 DOI: 10.35382/TVUJS.15.1.2025.114

DETERMINATION OF TOTAL ALKALOID, POLYPHENOL CONTENT AND ANTIOXIDANT ACTIVITY OF ETHANOLIC EXTRACT OF *ACANTHUS INTEGRIFOLIUS* T. ANDERS

Nguyen Xuan Thi Diem Trinh[1*], Huynh Thi Hong Hoa[2], Mai Thi Thuy Lam[3]

Abstract – *This study sought to evaluate the alkaloid and polyphenol content, as well as the antioxidant activity, of the ethanolic extract of Acanthus integrifolius T. Anders, collected from Hoa Thuan Commune in Chau Thanh District, Tra Vinh Province, Vietnam. It belongs to the Acanthaceae family and is also an ornamental plant. In addition, the plant is also a medicinal herb in traditional medicine that helps relieve joint pain. The results of preliminary phytochemical screening indicated the presence of various compounds within the ethanolic extract, including alkaloids, flavonoids, steroids, tannins, glycosides, and saponins. Total phenolic content (TPC) was determined spectrophotometrically by Folin Ciocalteu colorimetric method. Total alkaloid content (TAC) of ethanolic extracts was determined by spectrophotometric method based on the reaction with Bromocresol Green (BCG) with caffeine as a standard. The total alkaloid and polyphenol contents of the extract were quantified, yielding values of 0.84±0.01 mgCE/g and 373.86±1.08 mgGAE/g, respectively. Furthermore, the antioxidant activity of the extract was evaluated by DPPH (1,1-diphenyl-2-picrylhydrazil) radical assay, resulting in an IC_{50} value of 57.76±0.9 µg/mL. This research represents the inaugural report of the findings pertaining to the aerial parts of Acanthus integrifolius T. Anders.*

Keywords: Acanthus intergrifolius, alkaloid content, antioxidant activity, polyphenol content.

[1,2,3]Tra Vinh University, Vietnam
*Corresponding author: trinhnguyen@tvu.edu.vn
Received date: 26 December 2024; Revised date: 5 March 2025; Accepted date: 20 March 2025

I. INTRODUCTION

Acanthus is a genus of about 30 species of flowering plants in the family Acanthaceae, native to tropical and warm temperate regions, with the highest species diversity in the Mediterranean Basin and Asia, such as *Acanthus albus* Debnath, *Acanthus ebracteatus* Vahl, *Acanthus ilicifolius* L., *Acanthus hirsutus* Boiss., ... [1]. In Vietnam, excepting three species *A. ebracteatus*, *A. ilicifolius*, and *A. leucostachyus*, Pham Hoang Ho [2] noticed in 'An illustrated flora of Vietnam' a specie named 'Ac o', which scientific name is Acanthus integrifolius T. Anders (AI). It possesses a multitude of significant applications in human life. It is frequently cultivated as a hedge in various settings, including educational institutions and public parks. Furthermore, this plant is noteworthy in the realm of traditional medicine, where it has exhibited remarkable biological activities, particularly in the alleviation of rheumatism and joint pain [3]. However, research on this species in Vietnam remains limited, particularly in Tra Vinh Province. Consequently, this study seeks to identify the chemical components present in the ethanolic extract of the aerial parts of the plant and to assess its antioxidant activities.

II. LITERATURE REVIEW

In conventional medicine, *Acanthus* species are particularly used to treat illnesses of the respiratory, frightened, and reproductive system, gastrointestinal and urinary tract, and pores and skin illness. The maximum used species are *A. montanus*, *A. ilicifolius*, and *A. ebracteatus*. Chemical compounds (125) from one of the chemical instructions had been remoted and diagnosed in seven species, particularly from *A.*

ilicifolius, approximately 80, observed via way of means of *A. ebracteatus* and *A. montanus*, acting with a barely decreased variety with fewer phytochemical profile studies. This evidence bolsters the case for further investigation into the plants of this genus [4].

According to the three species recorded in Vietnam, preliminary investigations into the floral samples of *Acanthus ilicifolius* suggest its efficacy in inhibiting DPPH (1,1-diphenyl-2-picrylhydrazil) free radicals, evidenced by an LC_{50} value of 22 μg/mL [5]. Furthermore, research into the antibacterial properties of *Acanthus ilicifolius* has demonstrated that the minimum inhibitory concentration (MIC) of its ethanol, methanol, and chloroform extracts against five bacterial strains ranges from 2 to 64 μg/mL [6]. According to Chinnathambi et al. [7], *A. ilicifolius* methanolic extracts included a variety of bioactive components, including alkaloids, saponins, phenolics, flavonoids, steroids, cardiac glycosides, tannins, and terpenoids. Another study conducted by Tran et al. [8] echoed these findings regarding phytochemicals, that *A. ilicifolius* holds significant promise as a valuable natural medicinal resource. This plant not only boasts antioxidant properties but also exhibits the ability to inhibit both α-amylase and α-glucosidase. Moreover, in the study of Sravya et al. [9], leaf extracts from *A. ilicifolius* were evaluated for their in vitro activities. Among the four extracts examined including methanol, petroleum ether, aqueous and ethyl acetate extract, the methanol extract exhibited the most significant antibacterial effects, showing inhibition against *P. aeruginosa* (4$\pm$0.3 mm), A. hydrophila (5.9$\pm$0.5 mm), S. aureus (3.5$\pm$0.7 mm), and B. subtilis (2.9$\pm$0.5 mm). Additionally, this extract demonstrated impressive antioxidant activity, with DPPH values of 81.3$\pm$1.0 AAEμg/ml and FRAP values of 139.1$\pm$1.5 AAEμg/ml.

The second most studied species was *A. ebracteatus*, with a similar profile but fewer isolated [4]. *A. ebracteatus* is rich in chemical compounds with anti-inflammatory, neuroprotective, and wound-healing properties. These include fatty alcohols, glycosides, phenolic glycosides, sesquiterpenes, flavonoids, and lignin glycosides. In Chinese folk medicine, it is commonly used to treat various conditions such as rheumatism, cough, wound inflammation, hepatitis, and skin rashes [10]. According to Khamwut et al. [11], crude extract of *A. ebracteatus* protein hydrolysate significantly inhibited A431 cells with half inhibitory concentration equals to 425.9 ng protein/mL. In addition, Olatunji et al. [12] indicated in their research that extracts from the leaves and stem/root of *A. ebracteatus* identified several bioactive compounds in the extracts, including flavonoids, phenols, iridoids, and nucleosides. These results suggested that *A. ebracteatus* could have significant applications, particularly in the investigation of disorders related to oxidative stress. Another study about this genus in 2023, Mayuree et al.[13] confirmed that the phenolic-rich extracts of *A. ebracteatus* had in vitro antioxidant and anti-tyrosinase activities. Conclusions about the antioxidant and antibacterial activity of phenolic-rich plant species have also been confirmed in studies on other species such as *Tagetes erecta* (L.) [14] and *Camellia sinensis* L. [15].

About *A. leucostachyus*, Deepjyoti et al. [16] showed that treatment with *Acanthus leucostachyus* leaf extracts markedly improved the charge of wound contraction, tensile strength, the concentrations of protein, DNA, and hydroxyproline, and the expression of the boom factor, in addition to promoted epithelialization, in comparison to the control. In addition, *A. leucostachyus* leaf extracts considerably decreased the expression of pro-inflammatory cytokines.

These findings indicate that the *Acanthus* genus possesses considerable potential for therapeutic applications, attributable to its diverse phytochemical composition and antimicrobial properties. Therefore, further research must be conducted on AI to identify and characterize its specific bioactive compounds, as well as to evaluate their potential for pharmaceutical development.

III. MATERIALS AND METHODS

A. Materials

Regarding AI, the aerial parts of AI were collected from Tra Vinh Province, Vietnam, in August 2024. In this study, solvents and chemicals utilized for the extraction, determination of phytochemicals, and evaluation of total phenolic content (TPC), total alkaloid content (TAC), and antioxidant activity include ethanol, NaOH, H_2SO_4, Na_2HPO_4, $CuSO_4$, $Pb(CH_3COO)_2$, gallic acid, caffeine, and ascorbic acid. All of these were purchased from Sigma-Aldrich and Xilong Scientific Co., Ltd., China.

B. Methods

Preparation of the extract

The samples were subjected to drying and subsequently ground into a fine powder. Extraction was performed using 70% ethanol via a soaking method, adhering to a solid-to-solvent ratio of 1:20 (m/V) for 24 hours at ambient temperature. Thereafter, the filtrate was concentrated under reduced pressure at a temperature of 40^{o}C and subsequently stored at 4^{o}C.

Identification of chemical compositions

The extracts, comprising groups of organic compounds, namely alkaloids, flavonoids, steroids, tannins, glycosides, and saponins, were systematically identified through chemical reactions utilizing specific reagents. The qualitative results are indicated with a (+) symbol to signify the presence of phytochemicals, and a (–) symbol to denote their absence.

Determination of total phenolic content

The TPC was assessed using the Folin-Ciocalteu reagent method, following the protocol established by Singleton et al. [17]. 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^{o}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).

Determination of total alkaloid content

The method relies on the interaction between alkaloids and bromocresol green (BCG), leading to the formation of a yellow-colored product, as detailed by Shamsa et al. [18], with several modifications. To prepare the bromocresol green solution, BCG (69.8 mg) was heated with 3 mL of 2 N NaOH and 5 mL of distilled water until fully dissolved. The solution was diluted to 1,000 ml with distilled water. Next, a phosphate buffer solution with a pH of 4.7 is prepared by adjusting the pH of a 2 M sodium phosphate solution (71.6 g of Na_2HPO_4 in 1 L of distilled water) to 4.7 using 0.2 M citric acid (42.02 g of citric acid in 1 L of distilled water). The caffeine standard solution was made by dissolving 1 mg pure caffeine in 10 ml distilled water.

- Preparation of standard curve

Accurately measure aliquots of the caffeine standard solution in the following specified volumes: 8.0 mL, 6.0 mL, 4.0 mL, 2.0 mL, 1.0 mL, and 0.0 mL. Each aliquot was transferred into individual separatory funnels. Then, 5 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 the final volume was brought to 10 mL by adding additional chloroform. Finally, the absorbance of the complex in chloroform was measured at 470 nm.

- Extraction

The plant materials (100 grams) were subjected to grinding and subsequently underwent ethanol extraction via a 24-hour soaking process. Following this procedure, the resulting mixture was filtered, and the ethanol was evaporated using a rotary evaporator under a vacuum until dryness was achieved. A portion of the resultant residue was then dissolved in 2 N hydrochloric acid and subjected to filtration once more. From this solution, 1 mL was transferred to a separa-

tory funnel and washed three times with 10 mL of chloroform. The pH of the resultant solution was adjusted to neutrality through the addition of 0.1 N sodium hydroxide. Subsequently, 5 mL of bromocresol green solution and 5 mL of phosphate buffer were incorporated into the mixture, which was shaken vigorously to promote complex formation. The complex was then extracted using 1, 2, 3, and 4 mL of chloroform with vigorous shaking. The extracts obtained were combined in a 10 mL volumetric flask, and the volume was adjusted to mark with chloroform. Finally, the absorbance of the complex in chloroform was measured at a wavelength of 470 nm, The total alkaloid content was calculated using the following Formula (1).

$$A = \frac{C.V.a}{m} \quad (1)$$

In which:

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

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

V: Volume of extract (mL);

m: Mass of the material (g).

DPPH radical assay

The evaluation of antioxidant activity was conducted using the method developed by Schett [19]. Initially, a control series of vitamin C solutions was prepared, with concentrations ranging from 0 to 5 μg/mL. In parallel, a series of extract solutions was created, with concentrations varying from 0 to 100 μg/mL, using methanol as the solvent. To initiate the reaction, 0.5 mL of a 40 μg/mL DPPH solution was added to each sample. The mixtures were then allowed to react for 30 minutes under low light conditions, after which the absorbance was measured at 517 nm. The percentage of free radical inhibition by DPPH (I%) was calculated using the following Formula (2).

$$I\% = \frac{A_C - A_S}{A_C} \times 100 \quad (2)$$

In which: Ac: the absorbance of DPPH solution;

As: the absorbance of DPPH solution after reacting with antioxidant.

The antioxidant capacity of a substance, as evaluated through the DPPH method, is quantified by the IC_{50} value, or Half-Maximal Inhibitory Concentration [19]. This value reflects the concentration of an antioxidant required to inhibit 50% of DPPH free radicals. A lower IC_{50} value is indicative of enhanced antioxidant activity. Calculations were performed to ascertain the IC_{50} by utilizing the equation of the standard curve, which correlates the percentage of inhibition with the antioxidant concentration.

Statistical analysis

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

IV. RESULTS AND DISCUSSION

A. Identification of chemical compositions

The findings regarding the chemical composition of the extract are summarized in Table 1.

Table 1: The outcomes of the identification of chemical compounds

Compounds	Reagent	Observation	Ethanolic extract
Alkaloid	Mayer	creamy white precipitate	++
Flavonoid	H_2SO_4 conc	reddish brown precipitate	++
Steroid and Triterpenoid	Liebermann-Burchard Salkowski	reddish ring reddish brown color	+ +
Tannin	saturated $Pb(CH_3COO)_2$	precipitate	+
Glycoside	Fehling test	reddish brown precipitate	++
Saponin	Foam test	Foam produced persisted for 10mins	+

Table 1 reveals that the extract of the aerial parts of AI is abundant in a variety of compounds, such as alkaloids, flavonoids, steroids, tannins, and glycosides. The phytochemical analysis of the selected plant extract showed significant positive results, evidenced by striking color changes. Among these compounds, flavonoids,

alkaloids, and glycosides were found to be the most prevalent classes.

B. Determination of total polyphenol and alkaloid content

The antioxidant activity of plants is largely influenced by their secondary metabolite compounds, which exhibit various bioactivities. Previous studies have demonstrated that polyphenols found in many ethanolic extracts are crucial secondary metabolites that contribute significantly to antioxidant and anti-aging effects [14, 15, 20, 21]. Notably, gallic acid, a type of polyphenol, serves as a standard for the quantitative analysis of total phenolic content. On the other hand, the caffeine standard curve was established for measuring the total alkaloid content in the samples. As illustrated in Figure 1, the standard equation for gallic acid and caffeine was determined to be $y = 0.0094x - 0.0139$ ($R^2 = 0.9935$) and $y = 0.0036x + 0.019$ ($R^2 = 0.9961$), respectively. Consequently, the total polyphenol and alkaloid content in AI's aerial extract were determined to be 373.86±1.08 mg GAE/g and 0.84±0.01 mgCE/g. All measurements were conducted in triplicate, and the mean values were calculated.

Chinnathambi et al. [7] collected stem samples of *A. ilicifolius*, a plant of the same genus as AI, in Madras Presidency, India, and evaluated the concentration of polyphenols and alkaloids in them. The results demonstrated that the leaves of *A. ilicifolius* had polyphenol and alkaloid content of 42.3±0.08 mg GAE/g extract and 9.23±0.09 mg QE/g extract, respectively. From this study, the AI extract had a higher polyphenol content but a lower alkaloid content than *A. ilicifolius* stems. Another result from Tran et al. [8] showed that the above-ground parts of *A. ilicifolius* cultivated in Ca Mau Province (Vietnam) had a polyphenol content of 63.51±1.16 mg GAE/g extract. This value was also significantly lower than the results in this study.

Compared to another *Acanthus* genus, *A. ebracteatus*, Olatunji et al. [12] showed in their research that the polyphenol content of *A. ebracteatus* extract was 140.50 mg/GAE g extract. It was 2.66 times lower than this study. For the first time, this study has offered scientific findings concerning the phytochemicals of AI's components cultivated in Tra Vinh Province.

C. DDPH radical assay

The antioxidant activity of the evaluated extract was determined by its capacity to inhibit DPPH free radicals. After being diluted to suitable concentrations for testing its antioxidant activity, the extract demonstrated a robust ability to neutralize DPPH free radicals. Notably, this capacity to counteract the DPPH radicals increased with higher concentrations of the extract. Figure 2 illustrates the correlation between extract concentration and antioxidant activity.

The findings indicated that the IC_{50} value of the ethanolic extract of AI was 57.76±0.69 μg/mL. In comparison, the IC_{50} value for the control solution, ascorbic acid, was determined to be 3.72 μg/mL, thereby suggesting that the antioxidant activity of the AI extract is approximately 15 times lower. AI extract is a notion to have mild antioxidants inside the DPPH method.

Two studies about *A. ilicifolius* extract's antioxidant activity of Tran et al. [8] and Sravya et al. [9] showed that an aqueous extract from *A. ilicifolius*'s stems and leaves effectively neutralized DPPH free radicals with an IC_{50} value of 149.26±0.13 μg/mL and 164.8 μg/mL by aqueous extract, respectively. Thus, AI's aerial parts extract in this research exhibits approximately 2.5–2.8 times the DPPH free radical neutralizing activity of *A. ilicifolius* 's aqueous extract. Besides, the antioxidant activity result was recorded to be equivalent to the antioxidant activity of *A. ebracteatus*'s leaf extract, which was reported by Mayuree et al. [13] with an IC_{50} of 59.05±0.23 μg/mL. This study verified that the extract from the above-floor components of AI includes secondary metabolites, which can neutralize or lessen loose radicals and steel complexes to a much less poisonous state. Further research should be done to evaluate the antioxidant activity of this plant by using more methods such as ABTS•+, ferric reducing antioxidant power

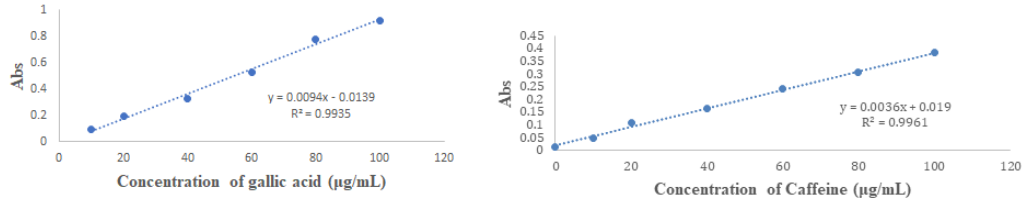

Fig. 1: Gallic acid (left) and caffeine standard curve (right)

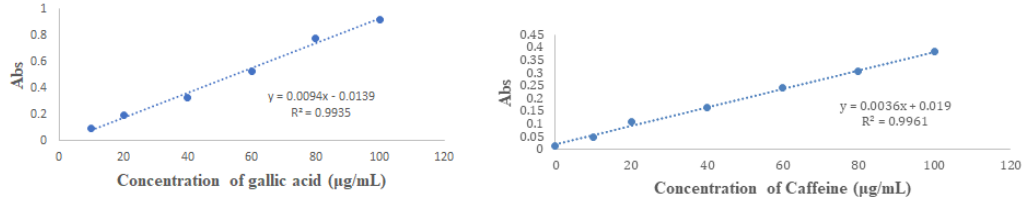

Fig. 2: Antioxidant activity (%) of ascorbic acid (a) and AI extract (b)

(FRAP) method, and the like to confirm its antioxidant activity.

V. CONCLUSION

The study conducted a comprehensive assessment of the total phenolic and alkaloid content, in addition to evaluating the antioxidant capacity of ethanolic extracts derived from the *A. integrifolius* plant, which was collected in Chau Thanh District, Tra Vinh Province, Vietnam. Specifically, the TPC and TAC were determined to be 373.86±1.08 mgGAE/g and 0.84±0.01 mgCE/g, respectively. Additionally, the ethanol extract of this species also demonstrated antioxidant activity with an IC_{50} value of 57.76±0.69 μg/mL. Based on the results obtained, that is a supply of essential secondary metabolites from the plant against oxidation. Further research is needed to investigate more about the antimicrobial and anti-inflammatory properties, mainly in the unexplored and precious aspects of *A. integrifolius*.

REFERENCES

[1] Umberto Q. *CRC world dictionary of plant names: common names, scientific names, eponyms, synonyms, and etymology*. Vol. 1 A-C. United States: CRC Press; 2000.

[2] Pham Hoang Ho. *An illustrated flora of Vietnam [Cây cỏ Việt Nam]*. Vol. III. Ho Chi Minh City, Vietnam: Tre Publishing House; 1999].

[3] Dak Nong Newspaper. *Acanthus integrifolius T. Anders: meaning, images, planting, caring at home [Cây Ắc Ó: Ý nghĩa, hình ảnh, cách trồng, chăm sóc tại nhà]*. https://baodaknong.vn/cay-ac-o-y-nghia-hinh-anh-cach-trong-cham-soc-tai-nha-171437.html. [Accessed 27 March 2025].

[4] Patrícia M, Maria TB, Artur F. A review of the ethnomedicinal uses, chemistry, and pharmacological properties of the genus *Acanthus* (Acanthaceae). *Journal of Ethnopharmacology*. 2022;293: 115271. https://doi.org/10.1016/j.jep.2022.115271.

[5] Muhamad F, Asep AP, Rahmi N. Antioxidant and cytotoxic activity of *Acanthus ilicifolius* flower. *Asian Pacific Journal of Tropical Biomedicine*. 2013;3(1): 17–21. https://doi.org/10.1016/S2221-1691(13)60017-9.

[6] Natarajan S, Rameshbabu V, Ariharasudhan S, Hayavadana J, Rajpurohit T, Prakash C. Antimicrobial properties of *Acanthus ilicifolius* and determination of phytochemical composition. *Biomass Conversion and Biorefinery*. 2024;14(23): 29719–29726. https://doi.org/10.1007/s13399-023-04723-7.

[7] Chinnathambi P, Paulra B, Ramkumar S, Muthukrishnan G, Rangasamy SB, Abdullah ADM, et al. Evaluating antimicrobial activities of *Acanthus ilicifolius* L. and *Heliotropium curassavicum* L against bacterial pathogens: an in-vitro study. *Journal of Infection and Public Health*. 2021;14: 1927–1934. https://doi.org/10.1016/j.jiph.2021.10.013.

[8] Tran CL, Do VM, Huynh VT, Nguyen VK, Chong KTD. Antioxidant and antidiabetic effects in vitro of extract from the above-ground parts of *Acanthus ilicifolius*. *Bionatura Journal*. 2024;1(3): 3. http://dx.doi.org/10.70099/BJ/2024.01.03.4.

[9] Sravya MVN, Simhachalam G, Kumar NSS. Anti-pathogenicity of *Acanthus ilicifolius* leaf extracts against A. hydrophila infection in Labeo rohita fingerlings. *AMB Expres*. 2023;13: 86. https://doi.org/10.1186/s13568-023-01595-y.

[10] Dongna M, Shiwei S, Liufeng W, Qiansu D, Hai-Lei Z. Comparative transcriptome analysis on the mangrove *Acanthus ilicifolius* and its two terrestrial relatives provides insights into adaptation to intertidal habitats. *Gene*. 2022;839: 146730. https://doi.org/10.1016/j.gene.2022.146730.

[11] Khamwut A, Jevapatarakul D, Reamtong O, Thienprasert T, Nattanan P. In vitro evaluation of anti-epidermoid cancer activity of *Acanthus ebracteatus* protein hydrolysate and their effects on apoptosis and cellular proteins. *Oncol Lett*. 2019;18(3): 3128–3136. https://doi.org/10.3892/ol.2019.10647.

[12] Olatunji OJ, Olatunde OO, Jayeoye TJ, Singh S, Nalinbenjapun S, Sripetthong S, et al. New insights on *Acanthus ebracteatus* Vahl: UPLC-ESI-QTOF-MS profile, antioxidant, antimicrobial and anticancer activities. *Molecules*. 2022;27(6): 1981. https://doi.org/10.3390/molecules27061981.

[13] Mayuree K, Puxvadee C, Mattaka K, Tawin I, Nattaya L. Anti-aging of phenolic-rich *Acanthus ebracteatus* Vahl. extracts. *Chemical and Biological Technologies in Agriculture*. 2023;10(1): 32. https://doi.org/10.1186/s40538-023-00403-w.

[14] Nguyen Trong Tuong, Huynh Duy Khang, Nguyen Quang Minh Hoc, Tri Kim Ngoc, Huynh Ngoc Trung Dung. Determination of polyphenol, flavonoid content and antioxidant activity in *Tagetes erecta* (L.) [Xác định hàm lượng Polyphenol, Flavonoid và hoạt tính kháng oxy hóa của cao chiết từ vạn thọ (*Tagetes erecta* L.) hoa vàng và hoa cam]. *Journal of Scientific Research and Economic Development [Tạp chí Nghiên cứu khoa học và Phát triển kinh tế]*. 2020;08: 188–198.

[15] Ren T, Zheng P, Zhang K, Liao J, Xiong F, Shen Q, et al. Effects of GABA on the polyphenol accumulation and antioxidant activities in tea plants (*Camellia sinensis* L.) under heat-stress conditions. *Plant Physiology and Biochemistry*. 2021;159: 363–371. https://doi.org/10.1016/j.plaphy.2021.01.003.

[16] Deepjyoti D, Ashish S, Bishnupada R. *Acanthus leucostachyus* leaf extracts promote excision wound healing in mice. *Asian Pacific Journal of Tropical Biomedicine*. 2022;12(11): 475–482.

[17] Singleton VL, Orthofer R, LamuelaRaventós RM. Analysis of total phenols and other oxidation substrates and antioxidants by means of folinciocalteu reagent. *Methods in enzymology*. United States: Academic Press; 1999. p.152–178. https://doi.org/10.1016/S0076-6879(99)99017-1.

[18] Shamsa F, Monsef H, Ghamooshi R, Verdian-Rizi M. Spectrophotometric determination of total alkaloids in some Iranian medicinal plants. *The Thai Journal of Pharmaceutical Sciences*. 2008;32: 17–20. https://doi.org/10.56808/3027-7922.2196.

[19] Sachett A, Gallas-Lopes M, Conterato GMM, Herrmann A, Piato A. *Antioxidant activity by DPPH assay: in vitro protocol*. Brazil: Lapcom UFRGS; 2021. https://dx.doi.org/10.17504/protocols.io.btbpnimn.

[20] Manach C, Williamson G, Morand C, Scalbert A, Rémésy C. Bioavailability and bioefficacy of polyphenols in humans. I. Review of 97 bioavailability studies. *The American Journal of Clinical Nutrition*. 2005;81(1): 230S–242S. https://doi.org/10.1093/ajcn/81.1.230S.

[21] Scalbert A, Manach C, Rémésy C, Morand C, Jiménez L. Dietary polyphenols and the prevention of diseases. *Critical Reviews in Food Science and Nutrition*. 2005;45(4): 287–306. https://doi.org/10.1080/1040869059096.