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Evaluation of Nutritional Value and Antioxidative Properties of The Medicinal Plant *Gynura procumbens* Extract

^{1,2,3}D. Puangpronpitag, ²S. Chaichanadee, ²W. Naowaratwattana, ²C. Sittiwet,
³K. Thammasarn, ³A. Luerang and ^{1,3}N. Kaewseejan

¹Center for Innovation in Chemistry, Department of Chemistry,

Faculty of Science, Maharakham University, Maharakham, 44150, Thailand

²Faculty of Medicine, Maharakham University, Maharakham, 44000, Thailand

³Department of Chemistry, Faculty of Science,

Maharakham University, Maharakham, 44150, Thailand

Abstract: The present study determined nutritional value in various parameters such as moisture content, carbohydrate, protein, lipid as standard methods and evaluated the antioxidative properties of the medicinal plant *Gynura procumbens* Extract by hydroxyl scavenging activity, ferrous ion-chelating activity and inhibition of lipid peroxidation. The highest extraction yield was obtained from extraction with distilled water (84.51%). While, Ethanolic *Gynura* extract (EGE) exhibited the highest in the antioxidative properties in every assay, $EC_{50} = 1.63$ in hydroxyl scavenging test, $EC_{50} = 2.17$ in chelating activity test and $EC_{50} = 2.75$ in inhibition of lipid peroxidation test. Although, the content still low (186.25 ± 0.00018 to 300.18 ± 0.0003 $\mu\text{g GAE g}^{-1}$ of extract). According determination the nutritional values was found that *Gynura procumbens* Extract were containing the contents of moisture was 7.08%, carbohydrate was 0.0537 to 0.1968 $\mu\text{g glucose equivalent/100 g dry weight}$ at EGE $0.1 \mu\text{g mL}^{-1}$, protein was 4.51 g/100 g dry weight and lipid was 0.023 g/100 g dry weight. These results suggest that *Gynura* Extract is a good protein source and that they may have positive effects on free radical scavenging and iron chelating which may use as preliminary information and develop further to be commercially useful in food industry or health products as medicinal food.

Key words: Chelating activity, herbaceous plant, medicinal food, phenolic antioxidant, scavenging effect

INTRODUCTION

Working on plants was a good start to a research career, since plants are of enormous importance in the free radical/antioxidant field. First, they supply us with the essential biradical, O_2 . Second, plants supply a range of antioxidants to humans. Third, diets rich in plants are associated with lower risk of developing many age-related diseases (e.g., some cancers, diabetes, atherosclerosis and dementia) (Halliwell, 2009). Because plant-based foods are complex mixtures of bioactive compounds, information on the potential health effects of individual polyphenols is linked to information on the health effects of foods that contain those polyphenols (Gharras, 2009). There is evidence that phenolic substances act as antioxidants by preventing the oxidation of LDL-lipoprotein, platelet aggregation and damage of red blood cells (Cheynier, 2005). Additionally, phenolics act as: (1) metal chelators, (2) antimutagens and anticarcinogens, (3) antimicrobial agents and (4) clarifying agents (Anli and

Vural, 2009; Proestos *et al.*, 2005). These compounds are a part of the everyday diet and also used as medicines or supplements. Research has shown that fruits and vegetables contain other antioxidant nutrients which significantly contribute to their total antioxidant capacity (Cao *et al.*, 1998; Wang *et al.*, 1996). *Gynura procumbens* (Compositae), a fast growing herbaceous plant is an annual evergreen shrub with a fleshy stem. *Gynura procumbens* is found in various parts of Asia and is widely used in Thailand and Southeast Asia as a traditional medicine. The plant has traditionally been used for treatment of eruptive fevers, rash, kidney disease, migraines, constipation, hypertension, diabetes mellitus and cancer (Perry, 1980). Recently, pharmacological studies have indicated that *G. procumbens* has antioxidant (Rosidah *et al.*, 2008, 2009), anti-herpes simplex virus (Nawawi *et al.*, 1999), anti-hyperglycemic (Li *et al.*, 2009; Akowuah *et al.*, 2002), anti-inflammatory (Iskander *et al.*, 2002), anti-hyperlipidaemic (Zhang and Tan, 2000) and blood hypertension reduction capabilities

(Hoe *et al.*, 2006; Kim *et al.*, 2006). The advantages of the traditional use of *G. procumbens* have also been supported by the isolation and identification of several possible active chemical constituents from this plant, including flavonoids, saponins, tannins, terpenoids and sterol glycosides (Akowuah *et al.*, 2002). Even though the various uses over long time periods, little information is available regarding nutritional value and the antioxidative properties of *G. procumbens* from Thailand. Therefore, the aim of the present study was to provide scientific data on this point, focusing on the nutritional value in various parameters such as moisture content, carbohydrate, protein, lipid and evaluated the antioxidative properties of the medicinal plant *Gynura procumbens* extract.

MATERIALS AND METHODS

Plant material: *Gynura procumbens* was collected from the local area of Mahasarakham Province, Mahasarakham, Thailand in February 2008. Fresh leaves (100-200 g) were air-dried and then 60°C oven. Each sample was extracted three times. The organic extract was then evaporated and stored at -20°C until ready to be tested.

Chemicals: The 1,1,3,3-tetraethoxypropane (MDA) and 2-thiobarbituric acid (TBA) were purchased from Sigma Chemical Co., USA. Ferric chloride hexahydrate (FeCl₃.6H₂O) and ferrous sulfate heptahydrate (FeSO₄.7H₂O) were obtained from Fluka, Germany. Sodium azide (NaN₃), absolute ethanol and trichloroacetic acid (TCA) were products of Merck, Germany. Hydrogen peroxide (H₂O₂) and chloroform was purchased from BDH, UK. All other chemicals used were in the purest form available commercially.

Moisture content: Fresh *G. procumbens* leaves (100-200 g) were air-dried and then oven drying at 60°C until a constant weight for determination of moisture content (AOAC, 1990, method 985.14).

***G. procumbens* extraction:** After drying *G. procumbens* leaves, the solids were extracted with various organic solvents (hexane, methanol, chloroform, acetonitrile, distilled water and ethanol). The concentration and solvent with the highest efficiency of extraction was used in subsequent experiments. The extraction protocol was done for 3 h (3 times) as modified method (Owen *et al.*, 2003). Organic solvent was removed by rotary evaporation at 35°C *in vacuo* (Buchi Rotavapor R-200, USA) until dry.

Determination of the yield of *G. procumbens* extracts:

The evaporative dried *G. procumbens* extracts were weighed for calculating the yield by the following Eq. 1:

$$\text{Yield (\% dry basis; \%db)} = \frac{(W_1 \times 100)}{W_2} \quad (1)$$

Equation 1 calculation of % yield.

where, W₁ is the weight of extracts after evaporation and W₂ is the solid weight of *G. procumbens*

Nutritional value determinations: All Nutritional value determinations were performed at least in triplicate. Values of different parameters were expressed as the Mean±Standard Deviation (SD). Quantitative analysis of carbohydrate content was done as standard method of Dubois *et al.* (1956). Total protein was determined by the Kjeldahl method. Protein was calculated using the general factor (6.25) (AOAC, 1990) and total lipid was measured by standard protocol of AACC (1987) method.

Determination of the antioxidative properties

Hydroxyl radical-scavenging activity assay: The effect of hydroxyl radical was assayed by using the 2-deoxyribose oxidation method (Chung *et al.*, 1997). 2-Deoxyribose is oxidized by hydroxyl radical that is formed by the Fenton reaction and degraded to malondialdehyde (MDA). The reaction mixture contained 0.45 mL of 0.2 M sodium phosphate buffer (pH 7.4), 0.15 mL of 10 mM 2-deoxyribose, 0.15 mL of 10 mM FeSO₄-EDTA, 0.15 mL of 10 mM hydrogen peroxide, 0.525 mL of distilled water and 0.075 mL of the *Gynura* extract solution in the tube. The reaction was started by the addition of hydrogen peroxide. After incubation at 37°C for 4 h, the reaction was stopped by adding 0.75 mL of 2.8% trichloroacetic acid and 0.75 mL of 1.0% of thiobarbituric acid. The mixture was boiled for 10 min, cooled in ice and then measured at 520 nm. The hydroxyl radical scavenging ability was evaluated by the following Eq. 2:

$$\text{Scavenging effect (\%)} = \frac{(A_{\text{control}} - A_{\text{sample}})}{A_{\text{control}}} \times 100 \quad (2)$$

Equation 2 Calculation of % hydroxyl radical scavenging effect.

Then the hydroxyl radical scavenging capacity was expressed as Median Effective Concentration (EC₅₀; mg mL⁻¹) which represented the concentration of sample having 50% hydroxyl radical scavenging ability.

Metal iron-chelating assay: The ferrous ion-chelating activity of the *Gynura* extract was measured according to

the modified method of Yen and Wu (1999). The absorbance of the ferrous iron-ferrozine complex at 562 nm was measured to determine the Fe²⁺-chelating ability of the extract. Briefly, the reaction mixture, containing the extract (1-80 mg mL⁻¹), FeCl₂ (2 mM) and ferrozine (5 mM), was adjusted to 5 mL with distilled water. The mixture was shaken well and incubated for 10 min at room temperature. The absorbance of the mixture was measured at 562 nm against a blank. EDTA was used as positive control. The ability of extracts to chelate ferrous ion was calculated using the following Eq. 3:

$$\text{Chelating activity (\%)} = \frac{(A_{\text{control}} - A_{\text{sample}})}{A_{\text{control}}} \times 100 \quad (3)$$

Equation 3 calculation of % metal iron chelating activity.

The ferrous ion-chelating activity was expressed as Median Effective Concentration (EC₅₀; mg mL⁻¹) which represented the concentration of sample at which the chelating activity was 50%.

Lipid peroxidation assay: The inhibition of Lipid peroxidation of the *Gynura* extract was assessed by modified method (Lo and Cheung, 2005). Briefly, 0.1 mL of linoleic acid in 0.2 M Na-phosphate buffer, pH 7.0 was mixed with 0.2 mL of the *Gynura* extract (1-80 mg mL⁻¹), followed by addition of 0.1 mL of 10 μM FeSO₄ and 0.1 mL ascorbic acid; the mixture was incubated at 37°C for 1 h. The reaction was terminated by adding 0.5 mL trichloroacetic acid (TCA, 28%, w/v), followed by 0.38 mL thiobarbituric acid (TBA, 2%, w/v), with heating at 100°C for 20 min. After centrifugation at 3000 g for 10 min to remove the precipitated protein, the absorbance of the supernatant containing the thiobarbituric acid-reactive substance (TBARS) was measured at 532 nm by a spectrophotometer. Malondialdehyde (MDA) was used as a standard and phosphate buffer was used instead of sample as the negative control. The inhibition percentage of lipid peroxidation was calculated by the following Eq. 4:

$$\text{Inhibition activity (\%)} = \frac{(A_{\text{control}} - A_{\text{sample}})}{A_{\text{control}}} \times 100 \quad (4)$$

Equation 4 calculation of % inhibition activity of lipid peroxidation.

The inhibition of lipid peroxidation was expressed as median effective Concentration (EC₅₀; mg mL⁻¹) which represented the concentration of sample having 50% inhibition effect on lipid peroxidation.

Statistical analysis: Data were expressed as Mean±SD. Paired samples test and ANOVA were used to perform statistical analysis for the comparison. Post hoc testing was carried out using the Bonferroni test. The criterion for statistical significance was p<0.05.

RESULTS

Extraction yield of the *G. procumbens* leaves: *Gynura procumbens* leaves were extracted using distilled water, hexane, methanol, chloroform, acetonitrile and ethanol. Distilled water produced the highest yield of *G. procumbens* leaves (84.51%) which was statistically different (p<0.01) from all of the *Gynura* extracts, following with ethanol and methanol, respectively (14.73 and 7.02%, respectively, Fig. 1).

Nutritional value of *G. procumbens* leaves: The moisture content of fresh *G. procumbens* leaves before drying was approximately 7.08 g/100 g dry weight. The chemical compositions of the *Gynura* extract possessed carbohydrate approximately 0.0537 to 0.1968 μg glucose equivalent/100 g dry weight (at the concentration of the ethanolic *Gynura* extract 0.1 μg mL⁻¹), protein approximately 4.51 g/100 g dry weight and lipid approximately 0.023 g/100 g dry weight (Fig. 2).

The antioxidative properties of the *Gynura* extract: We evaluated the antioxidative properties of the *Gynura* Extract by using distilled water, methanol and ethanol, which composing highest yield (Fig. 2), polyphenol and flavonoid (data not shown). The results indicated that Ethanolic *Gynura* Extract (EGE) exhibited the highest in the antioxidative properties in every assay, EC₅₀ = 1.63 in

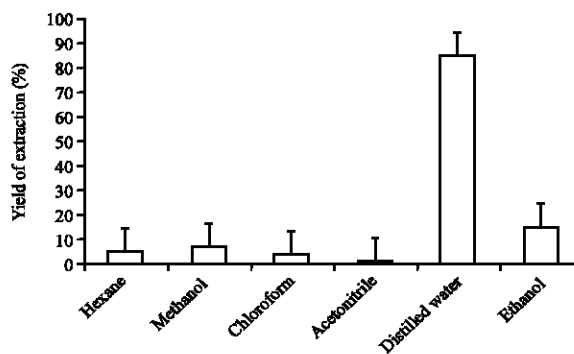
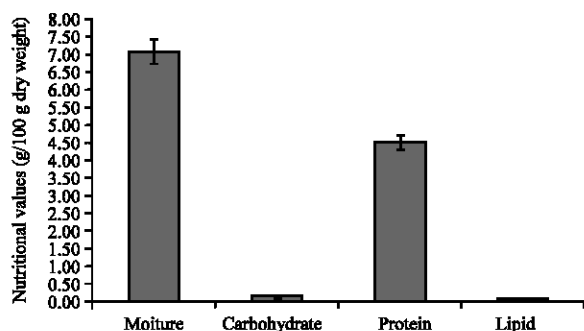
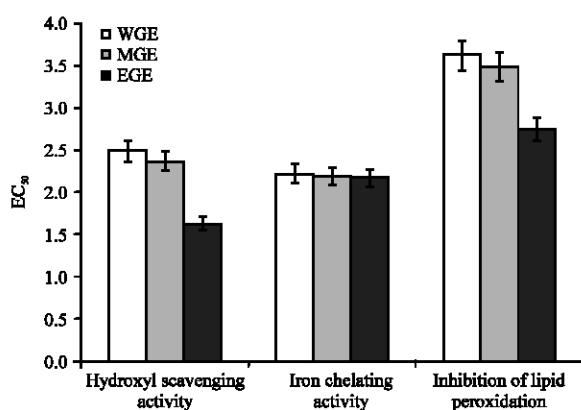


Fig. 1: Percent yield (dry basis, db) of the *Gynura* extracts. Data are representative of three repeated extractions

Fig. 2: Nutritional value of *G. procumbens* leavesFig. 3: The antioxidant properties in three assays of the *Gynura* extracts

hydroxyl scavenging activity, $EC_{50} = 2.17$ in iron chelating activity and $EC_{50} = 2.75$ in inhibition of lipid peroxidation (Fig. 3).

In this study reveals that the *Gynura* extract has a strong antioxidant properties by radical-scavenging action, iron metal chelating and lipid peroxidation inhibition. Anyway, the *Gynura* extracts showed the highest activity in hydroxyl radical-scavenging than the other activities (Fig. 3). These results suggested that consumption the *Gynura* extract may have health benefits.

DISCUSSION

Nowadays, herbaceous plant is gaining popularity in developing countries as medicinal food. Although, medicinal plants may cause several biological activities in humans, very little is known, such as *Gynura procumbens*. Although, previous studies have indicated that the important bioactive ingredients are present in methanol extract (Akowuah *et al.*, 2002) and the recently study which revealed that administration of the methanol

extract from leaves at 1000-5000 mg kg⁻¹ did not produce mortality or significant changes in the general behavior, body weight, or organ gross appearance of rats (Rosidah *et al.*, 2009). Contrary with our present extraction method which found that ethanolic *Gynura* extract (EGE) exhibited the highest in the antioxidative properties in every assay; this activity might be mediated directly by the phenolic compounds in the extracts. The phenolic mixtures in EGE extract might contain several different antioxidant activities or act in complex multiphase systems. Anyway, it agree well with the study of Iskander *et al.* (2002) which claimed that the crude ethanolic extract of *G. procumbens* has anti-inflammatory action, similar to Hoe *et al.* (2006) which investigated the hypotensive and angiotensin-converting enzyme (ACE) inhibitory activities of a partially purified fraction of the leaves of *G. procumbens* by the crude ethanolic extract. The findings also suggest that the putative hypotensive agents seem to be glycoconjugated or peptidal substances in nature. This evidence may support by our study which found that *Gynura* extract is a good protein source; however it showed a bit carbohydrate content. We believe the *Gynura* extract shows potential as a functional food or value-added ingredient. However, there are no studies of reactive oxygen species or anti-oxidation in relation to these *Gynura* extracts. Several investigations showed differences between the test systems for the determination of antioxidant assay (Gahler *et al.*, 2003; Schlesier *et al.*, 2002). It was recommended to use at least two methods. In this study, we use free radicals, such as hydroxyl radical in assayed the hydroxyl radical-scavenging activity. Phenolic compounds scavenge the H₂O₂ radicals that initiate lipid peroxidation (Cotelle *et al.*, 1996; Laughton *et al.*, 1991). Moreover, the ferrous ion-chelating activity and the inhibition of lipid peroxidation were investigated. A previous study reported that phenolic molecules may behave as metal chelators due to their catechol structure (Packer *et al.*, 1999). It is not clear whether the *Gynura* extract can chelate metal ions and inhibits lipid peroxidation, therefore we conducted the experiment and found that the *Gynura* extract showed antioxidative properties in all of the tested methods. Our present study is the first reveal that the mechanisms of antioxidant activities in the *Gynura* extract is likely free radical scavenging and ion chelating, this activity might be mediated directly by the polyphenolic compounds in the extracts. The polyphenolic mixtures in the extract might contain several different antioxidant activities or act in complex multiphase systems. This investigation suggested that the *Gynura* extract is a good source of natural antioxidant similar in the recent study of

Rosidah *et al.* (2008) which suggest that phenolics in these plants provide substantial antioxidant activity. Thus, consumption of foods containing various antioxidants has been recommended to prevent or slow the oxidative stress caused by free radicals (Shahidi and Wanasundara, 1992), especially, above preliminary information of the *Gynura* extract supported the benefits of the traditional use. The *Gynura* extract may further help strengthen the observations that this plant does indeed possess active principles and develop further to be commercially useful in food industry or health products as medicinal food. However, there is no study so far concerning the characterization of the active antioxidative components in Thai *G. procumbens* leaves. Since phenolic compounds might be a possible class of antioxidant in this the *Gynura*, future work on the isolation and structural characterization of the active compounds is needed.

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