doi.org/10.36721/PJPS.2024.37.2.SP.439-446.1

Antioxidant potentials of extracts from different parts of *Clinacanthus nutans* (Burm. f.) Lindau

Khanh Duy Dang*, Chi Hoang Minh Nguyen,
Thao Ngoc Nha Nguyen and Dai Thi Trang Nguyen
Faculty of Pharmacy, Can Tho University of Medicine and Pharmacy, Can Tho City, Vietnam

Abstract: The research aimed to explore the antioxidant potential of extracts from different parts of *Clinacanthus nutans* growing in Vietnam, a member of the Acanthaceae family. The plant's roots, stem, and leaves were extracted using 96% ethanol. The antioxidant actions of these extracts were evaluated by DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) assay on thin-layer plates and 96 well plates. The extract with the most potent activity was applied for distribution extraction with solvents with different polarities, including dichloromethane, ethyl acetate and water. Dry column vacuum chromatography was utilized to obtain the most antioxidant-potent extract fractions. The stem extract had the lowest IC_{50} value of 6.85µg/mL, showing the most potent antioxidant activity. The ethyl acetate fraction from the stem extract expressed the lowest IC_{50} value of 9.67µg/mL. Meanwhile, fraction 5, separated from the ethyl acetate fraction of the stem extract, had the lowest IC_{50} value of 9.89µg/mL. In conclusion, the extracts from different parts of *Clinacanthus nutans* all expressed antioxidant action at different levels, in which the stem extract, the ethyl acetate fraction and fraction 5 from the ethyl acetate fraction displayed the most effective actions. These findings highlight the promising potential of *Clinacanthus nutans* in treating oxidative stress-associated diseases, inspiring further research and exploration in this area.

Keywords: *Clinacanthus nutans*, free radicals, antioxidant activity, 2,2-diphenyl-1-picryl-hydrazyl-hydrate.

INTRODUCTION

The harmful effect of free radicals is one of the interests of scientists nowadays. Free radicals are continuously generated in the human body. Phagocytosis, cellular respiration, and liver detoxification pathways produce free radicals. Free radicals induce cell damage, attacking vital macromolecules and interfering with tissue homeostasis. Among them, proteins, nucleic acids and lipids are free radicals' main targets, altering the normal redox state and increasing oxidative stress. Oxidative stress induced by free radicals has been reported to be associated with many diseases, such as cardiovascular diseases (hypertension and atherosclerosis), neurodegenerative disorders (Parkinson's disease and Alzheimer's disease), diabetes, cataracts, rheumatoid arthritis, cancers and the aging process (Lobo *et al.*, 2010; Phaniendra *et al.*, 2015).

Clinacanthus nutans (Burm. f.) Lindau, an Acanthaceae family member, has been used for a long time in Asian countries as a traditional medicine. In Malaysia, fresh leaves were boiled in water and used as herbal tea. Fresh leaves were also extracted with alcohol and applied externally to cure skin rashes, *Varicella zoster* and *Herpes simplex virus* infection, snakebites and insect stings in Thailand (Lin *et al*., 2023). Meanwhile, the entire plant was used in China to treat inflammatory diseases such as rheumatism and hematoma. In addition, the plant was also utilized by Chinese herbalists to heal bone fractures, anemia and jaundice, relieve pain and regulate menstruation function (Alam *et al*., 2016).

The extracts from *Clinacanthus nutans* (*C. nutans*) have been indicated to express a wide range of biological activities and one of its outstanding effects is antioxidant action. Studies using a 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay illustrated that *C. nutans* extract, at a concentration of 1mg/mL, is a potential antioxidant (Chomnawang *et al.*, 2007; Yuann *et al.*, 2012). The strongest antioxidant activity among the polar and non-polar extracts of *C. nutans* was found in the study by Ghasemzadeh *et al.* (2014), in which the methanol extract from *C. nutans* grown for one year on a farm in Malaysia showed an IC_{50} value of 64.6µg/mL. Additionally, other tests like ferric reducing antioxidant power (FRAP) assay, hydrogen peroxide radical scavenging test, nitric oxide radical scavenging test, 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assay are also widely carried out. In general, *C. nutans* extracted with strongly polar and moderately polar solvents were more likely to present antioxidant potential at concentrations ranging from 0.0125 to 10mg/mL, higher than that extracted with non-polar solvents (Sarega *et al.*, 2016). A recent research evaluated the *in vivo* antioxidant capacity of *C. nutans* using a hyperlipidemia-associated oxidative stress model. The results showed that water and 80% methanol extracts from leaves at a maximum dose of 500mg/kg could reduce oxidative stress by improving

**Corresponding authors:* e-mails: ddkhanh@ctump.edu.vn

serum antioxidant enzyme activity and the liver's expression of antioxidant genes (Sarega *et al.*, 2016).

In addition, the protective effect of the extract against the action of 2,2′‐azobis(2‐amidinopropane) dihydrochloride (AAPH) on rat erythrocytes was also explored. The results revealed that the extract protected erythrocytes against AAPH-induced hemolysis with an IC_{50} value of 359.38±14.02mg/mL, indicating that *C. nutans* showed a powerful defense against oxidative damage of cellular components (Pannangpetch *et al.*, 2007). Another study by Yuan and colleagues (2012) demonstrated the antioxidant activity and protective effects of ethanol extract from *C. nutans* leaves on the integrity of plasmid DNA from *Escherichia coli*. The 70% ethanol extract was able to lessen the quantity of DNA cleavages, maintain the integrity of the super coiled plasmid DNA, and offer superior protection against super oxide-induced riboflavin inhibition reactions up to 50 min in comparison with the green tea extract (which only protected for up to 30 minutes). The research by Chiangchin *et al.* (2023) revealed that the leaf extracts of *C. nutans* from various sources in Thailand expressed anti-apoptotic activity against lipopolysaccharide-induced cell death.

According to many researchers, *Clinacanthus nutans* contains the following components: Alkaloids, flavonoids, saponins, tannins, triterpenoids, diterpenes, glycosides, phenolic compounds, carbohydrates, proteins, and amino acids (Khoo *et al.*, 2018). Therefore, it was supposed that this medicinal herb possessed a great potential for antioxidant activity. Important compounds isolated from *C. nutans* are stigmasterol, lupeol, β-sitosterol, betulin and myricyl alcohol. There were also six C-glycosyl flavones isolated from n-BuOH and the aqueous fraction of methanol extract from dried leaves and stems including vitexin, isovitexin, schaftoside, isomollupentin-7-O-b-glucopyranoside, orientin and isoorientin (Shim *et al.*, 2013; Yahaya *et al.*, 2015; Alam *et al.*, 2016; Zulkipli *et al.*, 2017; Khoo *et al.*, 2018).

Research on the biological activities of *C. nutans* growing in Vietnam is still limited, especially reports from the stems of this plant. In addition, the Vietnamese Pharmacopoeia V does not have a separate treatise on *C. nutans*. This plant was mainly used based on folk experience. Our study focused on clarifying the antioxidant properties of various extracts from different parts of the plant using different solvents. It would help to fig. out new natural resources with antioxidant potentials that contribute to protecting humans against oxidative stress-related disease.

MATERIALS AND METHODS

Plant materials

The whole plant of *Clinacanthus nutans* was harvested in Chau Doc, An Giang province, Vietnam. The plants were identified by comparing their morphological characteristics with descriptions in standard botanical documents and determining their gene sequence. The plants were separated into roots, stems and leaves, dried, ground to powder, extracted and tested for antioxidant activity.

Chemicals

Solvents used for extraction included ethanol and water (Vietnam), dichloromethane and ethyl acetate (China). Other chemicals were methanol (China), vanillin (India), sulfuric acid (China), ferric chloride (China), formic acid (China), ascorbic acid (Sigma-Aldrich, United States), dimethyl sulfoxide (DMSO) (Duchefa, Netherlands), and 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) (Sigma-Aldrich, United States).

Extraction

Roots, stems and leaves of *C. nutans* were extracted with hot 96% ethanol. Thin-layer chromatography was applied to determine the time for stopping extraction. The extracts were evaporated under reduced pressure at 40^{o}C to obtain the total extracts. The total extract was sequentially applied to liquid-liquid extraction with solvents of increasing polarity, including dichloromethane (DCM), ethyl acetate (EtOAc) and water. The fractionated extracts continued to be evaporated under reduced pressure at a temperature of 40^{o}C to obtain the corresponding fractions. The fractions were dried in a vacuum oven until unchanged weight, stored in colored glass bottles and stored in the refrigerator. The extraction efficiency was calculated for each extract.

Vacuum column chromatography

Vacuum chromatography was operated on a glass column (3cm × 60cm) with 150g of silica gels (40-63µm) as stationary phases. The column was filled with dry silica gel powder and stabilized with DCM for 6 hours. Test samples were prepared by mixing 15g of the extract with silica gel in a 1:1 ratio, then dried at reduced pressure at 40^{o}C to obtain a fine red-brown powder. 15g of test samples were also loaded into the column using the dry method. Column development was started with DCM, then the polarity of solvents was increased by adding EtOAc with the ratio of DCM: EtOAc was 9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8, 1:9 and 0:10. Finally, flushed the column with MeOH. The collection volume of each fraction was 150mL and the fractions were examined by thin-layer chromatography. The detection was achieved under UV254 and UV365 after spraying with VS reagent. The fractions sharing similar chromatograms, which display the same spots with similar retardation factor (R_f) values, were combined into one fraction for antioxidant activity assays.

Preliminary DPPH tests on thin-layer chromatography

Thin-layer plates were developed with the appropriate solvent system, allowed to dry and then spotted with

DPPH 0.2%/MeOH reagent to preliminarily screen the biological effects of the test samples, which are total and fractional extracts. The extract's antioxidant activities were preliminarily identified when the spots on the thin-layer plate caused DPPH to change from purple to yellow after dipping in the 0.2% DPPH/MeOH reagent.

DPPH tests on 96-well plates

0.08 mM DPPH reagents were prepared in MeOH, stored in a colored glass bottle at 4°C, and used only within the day. Ascorbic acid reference solutions were made at five concentrations of 5μg/mL, 3.75μg/mL, 2.5μg/mL, 1.25μg/mL and 0.25μg/mL. The total and fractional extracts were dissolved in MeOH with DMSO at a specific ratio if necessary. Each sample was examined at five different concentrations.

The experimental process was performed based on the procedure of Kulisic *et al.* (2004), including three main steps:

Step 1: 100μL of MeOH was added into each test well, followed by 50μL of test samples and 50μL of 0.08 mM DPPH reagents.

Step 2: the reaction mixture was incubated for 30 minutes at 25°C in the dark.

Step 3: the absorbance of the mixture was measured at 517 nm.

The experiment was simultaneously conducted on blank samples, control samples, blank control samples, and test samples on the same 96-well plate. Each sample type consists of different reaction components listed in table 1. The test was repeated at least three times, the average value was taken, and the relative standard deviation was determined.

The formula calculated the percentage of DPPH radical scavenging activity:

Blank control

$$\text{DPPH}(\%) = \left(1 - \frac{Abs_{test} - Abs_{\text{blank control}}}{Abs_{control} - Abs_{\text{blank}}}\right) \times 100$$

The IC_{50} of each sample was determined as the concentration at which the sample eliminated 50% of DPPH free radicals. Based on the concentrations of test samples and DPPH%, a regression equation (y = ax + b) representing the correlation between DPPH% (y) and the concentration (x) was created. Then, IC_{50} values were extrapolated by substituting y = 50 into the regression equation.

STATISTICAL ANALYSIS

Data were processed using SPPS 16.0, and statistical differences between samples were analyzed using the one-way ANOVA method with a statistically significant difference defined as $p < 0.05$.

RESULTS

Moisture measurement

The moisture of the roots, stems, and leaves of *C. nutans* was measured using a Moisture Analyzer MX-50. table 2 revealed that all parts of the plant had less than 13% moisture, within the allowable limit for medicinal plants.

Total extraction efficiency

After meeting moisture requirements, roots, stems and leaves of *C. nutans* were extracted with 96% ethanol at a temperature of 70°C. After extracting under the same conditions, the amount of extracts from roots, stems and leaves of *C. nutans* were 4.73g, 5.25g and 9.46g, respectively, in which the highest extraction efficiency was obtained from leaves with the value of 19.78% (table 3).

DPPH scavenging assay results of total extracts

The antioxidant effects of the total extracts were preliminarily examined to select the sample with the most potent activity. The antioxidant effect was evaluated based on the number of spots and the intensity of the yellow spots when dipping the thin-layer plates in 0.2% DPPH/MeOH solution. The extract is dotted uniformly using a graduated capillary to give more accurate and objective results. The chromatograms of the total root, stem and leaf extract samples of *C. nutans* revealed that the stem extract showed many antioxidant stains and had the darkest color intensity when dipping in 0.2% DPPH reagent. At the same time, when observing under UV254, the stem samples produced many dark spots and when dipping in ferric chloride reagent, the spots turned blue-black. The root and leaf extract also gave spots under UV254 and UV365, but when dipping in 0.2% DPPH reagent, the spots were faint and difficult to observe.

Similarly, the results from 96 plate experiments presented that the whole stem extract produced the lowest IC_{50} value of 6.85μg/mL, which possessed the strongest antioxidant activity. The leaf extract had the highest IC_{50} value of 157.01μg/mL and the root extract had an IC_{50} value of 66.9μg/mL, indicating that the antioxidant capacity of the stem extract was about ten times and 23 times more potent than the root extract and the leaf extract, respectively. This finding was consistent with the preliminary results on thin-layer plates, so the stem extract was chosen for further studies.

DPPH scavenging assay results of fractions from stem extracts

The results on thin-layer plates showed that when dipped in 0.2% DPPH/MeOH solution, the EtOAc extract produced clear antioxidant stains and had the darkest color intensity compared to the DCM extract and water extract. Similarly, the ethyl acetate extract produced many dard and blue-black spots when viewing under UV254 and dipping in ferric chloride reagent, respectively.

Table 1: Types of samples used for DPPH assays

	Extracts	DPPH	MeOH
Blank sample			+
Control sample		+	+
Blank control sample	+		+
Test sample	+	+	+

Table 2: Results of the moisture

	First time (%)	Second time (%)	Third time (%)	Average (%)
Roots	6.78	6.51	6.32	6.54
Stems	6.08	6.11	6.29	6.16
Leaves	4.1	4.61	4.34	4.35

Table 3: Total extraction efficiency

	Amount of materials (g)	Amount of extracts (g)	Efficiency (%)
Roots	50	4.73	10.12
Stems	50	5.25	11.19
Leaves	50	9.46	19.78

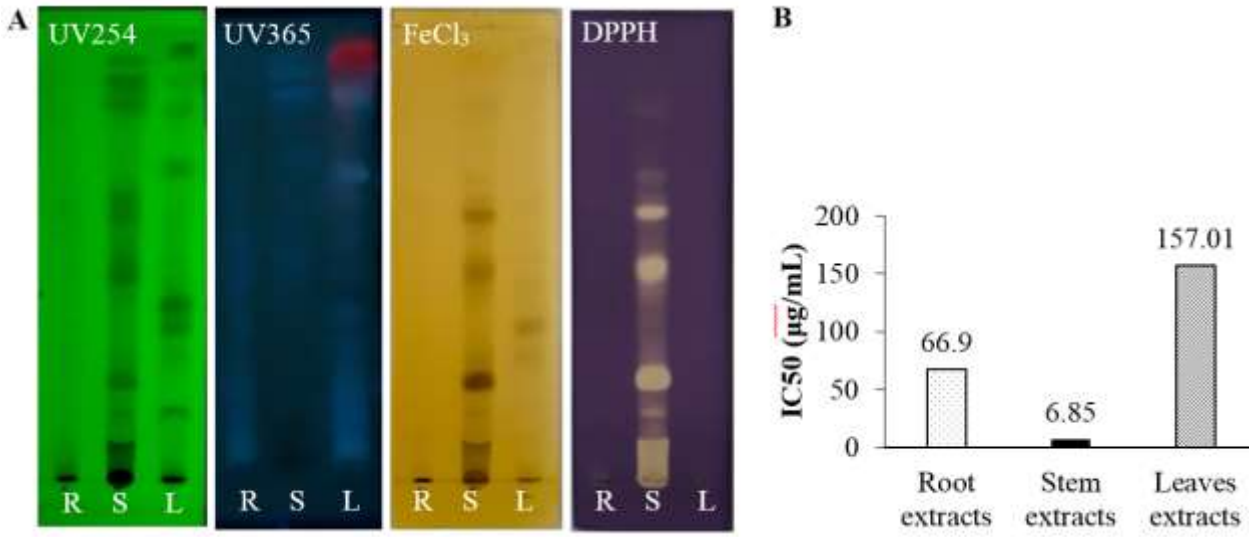

Fig. 1: DPPH assay results on TLC (A) and IC_{50} values (B) of total extracts (R: roots; S: stems; L: leaves)

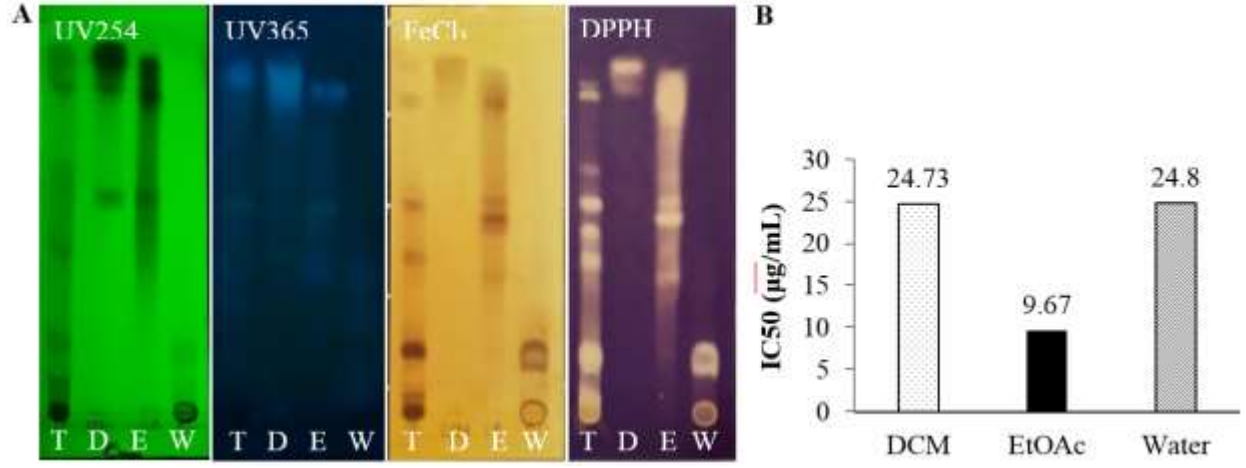

Fig. 2: DPPH assay results on TLC (A) and IC_{50} values (B) of fractions from stem extracts (T: total; D: DCM; E: EtOAc; W: water)

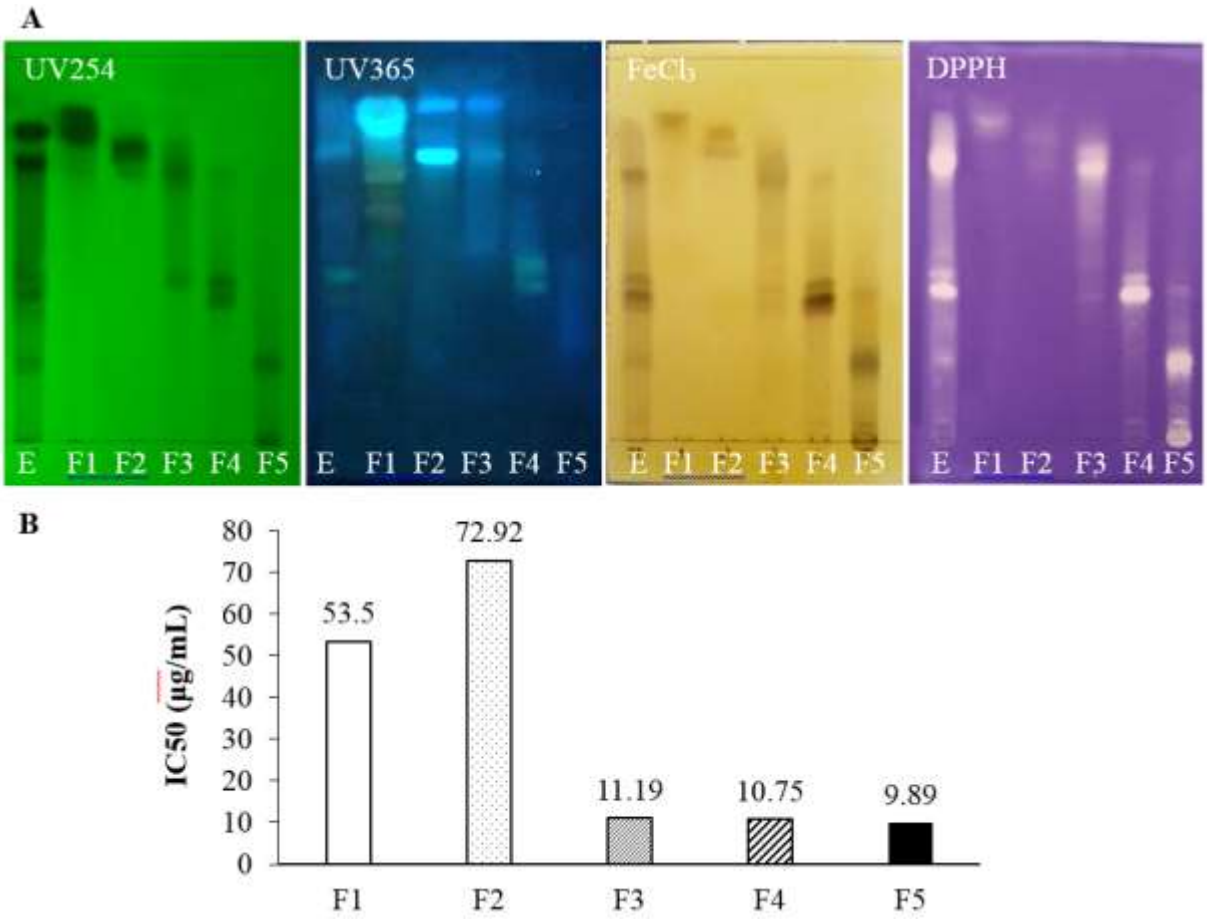

Fig. 3: DPPH assay results on TLC (A) and IC_{50} values (B) of fractions from EtOAc extracts (E: EtOAc; F: fraction)

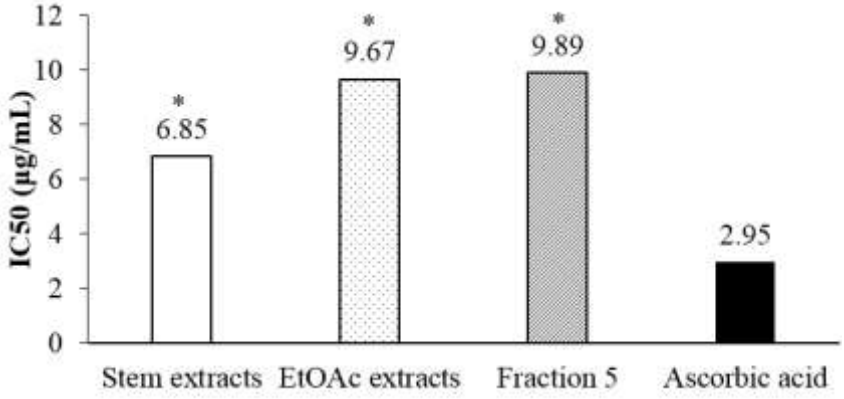

Fig. 4: IC_{50} values of the most effective extracts and ascorbic acid (*: $p<0.05$ compared to ascorbic acid)

DCM and water extracts also showed some antioxidant spots, but the number and color intensity were less evident than in ethyl acetate extracts.

Likewise, the EtOAc extract also had the lowest IC_{50} value of 9.67µg/mL, expressing the most potent antioxidant activity. For DCM extract and water extract, the IC_{50} values were nearly the same, 24.73µg/mL and 24.8µg/mL, respectively, about 2.5 times higher than the IC_{50} value of EtOAc extract.

DPPH scavenging assay results of fractions from EtOAc extracts

Among the fractions from the ethyl acetate extract, fractions 3, 4 and 5 showed spots with more intense color than other fractions, presenting higher antioxidant activities. The darkest spots were obtained from the fraction 5 chromatogram.

The same tendency was also achieved when performing the test on 96-well plates, in which fraction 5 had the

lowest IC50 value of 9.89µg/mL. Fractions 3 and 4 also gave relatively low IC_{50} values, 11.19µg/mL and 10.75 µg/mL, respectively, which is not significantly different from the fraction 5 value. Therefore, these two fractions may also be great resources in isolating substances with promising antioxidant activities.

Comparison of IC50 between the most effective extracts and ascorbic acid

All the extracts had higher IC_{50} values than ascorbic acid, showing less antioxidant effect than the reference standard. Indeed, ascorbic acid could eliminate free radicals with a very low IC_{50} value of 2.95µg/mL. Meanwhile, the whole stem extract, the EtOAc fraction extract, and the fraction 5 had potency approximately 0.43 times, 0.31 times and 0.30 times, respectively, compared with ascorbic acid.

DISCUSSION

The hot extraction method has many advantages, such as saving time and amount of solvent. An increase in temperature will reduce the solvent's surface tension and viscosity, thereby increasing the diffusion rate through the plant cell membrane, resulting in a better extraction process. According to research by Naczk and Shahidi (2006), the temperature of the solvent had an impact on the amount of phenolic compounds extracted. However, excessively high temperatures can increase the possibility of compound decomposition by hydrolysis, oxidation, or internal polymerization, leading to a decrease in the content of phenolic compounds (Alonso-Salces *et al.*, 2001). Therefore, choosing a temperature of 70ºC is appropriate for extraction. In addition, 96% ethanol was used because this is a safe solvent compared to other solvents, was very commonly used and was known as a powerful solvent, contributing to a high extraction efficiency and ensuring a more comprehensive investigation of antioxidant activity. Under the same extraction conditions, the leaves of *C. nutans* had the highest extraction efficiency at 19.78%.

Two methods were utilized to assess the samples' antioxidant properties: Color development on thin-layer plates and UV-Vis detection on 96-well plates to determine IC50 values. These are relatively simple tests that are easy to perform and do not take much time. At the same time, the tests give stable and highly repeatable results. In addition, DPPH free radical scavenging testing can be performed on thin-layer chromatography, thus allowing rapid preliminary identification of potential antioxidant compounds. However, the limitation of this test is that the reagent must be mixed immediately before use, refrigerated, protected from light and not left overnight because DPPH is easily affected by environmental conditions such as temperature and humidity. The absorbance of samples was measured at 517 nm because this is the maximum absorption wavelength of DPPH. The estimated time was 30 min after starting the reaction because, at this time, the absorbance of the product had stabilized or negligible changed (Sakdarat *et al.*, 2009; Nguyen *et al.*, 2023). Moreover, using the 96-well plate significantly decreased the number of measurements on the machine, the amount of test samples, solvents and chemicals and saved time and costs. The results on thin-layer chromatography and 96-well plates indicated that the stem extract presented a more potent antioxidant capacity than root and leaf extract. This study strongly confirmed the earlier study's results that only used thin-layer chromatography to estimate the antioxidant capacity of some extracts from *C. nutans* (Nguyen *et al.*, 2023).

The liquid-liquid extraction was applied to the stem extract with three solvents of increasing polarity to separate it into fractions with compounds that have the same polarity. DCM, EtOAc, and water were chosen to extract phenolic and flavonoid compounds because these substances often have moderate to very polar polarity. Moreover, compared to other solvents, these three have many outstanding advantages, such as being less toxic, causing less environmental pollution and being often used in laboratories.

The EtOAc fraction from stem extract showed the most apparent antioxidant spots on the thin layer and the lowest IC_{50} value compared to other fractions, illustrating the more substantial antioxidant effects. It is likely appropriate because natural antioxidants (like polyphenols) are known to be generally well soluble in moderately polar solvents. Similarly, in the research of Alam *et al.* (2017), who studied the antioxidant activity of ethyl acetate, butanol, hexane, methanol and water fractions of *C. nutans*, the ethyl acetate extract possessed the highest DPPH scavenging ability, with an IC_{50} equal to 269.1 µg/mL compared to the remaining extracts, and it had the highest content of flavonoid and phenolic compounds. As a result, the EtOAc extract continued to be utilized to conduct vacuum column chromatography to separate into simpler fractions.

The fractions obtained after partition extraction contain compounds with similar polarity, but the composition is still complex, making it difficult to isolate the pure substance. Vacuum column chromatography is a commonly used method to separate a total mixture into simpler segments, making the isolation of pure substances easier and faster. It was seen that fractions 3, 4 and 5 from the EtOAc extract had spots with darker color and better antioxidant abilities compared to the other fractions, in which the darkest areas came from the chromatogram of fraction 5. These fractions also had the same low IC_{50} values, with the lowest value belonging to fraction 5. Consequently, these fractions may be promising resources to help find substances with potent antioxidant effects.

In this study, the research team used ascorbic acid as a reference substance because it is a potent antioxidant that can inactivate free radicals by transferring two hydrogen atoms to the free radicals and becoming dehydroascorbic. All the extracts showed less antioxidant effect than ascorbic acid because they had higher IC_{50} values. At the same time, the extract from the whole stem, the EtOAc fraction extract and fraction 5 were about 0.43, 0.31 and 0.30, respectively, as potent as ascorbic acid.

The recent research was the first to use thin-layer chromatography and 96-well plate methods to demonstrate and compare the antioxidant potentials of different extracts from different parts of *C. nutans* growing in Vietnam. Currently, there is no comprehensive research on this plant in Vietnam. Moreover, research around the world mainly focused on leaf extracts. For the first time, our study proved that the stem extract possessed the most potent antioxidant activity. Further research may focus on extracting pure active substances with promising antioxidant effects from this plant stem.

CONCLUSION

The present study demonstrated considerable antioxidant effects of *Clinacanthus nutans* using DPPH scavenging assays on thin-layer and 96-well plates. The most pronounced actions were found in stem extracts, ethyl acetate fractions from the stem extract, and fraction 5 from the ethyl acetate fraction. Our following studies will evaluate the antioxidant effects of pure substances isolated from *C. nutans*. Moreover, further *in vivo* studies should be performed to strongly approve its valuable activities and develop new products from this plant.

ACKNOWLEDGEMENT

The authors sincerely appreciate the assistance provided by Can Tho University of Medicine and Pharmacy.

REFERENCES

Alam A, Ferdosh S, Ghafoor K, Hakim A, Juraimi AS, Khatib A and Sarker ZI (2016). *Clinacanthus nutans*: A review of the medicinal uses, pharmacology and phytochemistry. *Asian. Pac. J. Trop. Med.*, **9**(4): 402-409.

Alam A, Zaidul ISM, Ghafoor K, Sahena F, Hakim MA, Rafii MY, Abir HM, Bostanudin MF, Perumal V and Khatib A (2017). *In vitro* antioxidant and α-glucosidase inhibitory activities and comprehensive metabolite profiling of methanol extract and its fractions from *Clinacanthus nutans*. *BMC Complement. Altern. Med.*, **17**(1): 181.

Alonso-Salces RM, Korta E, Barranco A, Berrueta LA, Gallo B and Vicente F (2001). Determination of polyphenolic profiles of Basque cider apple varieties using accelerated solvent extraction. *J. Agric. Food. Chem.*, **49**(8): 3761-3767.

Chiangchin S, Thongyim S, Pandith H, Kaewkod T, Tragoolpua Y, Inta A, Watthana S, Pongamornkul W, Jangsutthivorawat S and Panya A (2023). *Clinacanthus nutans* genetic diversity and its association with anti-apoptotic, antioxidant, and anti-bacterial activities. *Sci. Rep.*, **13**(1): 19566.

Chomnawang MT, Surassmo S, Nukoolkarn VS and Gritsanapan W (2007). Effect of *Garcinia mangostana* on inflammation caused by *Propionibacterium acnes*. *Fitoterapia*, **78**(6): 401-408.

Ghasemzadeh A, Nasiri A, Jaafar HZE, Baghdadi A and Ahmad I (2014). Changes in phytochemical synthesis, chalcone synthase activity and pharmaceutical qualities of sabah snake grass (*Clinacanthus nutans* L.) in relation to plant age. *Molecules*, **19**(11): 17632-17648.

Khoo LW, Kow SA, Lee MT, Tan CP, Shaari K, Tham CL and Abas F (2018). A comprehensive review on phytochemistry and pharmacological activities of *Clinacanthus nutans* (Burm.f.) Lindau. *Evid. Based. Complement. Alternat. Med*.

Kulisic T, Radonic A, Katalinic V and Milos M (2004). Use of different methods for testing antioxidative activity of oregano essential oil. *Food. Chem.*, **85**(4): 633-640.

Lin CM, Chen HH, Lung CW and Chen HJ (2023). Antiviral and immunomodulatory activities of *Clinacanthus nutans* (Burm. f.) Lindau. *Int. J. Mol. Sci.*, **24**(13): 10789.

Lobo V, Patil A, Phatak A and Chandra N (2010). Free radicals, antioxidants and functional foods: Impact on human health. *Pharmacogn. Rev.*, **4**(8): 118-126.

Naczk M and Shahidi F (2006). Phenolics in cereals, fruits and vegetables: occurrence, extraction and analysis. *J. Pharm. Biomed. Anal.*, **41**(5): 1523-1542.

Nguyen TTD, Cao NNA and Le TTY (2023). Study on antioxidant activity of *Clinacanthus nutans* (Burm. F.) Lindau, Acanthaceae on some *in vitro* models. *Can Tho J. Med. Pharm.*, **57**: 101-108.

Pannangpetch P, Laupattarakasem P, Kukongviriyapan V, Kukongviriyapan U, Kongyingyoes B and Aromdee C (2007). Antioxidant activity and protective effect against oxidative hemolysis of *Clinacanthus nutans* (Burm.f) Lindau. *Songklanakarin. J. Sci. Technol.*, **29**(1): 1-9.

Phaniendra A, Jestadi DB and Periyasamy L (2015). Free radicals: Properties, sources, targets and their implication in various diseases. *Indian. J. Clin. Biochem.*, **30**(1): 11-26.

Sakdarat S, Shuyprom A, Pientong C, Ekalaksananan T and Thongchai S (2009). Bioactive constituents from the leaves of *Clinacanthus nutans* Lindau. *Bioorg. Med. Chem.*, **17**(5): 1857-1860.

Sarega N, Imam MU, Ooi DJ, Chan KW, Esa NM, Zawawi N and Ismail M (2016). Phenolic rich extract from *Clinacanthus nutans* attenuates hyperlipidemia-

associated oxidative stress in rats. *Oxid. Med. Cell. Longev*., 1-16.

Shim YS, Aziana I and Khoo BY (2013). Perspective and insight on *Clinacanthus nutans* Lindau in traditional medicine. *Int. J. Integr. Biol.*, **14**(1): 7-9.

Yahaya R, Dash GK, Abdullah MS and Mathews A (2015). *Clinacanthus nutans* (burm. F.) Lindau: An useful medicinal plant of South-East Asia. *Int. J. Phcog. Phyto. Res.*, **7**(6): 1244-1250.

Yuann JMP, Wang JS, Jian HL, Lin CC and Liang JY (2012). Effects of *Clinacanthus nutans* (Burm.f) Lindau leaf extracts on protection of plasmid DNA from riboflavin photoreaction. *MC-Transaction. Biotechnol.*, **4**(1): 45-58.

Zulkipli IN, Rajabalaya R, Idris A, Sulaiman NA, David SR (2017). *Clinacanthus nutans*: A review on ethnomedicinal uses, chemical constituents and pharmacological properties. *Pharm. Biol.*, **55**(1): 1093-1113.