DETERMINATION OF THE TOTAL POLYPHENOL AND ALKALOID CONTENT AND ANTIOXIDANT ACTIVITIES OF SOME PLANTS IN TRA VINH PROVINCE

Xuan Thi Diem Trinh Nguyen*, Ngoc Tram Nguyen Thi,

Huynh Thi Hong Hoa, Mai Thi Thuy Lam

TraVinh University

*Email: *trinhnguyen@tvu.edu.vn*

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ABSTRACT

This study aimed to determine the total polyphenol and alkaloid contents, as well as the antioxidant activity of ethanolic extracts from five plant species collected in Tra Vinh province. The total polyphenol content (TPC) was measured using the Folin-Ciocalteu colorimetric method, and the total alkaloid content (TAC) was determined using UV-vis spectroscopy. Antioxidant activity was assessed via the DPPH radical scavenging assay. Results showed that TPC ranged from 91.60 to 512.67 mg GAE/g extract and TAC from 0.36 to 3.68 mg CE/g. The aerial part of Galphimia gracilis exhibited the strongest antioxidant activity (IC_{50} = 69.88 ± 0.63 µg/mL). A positive correlation was observed between polyphenol content and antioxidant activity, suggesting that polyphenol compounds significantly contribute to radical scavenging capacity. This is the first report on these species from Tra Vinh province, providing valuable data for future ethnobotanical and phytochemical studies.

Keywords: Alkaloid content, antioxidant activity, polyphenol content, Tra Vinh province.

1. INTRODUCTION

Since ancient times, humans have utilized plants as natural remedies for treating illnesses and maintaining health. With the advancement of modern scientific research, it has been confirmed that plants are rich sources of bioactive compounds exhibiting various pharmacological properties, including antioxidant, anti-inflammatory, and antibacterial activities. Among these bioactive substances, secondary metabolites such as polyphenols, flavonoids, and alkaloids have attracted significant attention due to their potent antioxidant potential. These compounds are widely distributed in different parts of plants, including roots, stems, leaves, and bark, and are regarded as valuable natural agents for promoting human health and preventing oxidative stress-related diseases [1].

Tra Vinh, a province located in the Mekong Delta region of Vietnam, is characterized by a rich and diverse ecosystem with a wide variety of plant species. Notably, several native and cultivated plants are traditionally used by the Khmer ethnic community as medicinal herbs. Species such as *Limonia acidissima* L., *Ricinus communis* L., *Talinum fruticosum*, and *Cleome chelidonii* are commonly employed in traditional medicine, contributing to the unique botanical profile of the region [2]. Recent studies have focused on the biological characteristics, phytochemical compositions, and bioactive properties of several plant species found in Tra Vinh, including *Nymphaea rubra* Roxb. ex Andrews [3], *Achyranthes aspera* L.

[4], and *Limonia acidissima* L. [5]. These findings highlight the biodiversity and medicinal potential of the local flora, underscoring its significance in both traditional healthcare practices and modern phytopharmacological research.

This study aimed to quantify the total polyphenol and alkaloid contents and assess the antioxidant activity of five plant species collected from various locations in Tra Vinh province. The selected species included *Galphimia gracilis, Leucophyllum frutescens, Alternanthera tenella, Urtica dioica L.,* and *Breynia vitis-idaea* (Burm.f.) C. Fisch. The findings of this research are intended to serve as a scientific foundation for future investigations into the medicinal potential of these and other regional plant species.

2. LITERATURE REVIEW

For thousands of years, traditional medicine systems have relied on medicinal plants to address a wide range of ailments. Even with the rise of modern medicine, the use of plants for therapeutic purposes continues to thrive in many regions around the globe [6]. The growing interest in medicinal plants highlights a renewed appreciation for the validity of many traditional beliefs about the benefits of natural products in healthcare. Plants generate a wide array of secondary metabolites, such as alkaloids, flavonoids, lignans, and tannins, as part of their defense against microbial infections. This makes medicinal plants promising sources for discovering new antimicrobial compounds. Blessed with a tropical climate and abundant rainfall, Tra Vinh boasts a diverse and thriving botanical richness.

This study aims to evaluate five plant species in Tra Vinh province for phytochemistry and antioxidant activity. The plants tested were *Galphimia gracilis*, *Leucophyllum frutescens*, *Alternanthera tenella*, *Urtica dioica* L., and *Breynia vitis-idaea* (Burm.f.) C. Fisch. These plants have been reported to possess various biological activities, including anti-inflammatory, antioxidative, antidiabetic, alpha-glucosidase inhibitory activity, urease inhibitory activity, and anticancer activities [7-10]. Specifically, the report by Md. Rakib Hasan *et al*. [11] stated that the ethyl acetate extract of *G. gracilis* leaves has a TPC of 934.04 ± 3.21 mg/g GAE, total flavonoid content (TFC) of 236.88 ± 2.66 mg/g CE, and lipid peroxidation in human erythrocyte cells with an IC_{50} of 10.38 ± 0.34 µg/ml. However, the extract from *G. gracilis*'s leaves showed relatively weak antibacterial activity when evaluated against *E. coli*, *S. typhi,* and *P. aeruginosa* [7].

Similarly, the research results on phytochemistry for the species *A. tenella* by the author M. Sabitha [12] indicated that the TPC is 61.45 mg/g GAE. Additionally, the analysis using LC ESI-QTOF-MS/MS identified a total of 24 polyphenol compounds, which were categorized into various classes. These included 6 polyphenol acids, 9 flavonoids (comprising both flavonols and flavones), 2 diterpenoids, 1 coumarin, 1 saponin, 3 alkaloids, and 1 glycoside. Another study about the bioactivity of *L. frutescens* revealed that verbascoside (Vb), which was isolated in the aerial extract of *L. frutescens,* possessed strong hepatoprotective properties against TA-induced liver damage in rats and was non-toxic. These findings suggested that Vb, derived from *L. frutescens*, could serve as a promising new therapeutic option for treating liver injuries [13].

However, very few reports are available regarding their information about total alkaloid content, especially in the unique ecological conditions of Tra Vinh. Therefore, this study was conducted to further evaluate the phytochemicals of these plant species in Tra Vinh. At the same time, it aims to provide additional scientific information about local herbal species.

3. MATERIALS AND METHODS

3.1. Materials

Plant materials: aerial parts of *Galphimia gracilis*, *Leucophyllum frutescens*, *Alternanthera tenella*, *Urtica dioica* L, *Breynia vitis-idaea* (Burm.f.) C. Fisch was collected from Tra Vinh province in June 2024.

Solvents and chemicals utilized for the extraction and determination of phytochemicals include ethanol, NaOH, H_2SO_4, Na_2HPO_4, $CuSO_4$, and $Pb(CH_3COO)_2$. All of these were purchased from Xilong Scientific Co., Ltd., China.

Gallic acid (China), caffeine (Sigma), and ascorbic acid (Sigma-Aldrich) were used as standards for evaluation of alkaloid content, polyphenol content, and antioxidant activity in this study.

3.2. Preparation of the extracts

The air-dried aerial parts were ground into a coarse powder using a mechanical grinder. This powdered plant material was subsequently treated with successive solvent extractions, utilizing ethanol as the solvent. The extraction process involved maceration, a technique that entails soaking the plant material in a solvent for an extended period while frequently agitating the mixture to enhance the dissolution of soluble phytoconstituents. Specifically, 50 grams of powdered leaf material was combined with 1000 mL of ethanol in an amber bottle and left to macerate for 24 hours at room temperature. The mixture was stirred at regular intervals, and after 24 hours, it was filtered through Whatman No. 1 filter paper. The resulting filtrate was then concentrated with a rotary evaporator, using reduced pressure and controlled temperature, to yield a semi-solid ethanolic extract. The obtained ethanolic extracts were stored in airtight containers at 4°C until further use in phytochemical screening.

3.3. Method for total polyphenol determination

The total polyphenol content (TPC) was assessed using the Folin-Ciocalteu reagent method, following the protocol established by Singleton *et al*. [14]. The procedure entailed the incorporation of 250 µL of the extract with 250 µL of Folin-Ciocalteu reagent, ensuring thorough homogenization. The resultant mixture was then subjected to incubation in a dark environment at room temperature for five minutes. Subsequently, 250 µL of a 10% sodium carbonate solution (Na_2CO_3) was introduced, after which the mixture was incubated in a water bath maintained at 40 °C for thirty minutes. The absorbance of the reaction mixture was ultimately measured at a wavelength of 765 nm. The results were expressed as gallic acid equivalents per gram of extract (mg GAE/g extract).

3.4. Method for total alkaloid determination

The analytical method is based on the interaction between alkaloids and bromocresol green (BCG), resulting in the formation of a yellow-colored complex, as previously described by Fazel Shamsa (2008) [15] with minor modifications. Caffeine is employed as a reference standard in the quantification of total alkaloid content due to its nature as a naturally occurring alkaloid commonly present in various plant species [16].

Bromocresol green solution: Bromocresol green (69.8 mg) was warmed with 3 mL of 2 N NaOH and 5 mL of distilled water until completely dissolved and diluted to 1000 mL with distilled water.

Phosphate buffer (pH 4.7): pH of 2 M sodium phosphate (71.6 g Na_2HPO_4 in 1 L distilled water) was adjusted to pH 4.7 with 0.2 M citric acid (42.02 g citric acid in 1 L distilled water).

Caffeine standard solution: 1mg pure caffeine was dissolved in 10 mL of distilled water.

3.4.1. Preparation of a calibration curve

Accurately measured aliquots (0.0, 1.0, 2.0, 4.0, 6.0, 8.0 mL) of caffeine standard solution were transferred to different separatory funnels. Five mL phosphate buffer (pH 4.7) and 5 mL BCG solution were added. The mixture was shaken with 1, 2, 3, and 4 mL of chloroform. The extracts were collected in a 10 mL volumetric flask and then diluted to volume with chloroform. The absorbance of the complex in chloroform was measured at 470 nm.

3.4.2. Preparation of samples

Ethanolic extracts (30 mg) were dissolved in 2 N HCl and then filtered. A 1 mL aliquot of this solution was transferred to a separatory funnel and washed with 10 mL of chloroform (3 times). The pH of this solution was adjusted to neutral with 0.1 N NaOH. Then, 5 mL of BCG solution and 5 mL of phosphate buffer were added to this solution. The mixture was shaken, and the complex formed was extracted with 1, 2, 3, and 4 mL of chloroform by vigorous shaking. The extracts were collected in a 10 mL volumetric flask and diluted to volume with chloroform. The absorbance of the complex in chloroform was measured at 417 nm vs. a similarly prepared blank.

The absorbance of the final solution was measured at 470 nm using a UV-Vis spectrophotometer.

$$A = \frac{C.V.a}{m}$$

in which,

A: Total alkaloid content (mg CE/g);

C: Concentration of caffeine from standard curve (µg/mL);

V: Volume of extract (mL);

m: Mass of the material (g).

3.5. DPPH radical assay

The evaluation of antioxidant activity was conducted using the method developed by Adrieli Schett [17]. Different concentrations of extracts (from 0 - 100 µg/mL) and vitamin C (from 0-5 µg/mL) were prepared in test tubes, each containing 1920 µL of the respective concentration and clearly labeled. To each test tube, 80 µL of a 1000 µg/mL DPPH solution was added, bringing the total volume to 2 mL. The mixtures were then incubated at room temperature for 30 minutes in a dark environment. After incubation, the absorbance was measured at 517 nm, and the IC_{50} value was determined using linear regression analysis.

3.6. Statistical analysis

Experimental results were expressed as mean ± standard deviation. All measurements were replicated three times. The IC_{50} values were calculated using linear regression analysis.

4. RESULTS AND DISCUSSION

4.1. Determination of total polyphenol and alkaloid content

Numerous studies have demonstrated that polyphenols found in various ethanolic extracts function as important secondary metabolites, contributing significantly to antioxidant and anti-aging activities [18-20]. Among these, gallic acid—a representative polyphenol—is commonly used as a standard for the quantitative determination of total polyphenol content. In contrast, a caffeine standard curve was employed to assess the total alkaloid content in the samples. To construct the calibration curves, six standard solutions of gallic acid and caffeine were prepared at concentrations ranging from 0 to 100 µg/mL. Both calibration curves exhibited good linearity, with correlation coefficients (r) of 0.9935 for gallic acid and 0.9961 for caffeine. The regression equations were derived from the linear relationship between absorbance (y) and concentration (x), using the formula $y = ax + b$. The resulting regression equation for gallic acid was $y = 0.0094x – 0.0139$, and for caffeine, $y = 0.0036x + 0.019$. These equations were subsequently used to determine the TPC and TAC values in the plant extracts.

The findings from Table 1 revealed considerable variation in both total polyphenol content (TPC) and total alkaloid content (TAC) among the five analyzed ethanolic extracts. The TPC values, ranging from 91.60 to 512.67 mg GAE/g extract, align with previous literature yet also showed notable differences, potentially due to factors such as extraction method, plant maturity, and growing conditions.

Table 1. The total polyphenol (TPC) and alkaloid content (TAC) of five extracts

No.	Samples	TPC (mg GAE/g extract)	TAC (mg CE/g extract)
1	*Galphimia gracilis*	512.67 ± 1.36	1.25 ± 0.06
2	*Leucophyllum frutescens*	91.60 ± 0.21	0.71 ± 0.11
3	*Alternanthera tenella*	354.10 ± 0.72	3.68 ± 0.17
4	*Urtica dioica L.*	388.68 ± 0.31	0.66 ± 0.03
5	*Breynia vitis-idaea* (Burm.f.) C. Fisch	374.82 ± 0.45	0.95 ± 0.14

For instance, the TPC of *G. gracilis* in this study (512.67 mg GAE/g) was lower than the value reported by Md. Rakib Hasan *et al*. (2014) [7] found 934.04 ± 3.21 mg GAE/g in the ethyl acetate extract. Similarly, the results also revealed considerable variation in the total alkaloid content (TAC) among the five investigated plant species, with values ranging from 0.66 to 3.68 mg CE/g extract. The highest alkaloid concentration was observed in *A. tenella* (3.68 ± 0.17 mg CE/g extract), followed by *G. gracilis* (1.25 ± 0.06 mg CE/g extract), *B. vitis-idaea* (Burm.f.) C. Fisch (0.95 ± 0.14 mg CE/g extract), *L. frutescens* (0.71 ± 0.11 mg CE/g extract), and *U. dioica* L. (0.66 ± 0.03 mg CE/g extract). These findings indicate that the studied plant extracts contain moderate levels of alkaloids, an important class of secondary metabolites known for their diverse pharmacological effects. Previous studies have shown that alkaloids possess a wide range of biological activities, including analgesic, anti-inflammatory, and sedative properties, particularly due to their influence on the central nervous system [21].

Notably, there is a paucity of scientific literature specifically quantifying alkaloid content in the plant species examined in this study. Existing research has primarily focused on other classes of phytochemicals, such as polyphenols and flavonoids. Therefore, the present study contributes novel quantitative data on alkaloid levels in these species, particularly for *A.*

tenella and *G. gracilis*, which demonstrated relatively high TAC values. These results not only provide valuable phytochemical insights but also support the traditional medicinal use of these species in Tra Vinh province, highlighting their potential as promising sources of bioactive compounds for future pharmacological applications.

4.2. DPPH radical assay

The antioxidant activity of the evaluated extract was assessed through its ability to inhibit DPPH free radicals. Once diluted to appropriate concentrations for testing, the extract exhibited a strong capacity to neutralize these radicals. Interestingly, its effectiveness in counteracting DPPH radicals increased with higher concentrations of the extract. Table 2 shows the antioxidant activity of the 5 extracts in our study.

Table 2. Antioxidant activity (%) of five extracts

No.	Samples	IC_{50} (µg/mL)
1	*Galphimia gracilis*	69.88 ± 0.63
2	*Leucophyllum frutescens*	201.54 ± 1.96
3	*Alternanthera tenella*	105.47 ± 2.06
4	*Urtica dioica L*	116.86 ± 2.31
5	*Breynia vitis-idaea* (Burm.f.) C. Fisch	97.25 ± 0.58
6	Ascorbic acid	3.72 ± 0.02

Based on the data presented in Table 2, all five ethanolic plant extracts demonstrated measurable antioxidant activity, as indicated by their IC_{50} values, which ranged from 69.88 to 201.54 µg/mL. Notably, *Galphimia gracilis* emerged as the most potent antioxidant among the tested samples, with an IC_{50} of 69.88 ± 0.63 µg/mL. This is in line with findings by Md. Rakib Hasan *et al*. [7] reported a remarkably low IC_{50} of 22.82 ± 0.17 µg/mL for the ethyl acetate extract of the same species in the DPPH assay. Furthermore, a 2024 study by Narashimachar Joshi [22] confirmed the presence of multiple bioactive constituents in *G. gracilis*, including flavonoids, terpenoids, and alkaloids—compounds known to contribute to antioxidant and pharmacological activities such as anti-inflammatory, analgesic, anxiolytic, and anticonvulsant effects.

In contrast, *L. frutescens*, *A. tenella*, and *U. dioica L.* displayed moderate antioxidant activity, with IC_{50} values substantially higher than that of ascorbic acid, by factors of approximately 54, 28, and 31, respectively. These results are consistent with previous studies. For instance, Imtiaz Ahmad [10] reported an IC_{50} of 209.59 ± 8.50 mg Trolox equivalents/g for the chloroform extract of *L. frutescens*, supporting the observed moderate activity in this study.

Interestingly, stronger antioxidant activity has been reported for *A. tenella* in a study by M. Sabitha *et al*. [12], where the extract showed an IC_{50} of only 8.53 µg/mL. Their LC ESI-QTOF-MS/MS analysis also identified 24 distinct polyphenol compounds, further substantiating its antioxidant potential. Likewise, *Urtica dioica* L. was shown by Hari Prasad Devkota [9] to possess notable antioxidant activity, with an IC_{50} of 88.33 ± 2.88 µg/mL, which aligns well with the present findings.

On the other hand, the Pearson correlation coefficient $r = -0.97$ between TPC and IC_{50} value indicates a very strong inverse relationship, supporting the hypothesis that polyphenols are the principal contributors to the antioxidant capacity of the samples. This result provides

valuable reference data for both research and practical applications related to natural antioxidant compounds.

In addition, there was no clear correlation between the total alkaloid content and the antioxidant activity of the analyzed samples. This may be explained by the presence of other chemical constituents (such as polyphenols, flavonoids, etc.) in the plant samples that exhibit more significant antioxidant activity compared to alkaloids. Furthermore, future studies should be conducted to thoroughly evaluate specific alkaloid compounds in these samples in order to draw clearer conclusions about the relationship between their activity and chemical structure.

Collectively, these comparisons highlight both the consistency and uniqueness of the antioxidant profiles observed in plant species native to Tra Vinh province. The results underscore the value of local flora as a promising source of natural antioxidants and warrant further phytochemical and pharmacological investigation.

5. CONCLUSION

This study provides new insights into the phytochemical composition and antioxidant capacity of five ethanolic plant extracts collected from Tra Vinh province. The results reveal considerable variation in total polyphenol content (TPC), total alkaloid content (TAC), and antioxidant activity among the species analyzed. Notably, *Galphimia gracilis* exhibited the highest TPC and antioxidant capacity, followed by *Breynia vitis-idaea* (Burm.f.) C. Fisch, highlighting their potential as rich sources of natural antioxidants. A moderate correlation between TPC, TAC, and antioxidant activity was observed, suggesting that both polyphenol and alkaloid compounds contribute to the overall bioactivity of these plants.

These findings not only underscore the medicinal potential of native plant species in Tra Vinh but also support their use in traditional remedies and future applications in pharmaceutical and nutraceutical development. Further investigations are essential to isolate, identify, and characterize the individual bioactive constituents responsible for these effects and to validate their therapeutic efficacy through in vivo and clinical studies.

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TÓM TẮT

ĐÁNH GIÁ HÀM LƯỢNG POLYPHENOL, ALKALOID TOÀN PHẦN VÀ HOẠT TÍNH KHÁNG OXY HÓA CỦA MỘT SỐ LOÀI THỰC VẬT TRÊN ĐỊA BÀN TỈNH TRÀ VINH

Nguyễn Xuân Thị Diễm Trinh*, Nguyễn Thị Ngọc Trăm,
Huỳnh Thị Hồng Hoa, Mai Thị Thùy Lam
Trường Đại học Trà Vinh
*Email: *trinhnguyen@tvu.edu.vn*

Mục tiêu của nghiên cứu là xác định tổng hàm lượng polyphenol và alkaloid, cũng như hoạt tính chống oxy hóa của các cao chiết ethanolic từ năm loài thực vật được thu thập ở tỉnh Trà Vinh. Hàm lượng polyphenol tổng (TPC) được đo bằng phương pháp Folin-Ciocalteu, và hàm lượng alkaloid tổng (TAC) được xác định bằng quang phổ UV-vis. Hoạt tính chống oxy hóa được đánh giá thông qua thử nghiệm khử gốc tự do DPPH. Kết quả cho thấy TPC dao động từ 91,60 đến 512,67 mg GAE/g cao chiết và TAC từ 0,36 đến 3,68 mg CE/g. Phần trên mặt đất của *Galphimia gracilis* thể hiện hoạt tính chống oxy hóa mạnh nhất (IC50 = 69,88 ± 0,63 µg/mL). Mặt khác, các kết quả phân tích cũng thể hiện mối tương quan giữa hoạt tính chống oxy hóa và hàm lượng polyphenol trong các mẫu thử nghiệm, cho thấy rằng các hợp chất polyphenol đóng góp đáng kể vào khả năng loại bỏ gốc tự do. Đây là báo cáo đầu tiên về các loài này từ tỉnh Trà Vinh, cung cấp dữ liệu quý giá cho các nghiên cứu về dược liệu tại địa phương và các thành phần các hợp chất thứ cấp trong thảo dược trong tương lai.

Từ khóa: Hàm lượng alkaloid toàn phần, polyphenol toàn phần, hoạt tính kháng oxy hóa, tỉnh Trà Vinh.