# TOTAL PHENOLIC CONTENT AND ANTIOXIDANT ACTIVITY OF GYNURA PROCUMBENS LEAVES GROWN IN VIETNAM

● TRAN BUI PHUC - VU THI KIM NGOC

**ABSTRACT:**

Gynura procumbens plant, widely known as an edible plant in Vietnam, has been used as a traditional medicine in many regions of Asia. In this study, an affordable and convenient method of soaking dry leaves of Gynura procumbens in water was employed to obtain extracts. The extraction conditions were optimized, including the dried plant weight/solvent volume ratio of 1/30 g/mL, the extraction temperature of $80^0$C, and the extraction time of 90 minutes. The results indicated that the total phenolic content (TPC) of the extracts varied from 3.3 to 5.8 mgGAE/gdry. All the extracts possessed DPPH radical scavenging activity. Compared to $IC_{50}$ of Trolox (43.5 mg/L), the optimum extract showed significant antioxidant activity with $IC_{50}$ of 7.6 mg/L and the antioxidant capacity equivalent to Trolox of 5.7 mgTE/gdry. The study results suggest that the Gynura procumbens leaf extract demonstrates its potential to be used as a natural antioxidant, which is capable of being used in food industry, medicine, and cosmetics.

**Keywords:** Gynura procumbens, total phenolic content, antioxidant activity, extraction, Trolox.

## 1. Introduction

Free radicals are atoms or molecules that lose an electron in the outer shell continuously generated. During the body's metabolism or under the influence of factors such as environmental pollution, stress, alcohol, tobacco ... free radicals are formed. They are harmful substances, causing damaging change, dysfunction, aging and diseases [1]. Free radicals are harmful substances, causing damaging changes, dysfunction, aging, and most diseases. Over time, free radicals are constantly being produced and harmful while the body's defenses gradually weaken. Therefore, to protect health - especially the brain, it is necessary to limit the free radical proliferation factors, and add anti-free radicals to the body. With the concern of some side effects causing pneumonia, impaired blood clotting... of synthetic antioxidants such as butylated [2], nowadays, scientists have paid more attention to the study of compounds. antioxidants from nature. Previous studies have shown that foods such as fruits, chocolates, vegetables, tea, wine,... contain more than 8.000 polyphenols - nutrients that play a role in protecting cells from the harmful effects of radicals [3]. The antioxidant potential and phenolic composition of berries such

as blueberries, blackberries and strawberries in Nanjing were investigated in 2012 [4]. In this study, blueberries were believed to contain antioxidants such as proanthocyanidins and anthocyanidins. Extracts of 20 locally available Sri Lankan fruits were investigated for their antioxidant activity as reported by Silva and Sirasa [5]. The results showed that gooseberry showed the highest DPPH removal activity (111.25 mg ascorbic acid equivalent antioxidant capacity (AEAC)/g). Sugar apple and starfruit had the second and third highest antioxidant activity in terms of DPPH, TPC, TFC activity ratings, and vitamin C content.

In Vietnam, Gynura procumbens (GP) is widely grown in rural areas. The locals use it as a daily nutritional supplement. Many studies show that GP is a nutritious plant [6]. In addition, it is also used as a medicine in traditional medicine to cure diseases: detoxification, heat treatment, diuretic, anti-aging, liver, kidney, intestinal, cardiovascular or blood and sugar diseases... [7-9]. Much research has been done on extracting polyphenol compounds from Gynura procumbens leaves [6, 10]. Conditions such as soil, climate and extraction methods also significantly affect the content of beneficial active ingredients in the plant [11]. With the purpose of not using harmful chemicals, this study is conducted to investigate the conditions of Gynura procumbens (GP) leaves extraction with aqueous solvents.

**2. Materials and methods**

***2.1. Materials***

The GP leaves used in the study were collected at garden in Ho Chi Minh City in February 2021. The GP leaves were dried to reach the moisture below 10%. The dry materials were crushed and sieved through a 0.1 mm diameter screen. The dried potting medium powder was vacuum packed in bags and stored at room temperature until the experiment is carried out.

Chemicals used for this study included: 2,2 '-diphenyl - 1 - picrylhydrazyl (DPPH), gallic acid, Folin reagent - Ciocalteu, Sodium carbonate ($Na_2CO_3$) made in India; methanol purchased from Merck (Germany).

***2.2. Extraction method***

Single factor method is used in this study. A mass of 1 g of GP leaves was used for extraction. The survey parameters of extraction time, temperature and the ratio of raw materials / extraction solvent were shown in the Table 1:

**Table 1. Survey parameters of Gynura procumbens leaf extract**

| | Ratio material/solvent (g/mL) | Time (minute) | Temperature ($^0$C) |
|---|---|---|---|
| Survey parameters | 1/25; 1/30; 1/35; 1/40 | 60, 90, 120, 150 | 70; 80; 90; 100 |
| Solvent | | Distilled water | |
| Fixed parameters | 80$^0$C; 60 minutes | 80$^0$C; Optimal ratio | Optimal ratio, time |

Appropriate extraction parameters were selected based on the total polyphenol content (TPC) and DPPH-free radical scavenging capacity of the extract (DPPH).

***2.3. Total Phenolic Content (TPC) Measurement***

The total polyphenol content was determined according to the method of Singleton et al. (1999) [12]. Exactly 1 ml of extract to remove 5 ml of reagent Folin - Ciocalteu 10% and 4 ml of 7.5% $Na_2CO_3$ solution. The mixture was shaken by Vortex machine for 30 seconds before being kept at room temperature and dark for 60 minutes. The mixture was then measured at 765 nm on a Carry 50 spectrometer (Spectrophotometry, Varian, Australia). Gallic acid was used to construct the calibration curve and results were expressed in milligrams equivalent gallic acid (mg GAE)/g dry extract. Total polyphenol content (TPC) with substance standard was gallic acid in the

concentration range 1 to 10 µg/mL had a linear regression equation y = 0.0125x + 0.0249 ($R^2$ = 0.9905).

***2.4. DPPH Radical Scavenging Activity Measurement***

DPPH free radical reduction capacity of extracts was determined according to the method of Fu and Shieh (2002) [13] with some minor corrections. Take 0.5 ml of extract, then add 3.5 ml of 0.1 mM DPPH solution in 80% methanol, mix and allow to stand in the dark for 30 minutes. The optical absorbance was measured at a wavelength of 517 nm. DPPH free radical reduction was determined by the following formula:

$$\text{DPPH}\,(\%) = \frac{A_{CT} - A_{SP}}{A_{CT}}\,100$$

Whereas:

$A_{CT}$: Absorption of blank sample without extract;

$A_{SP}$: Absorption of the sample containing the extract.

DPPH radical scavenging activity of extracts counted Trolox equivalent (mg TE/g) based on method normal curve y = 0.8879x + 0.7889 ($R^2$ = 0.9961).

**3. Results**

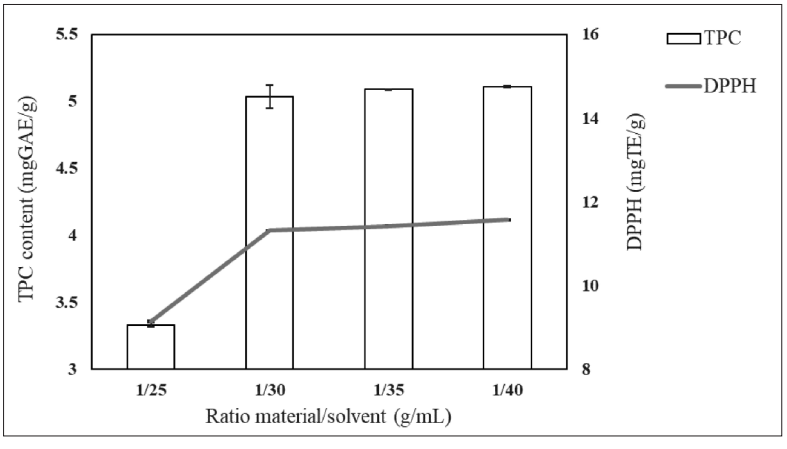
***3.1. Effects of ratio material/solvent on polyphenol content (TPC)and DPPH radical scavenging activity***

The effect of the raw material/solvent ratio (g/mL) on the total polyphenol content and DPPH radical scavenging activity of the GP leaf extract was depicted in the figure 1. When the ratio decreased from 1/25 to 1/30 (g/mL), the TPC increased significantly. However, further reducing the ratio to 1/35 and 1/40 (g/mL), the TPC did not change significantly. Free radical scavenging activity increased significantly from 1/25 to 1/30 (g/mL). But if the ratio continued to decrease, the free radical reduction value increased very little. Selecting an appropriate material/solvent ratio requires taking into account the overall efficiency of the process as it relates to energy, equipment, and solvent costs. Therefore, depending on the specific purpose and the amount of extract to be collected, choose the appropriate extraction ratio material/solvent. The result was consistent with previous study of Hainorita et al. [14]. With the results of the current study, the extraction rate of ratio material/solvent of 1/30 (g/mL) was selected for the further study.

***3.2. Effects of extraction time on polyphenol content (TPC) and DPPH radical scavenging activity***

Figure 2 showed the effect of extraction time on the total polyphenol content and the DPPH free radicals scavenging activity of the GP extract. When the extraction time was changed from 60 to 150 minutes, the TPC content reached the maximum value at 90 minutes (5.8 mgGAE/g). After a 90-minute extraction time, the values of TPC began to decrease. However, for DPPH, the free radicals scavenging activity increased sharply from 60 to 90 minutes. After 90 minutes, the DPPH free radicals scavenging activity had barely changed much. Results showed similarities with extracts of Limnophila aromatic [15]. Based on the

***Figure 1: The effect of the ratio material/solvent (g/mL) on the total polyphenol content TPC and DPPH radical scavenging activity of the GP leaves extract***



results achieved, time 90 minutes was selected for the next study.

***3.3. Effects of extraction temperature on TPC and DPPH radical scavenging activity***

Temperature is one of the important parameters affecting the extraction of polyphenol compounds from plant materials. In this study, the effect of extraction temperature (70, 80 and 90$^0$C) on polyphenol content and DPPH free radical absorption was studied (Figure 3). The study results showed that the total polyphenol content and the ability to DPPH free radicals scavenging activity in the GP leaf extract increased with the increasing direction of temperature in the range from 70 to 80$^0$C. However, when the temperature increased from 80 to 90$^0$C, the values of polyphenol compounds and the ability to DPPH free radicals had a tendency of decreasing. When the extraction temperature increases, the solubility and diffusion capacity of polyphenol compounds increase; increasing the transfer and wetting of the extraction materials, thereby increasing the extraction efficiency of polyphenols [16]. From that, temperatures of 80$^0$C were selected to extract GP leaves for water-extraction method.

***Figure 2: The effect of the extraction time (minute) on the total polyphenol content TPC and DPPH radical scavenging activity of the GP leaves extract***

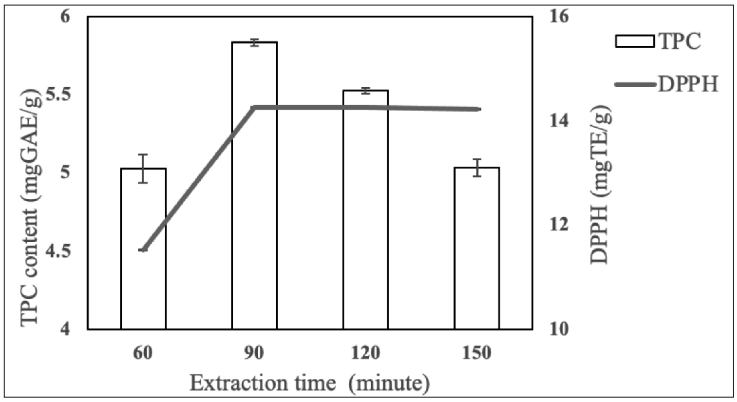
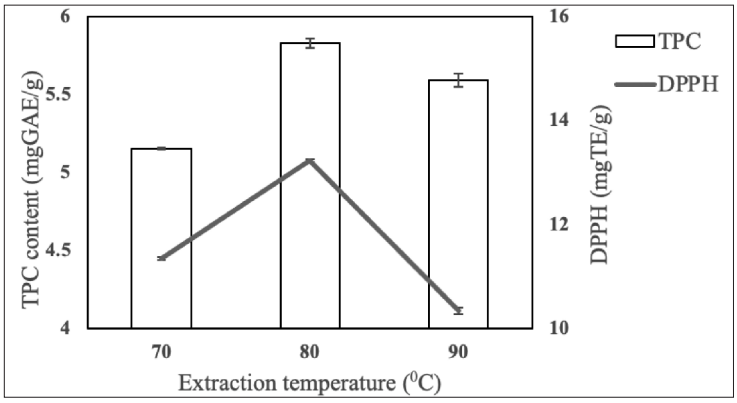



***Figure 3: The effect of the extraction temperature ($^0$C) on the total polyphenol content TPC and DPPH radical scavenging activity of the GP leaves extract***




The DPPH free radical capacity of GP leaves extract was compared based on the absorption value of Trolox $IC_{50}$. Survey results showed that $IC_{50}$ of Trolox (43.5 mg/L) was higher than $IC_{50}$ of optimal extract of GP leaf (7.6 mg/L) and Trolox equivalent antioxidant capacity TEAC was 5.7 mgTE/gdry. The aqueous GP extract in this study had a TPC value (5.8 mgGAE/g) close to the TPC of Limnophila aromatic aqueous extract (6.2 mgGAE/g) and $IC_{50}$ value (7.6 mg/L) was higher than Limnophila aromatic (679µg/mL) [15].

**4. Conlusion**

The study showed that Gynura procumbens leaves have the potential to extract natural antioxidants. The extraction conditions were obtained with the highest value of polyphenol content (5.8 mgGAE/g) and antioxidant capacity equivalent to Trolox (5.7 mgTE/gdry) from Gynura procumbens leaves in 1/30 g/mL, 80$^0$C, and 90 minutes ■

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# KHẢO SÁT HÀM LƯỢNG POLYPHENOL VÀ KHÁNG OXY HÓA CỦA LÁ BẦU ĐẤT

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**TÓM TẮT:**

Cây bầu đất, được biết đến rộng rãi như một loại cây ăn được ở Việt Nam, đã được sử dụng làm thuốc cổ truyền ở nhiều vùng của châu Á. Trong nghiên cứu này, phương pháp ngâm nóng đã được sử dụng để thu hồi dịch chiết. Các điều kiện chiết xuất được tối ưu hóa, bao gồm tỷ lệ khối lượng nguyên liệu khô/dung môi là 1/30 g/mL, nhiệt độ chiết $80^0$C và thời gian chiết là 90 phút. Kết quả chỉ ra rằng, tổng hàm lượng phenolic (TPC) của các chất chiết xuất thay đổi từ 3,3 đến 5,8 mgGAE/g. Tất cả các chất chiết xuất đều có khả năng loại bỏ gốc DPPH. So với $IC_{50}$ của Trolox (43,5 mg/L), chiết xuất tối ưu cho thấy hoạt động chống oxy hóa đáng kể với $IC_{50}$ là 7,6 mg/L và khả năng chống oxy hóa tương đương với Trolox là 5,7 mgTE/g. Kết quả nghiên cứu cho thấy, chiết xuất lá bầu đất có tiềm năng được sử dụng như một chất chống oxy hóa tự nhiên, có khả năng được sử dụng trong công nghiệp thực phẩm, y học và mỹ phẩm.

**Từ khóa:** cây bầu đất, tổng hàm lượng phenolic, hoạt tính chống oxy hóa, chiết xuất, Trolox.