrified by dislocation of their necks. The abdomen was opened, and the liver was removed. The liver was homogenized in 150 mM Tris-HCl buffer (pH 7.2). Total protein concentration was estimated by the method of Lowry *et al.* (1951) using bovine serum albumin (BSA) as a standard. The reaction mixture was prepared composing of 0.2 mL of rat liver homogenate in 150 mM Tris-HCl buffer (pH 7.2), 0.5 mM ferrous chloride solution, 0.06 mM ascorbic acid solution, and various concentrations of the GDE in a final volume of 1.0 mL. The mixture was incubated at 37°C for 1 h. The incubated reaction mixture (0.4 mL) was mixed with 0.2 mL of 0.8% (w/v) TBA solution, 1.5 mL of 20% acetic acid and 0.4 mL of deionized water. Then, the mixture was heated in a water bath at 100°C for 1 h. After the mixture was cooled, 1 mL of deionized water and 5 mL of *n*-butanol were added, followed by vigorously shaking for 1 min. After centrifugation at 4000 rpm for 10 min, the organic layer was taken and its absorbance at 532 nm was measured. Trolox was used as a positive control.

Preparation of obtained results

All determinations were carried out in triplicate. Percent inhibition of free radical formation, and lipid peroxide formation were determined from the following equation:

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

Where $A_{control}$ is the absorbance of the control and A_{sample} is the absorbance in presence of the GDE or positive control.

The EC_{50} value (concentration that caused 50% inhibition) was determined from the concentration-response (% inhibition) curve. The $ABTS^{+\bullet}$, hydroxyl radicals scavenging activity, and inhibition of lipid peroxidation were expressed as Trolox equivalent antioxidant capacity (TEAC) which represented the concentration (mM) of Trolox per mg of the GDE. For the superoxide anion scavenging activity was expressed as gallic acid equivalent (GAE) in g of the GDE per mg of gallic acid.

Determination of total phenolic contents of the GDE

The phenolic content in the GDE was determined according to the method of Hammerschmidt and Pratt (1978) with minor modifications. Briefly, 0.2 mL of the GDE was mixed with 1.0 mL of 10% Folin-Ciocalteu solution and 0.8 mL of 7.5% sodium carbonate solution. The mixture was allowed to stand for 1 h at room temperature, and the absorbance was measured at 765 nm. Gallic acid was used as the standard for the calibration curve, and the total phenolic content was expressed as gallic acid equivalent (GAE) in g of the GDE per mg of gallic acid.

Statistical Analysis

The data from the experiment were expressed as mean ± standard deviation of 3 replicates. Experimental results were analyzed by SPSS version 16.0 (SPSS Inc. Chicago, IL). Statistical comparisons between groups were analyzed by one-way analysis of variance (ANOVA) and post hoc least-significant difference (LSD) test. *P*-values less than 0.05 were considered significant.

RESULTS AND DISCUSSION

Evaluation of the GDE for antioxidant properties

1. $ABTS^{+\bullet}$ radical scavenging activity

The $ABTS^{+\bullet}$ radical scavenging assay is widely used to evaluate the antioxidant properties of compounds due to the simple, rapid, sensitive, and reproducible procedure (Ozcelik *et al.*, 2003). In this assay, ABTS is converted to its radical cation ($ABTS^{+\bullet}$) by an addition of potassium persulfate. The $ABTS^{+\bullet}$ radical is blue-green in color and absorbs light at 734 nm (Re *et al.*, 1999). The $ABTS^{+\bullet}$ is reactive towards most antioxidants including phenolics, thiols and vitamin C (Walker *et al.*, 2009). During this reaction, the blue-green $ABTS^{+\bullet}$ is converted back to its colorless neutral form. The reaction may be monitored spectrophotometrically. This assay is often referred to as the Trolox equivalent antioxidant capacity (TEAC) assay. The GDE showed $ABTS^{+\bullet}$ radical scavenging activity with the EC_{50} value of 0.476 ± 0.017 mg/mL and TEAC value of 764.74 ± 8.24 (Table 1).

2. Superoxide anion ($O_2^{-\bullet}$) scavenging activity

Superoxide anion, a highly reactive radical is a common intermediate in numerous biological oxidations and an important killing mechanism generated in lysosomes of phagocytes after having phagocytosed microorganisms and also deleteriously produced as a byproduct of mitochondrial respiration (Muller *et al.*, 2007). It can generate more dangerous species in living system, including hydrogen peroxide, hydroxyl radical

or singlet oxygen (Lee *et al.*, 2004) which induces oxidative damage in lipids, proteins, and DNA. $O_2^{-\bullet}$ is converted to hydrogen peroxide by superoxide dismutase (Pietta *et al.*, 2000). In the assay for $O_2^{-\bullet}$ radical scavenging activity, $O_2^{-\bullet}$ is induced in the PMS/NADH-NBT system. The $O_2^{-\bullet}$ then reduces the yellow dye (NBT^{2+}) to produce the blue formazan, which can be measured spectrophotometrically at 560 nm. The decrease of absorbance with antioxidants thus indicates the consumption of $O_2^{-\bullet}$ in the reaction mixture. The GDE exhibited superoxide anion scavenging activity, the EC_{50} and GAE values were found to be 0.539 ± 0.006 mg/mL and 0.0021 ± 0.00005, respectively (Table 1).

3. Hydroxyl radical ($^{\bullet}$OH) scavenging activity

In living organisms, two major reactive oxygen species, $O_2^{-\bullet}$ and $OH^{\bullet}$ are being continuously formed in a process of reduction of oxygen to water. The $OH^{\bullet}$ is highly reactive, very dangerous, and it can damage virtually all types of macromolecules such as carbohydrates, nucleic acids (mutations), lipids (lipid peroxidation) and amino acids (Reiter *et al.*, 1995; Reiter *et al.*, 1997). In the assay method, hydroxyl radicals were produced by incubating ferric-nitrilotriacetic acid and H_2O_2 at pH 7.4 and reacted with deoxyribose to degrade it into fragments that forms a pink chromogen upon heating with TBA at low pH. The GDE showed hydroxyl radicals scavenging activity with EC_{50} and TEAC values of 0.014 ± 0.001 mg/mL and 998.25 ± 28.37, respectively (Table 1). Similarly, another plant of the same genus: *G. procumbens* has been reported to have hydroxyl radicals scavenging activity with EC_{50} value of 1.63 mg/mL (Puangpronpitag *et al.*, 2010).

4. Inhibition of lipid peroxidation

Lipid peroxidation referring to the oxidative degradation of lipids is the process in which free radicals steal electrons from the lipids in cell membranes (mostly, polyunsaturated fatty acid), resulting in cell damage. The anti-lipid peroxidation assay is often used as the first parameter to prove the involvement of free radicals in cell damage. In the present study, the liver of rat was used as a source of polyunsaturated fatty acid for determining the extent of lipid peroxidation, and malondialdehyde (MDA) is the specific end-products of lipid peroxidation. (Rice-Evans *et al.*, 1996; Marnett, 1999). In the assay, thiobarbituric acid reacts with malondialdehyde to yield a fluorescent product (Maurizio *et al.*, 2001), a pinkish red chromogen, which is measured at 532 nm, and this TBARS assay (thiobarbituric acid reactive substances assay) is most commonly used. The GDE exhibited an ability to inhibit lipid peroxidation with the EC_{50} value of 16.134 ± 0.461 mg/mL and TEAC value of 52.78 ± 3.00 (Table 1). Other plants of the same genus: *G. procumbens* (ethanol extract), *G. pseudochina* (methanol extract) and *G. pseudochina var. hispida* (methanol extract) showed EC_{50} values of 2.75 mg/mL, 93.56 and 73.63 µg/mL, respectively (Puangpronpitag *et al.*, 2010; Nisarat *et al.*, 2010).

Table 1 EC_{50}, GAE and TEAC values of the GDE and standard antioxidants obtained from various assays for antioxidant activity

Assays for antioxidant activity	EC_{50} (mg/mL)			GAE[a] or TEAC[b] values
	The GDE	Trolox	Gallic acid	of the GDE
$ABTS^{+\bullet}$ scavenging activity	0.476 ± 0.017	0.364 ± 0.008	–	764.74 ± 8.24[b]
$O_2^{-\bullet}$ scavenging activity	0.539 ± 0.006	–	0.255 ± 0.006	0.0021 ± 0.00005[a]
$^{\bullet}OH$ scavenging activity	0.014 ± 0.001	0.014 ± 0.0001	–	998.25 ± 28.37[b]
Anti-lipid peroxidation	16.134 ± 0.461	0.851 ± 0.031	–	52.78 ± 3.00[b]

Data expressed as mean ± S.D. of triplicate measurements

[a]GAE (gallic acid equivalent) expressed as g of the GDE per mg of gallic acid

[b]TEAC (Trolox equivalent antioxidant capacity) expressed as mM Trolox per g of the GDE

Determination of phenolic contents in the GDE

The most important class of phytochemicals with antioxidant properties in plant food sources is the group of phenolic compounds (Demo *et al*., 1998) because of their redox potential, which allow them to act as reducing agents, hydrogen donators, metal chelators and singlet oxygen quenchers (Rice-Evans *et al*., 1996). The estimation of phenolic contents of the GDE was done by using Folin-Ciocalteu reagent that produced blue color by reducing yellow hetero polyphosphomolybdatetungstate anions (Huang *et al.*, 2005). The GDE showed the presence of phenolic compounds with the GAE value of 0.017 ± 0.0003. Other study (Daduang *et al.*, 2011) reported the phenolic content of the ethanolic extract from *G. divaricata* of which GAE value was 5.41 ± 0.04. The extract of the present work (the GDE) seems to contain higher phenolic contents. The phenolic contents in plants of the *Gynura* species have been found, the methanol extract of *G. pseudochina* and *G. pseudochina var. hispida* in an amount 1 µg contained 10.82 ± 0.25 and 5.27 ± 1.08 µmol of caffeic acid, respectively (Nisarat *et al.*, 2010).

CONCLUSION

The present study has demonstrated the antioxidant properties of the GDE by showing the free radicals ($ABTS^{+\bullet}$, superoxide anion, hydroxyl) scavenging and anti-lipid peroxidation properties. According to the EC_{50} values (low EC_{50} is high efficacy), the order of efficacy is: hydroxyl radicals scavenging > $ABTS^{+\bullet}$ radical scavenging > superoxide anion scavenging > anti-lipid peroxidation. The presence of phenolic contents in the GDE is likely to play roles in the antioxidant activity. It is also suggested that *G. divaricata* is possibly be the potential sources for natural antioxidants to be further developed as medicinal plant products, food supplements or nutraceuticals which will be beneficial in healthcare system.

ACKNOWLEDGEMENTS

The financial supports from the Thailand Research Fund, Chiang Mai University, and Faculty of Medicine are greatly appreciated.

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