Evaluation of total polyphenol content, total flavonoid content, and antioxidant activity of *Plectranthus amboinicus* leaves

N Q Nguyen\(^1\), L V Minh\(^4\), L H Trieu\(^4\), L M Bui\(^2\), T D Lam\(^2\), V Q Hieu\(^2\), T V Khang\(^3\), L N Y Trung\(^6\)

\(^1\)Department of Pharmacy, Nguyen Tat Thanh University, Ho Chi Minh City, Vietnam
\(^2\)NTT Hi-Tech Institute, Nguyen Tat Thanh University, Ho Chi Minh City, Vietnam
\(^3\)Center of Excellence for Biochemistry and Natural Products, Nguyen Tat Thanh University, Ho Chi Minh City, Vietnam
\(^4\)Research Center of Ginseng and Medicinal Materials, National Institute of Medicinal Materials, Ho Chi Minh City, Vietnam
\(^5\)The Center for Application of Technological Progress, Ninh Thuan Province, Vietnam
\(^6\)BKU Institute of Advanced Applied Science and Technology (BKIST), Ho Chi Minh City, Vietnam

* labasm2013@gmail.com, ngocquynguyen1995@gmail.com.

**Abstract.** *Plectranthus amboinicus* (Lour.) Spreng is a valuable medicinal plant that has been used for a long time in Vietnam. Previous studies have shown that *Plectranthus amboinicus* exhibited several useful pharmacological effects. This study evaluated total polyphenol, flavonoid contents and anti-oxidant potential of the leaf extract of *Plectranthus amboinicus*. The phenolic content and flavonoid content achieved 26.84 ±0.91 µg GAE/mg and 12.14 ±0.42 µg QE/mg, respectively. Ethanol extract (IC50 = 48.23 µg/ml) showed potent antioxidant activity. According to the results of the present investigation, the plant showed significant antioxidant activity that is applicable for the treatment of various diseases.

1. Introduction

Nowadays, metabolites including triterpenoids and phenolic compounds have been receiving a great deal of public attention in the food and medical industries due to their advantageous beneficial effect on human health [1-3]. *Plectranthus* belong to the *Lamiaceae* class which cultivated mainly in tropical and subtropical countries [0]. The main group of secondary metabolites in *Plectranthus* is Diterpenoids 0. Phenolic acid esters, phenolic acids, and flavonoids are isolated from various Plectranthus species [6-8]. Different pharmacological properties are shown including urolithiasis, antitumor, neuropharmacological, radioprotective effect, antioxidant, antimicrobial, antibacterial, antifungal properties. The use of medicinal plant extracts for the treatment of human disease is an ancient practice that has been significantly increasing in recent years. Free radicals trigger degenerative disease such as cancer. Cancer has become one of the most prevalent and distressing diseases with increasing sufferer in the last 50 years 0. Phenolic compounds extracted from plants have been receiving a great deal of public attention during recent years. In facts, phenolic compound effects on diet health interaction in
the human body, antioxidants can reduce oxidative stress due to the high antioxidant activity [15, 16]. In DPPH assay, the ability of the compound like donor for a hydrogen atom or electron was calculated spectrophotometrically. The DPPH scavenging assay is broadly applied to assess the free radical scavenging of plant extracts thanks to its sensitive, simple, rapid. Antioxidants can remove the radical by hydrogen donation, which results in a decrease of DPPH absorbance at 517 nm. Many researchers have focused on the identification and isolation of natural antioxidants from medicinal plants. The present study was carried out to evaluate chemical components, total polyphenol, flavonoids content, and antioxidant activity of the leaves of *Plectranthus amboinicus*.

2. Materials and methods

2.1. Materials and chemicals
The leaves of *Plectranthus amboinicus* were collected in An Giang provide, Vietnam in February 2019. The *Plectranthus amboinicus* leaves were washed, following dried at 40˚C.

2.2. Procedures

2.2.1 Total polyphenol content determination (TPC)
First, 0.5 mL of extract was pipetted into a test tube, which contained 2.5 mL Folin-Ciocalteu reagent 10%. (v/v). After 5 minutes, 1 mL Na₂CO₃ 20% (w/v) was added to the sample. Next, the mixture was vigorously shaken and incubated for 60 minutes in the dark. Finally, the absorbance was spectrophotometrically measured at 765 nm and the results were shown in µg of gallic acid equivalents per dry weight of sample (µg GAE/mg).

2.2.2 Total Flavonoid Content (TFC)
Base on the aluminum chloride colorimetric method, the total flavonoid content was determined by the aluminum chloride colorimetric method 0. 0.5 mL aliquot of the extract was mixed with 0.3 mL of 5% sodium nitrite solution in a test tube. After 5 min, 0.3 mL of 10% aluminum chloride solution was added. Then, 2 mL 1 M sodium hydroxide solution was added to the mixture. Immediately, the solution was diluted with 1.9 mL of distilled water and vigorously shaken. The absorbance was spectrophotometrically measured at 510 nm against a blank reaction. Total flavonoid content of extracts was expressed as equivalent of quercetin (QE) in mg per 100 g quinoa seeds in dry weight basis.

2.2.3 DPPH Scavenging Activity.
The antioxidant activity of the individual essential oil was tested by means of 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay. 1.5 mL DPPH (OD 517 nm = 1.1 ± 0.02) was mixed with500 µL solution sample. After agitation, the mixture was incubated in the dark for 37 min and the absorbance was measured at 517 nm. The optical measurement of mixture by UV/VIS - 1800 Shimadzu Spectrometer at 517 nm. Blank sample500 µL solution replaced EtOH 99.7%. Standard sample: Vitamin C (0.1g ÷ 0.01) was dissolved EtOH 99.7% into volume flask 100mL, in the dark (C = 100 µL/mL).

The percent DPPH scavenging effect was calculated by using following equation: DPPH scavenging effect (%) or percent inhibition (%I) 0 %I = (Ab-As)/Ab× 100
In there: Ab - Absorbance of blank sample, As - Absorbance of sample, %I - Percent inhibition

2.2.4 Statistical analysis
All determinations were carried out in triplicate and the results were expressed as mean values and standard deviation. One-way analysis of variance (ANOVA) was performed using SPSS 23 (Statistical
Package for the Social Sciences) and differences between samples were compared using Tukey’s test (P < 0.05).

3. Results and discussions

3.1. Total phenolic and flavonoid content

Phenolic components play a vital role in plants due to their scavenging ability. Total phenolic contents were measured by Folin–Ciocalteu method. Total polyphenol content achieved 26.84 ± 0.91 µg GAE/mg extract (figure 1). The total flavonoid content was performed as µg quercetin equivalents per milligram of dried sample (µg QE/mg). Flavonoid contents achieved 12.14 ± 0.42 µg QE/mg dry weight ethanol extract in P. fruticosa leaves (figure 2).

![Figure 1. Standard gallic acid solution (µg/ml)](image)

![Figure 2. Standard quercetin solution (µg/ml)](image)
3.2. DPPH radical scavenging activity

The percentage inhibition of DPPH radicals was increased as concentration of extracts was increased in figure 3 and 4. Ethanol extract (IC$_{50}$ = 48.23 µg/ml) showed potent antioxidant activity. This activity might be due to the presence of phenolic and flavonoids compounds. The IC$_{50}$ value of standard ascorbic acid was 4.80 µg/ml.

![Graph showing DPPH radical scavenging activity](image)

**Figure 3.** DPPH radical scavenging activity of ethanolic extracts of *Plectranthus amboinicus* leaves.

![Graph showing IC 50 values](image)

**Figure 4.** IC 50 of Vitamin C and ethanol extract from *Plectranthus amboinicus* leaves.

4. Conclusions

*Plectranthus amboinicus* (Lour.) Spreng essential oil has been widely used in folk medicine for treatment of anxiety, memory deficit, and cancer thanks to its high antioxidant activity and antibacterial properties. In this study, we carried out the total polyphenol, flavonoids content, and antioxidant activity of the leaves of *Plectranthus amboinicus*. The phenolic content was found 26.84 ±0.91 µg GAE/mg extract. Flavonoid content was 12.14 ± 0.42 µg QE/mg dry weight ethanol extract in *Plectranthus amboinicus* leaves. Ethanol extract (IC$_{50}$ = 48.23 µg/ml) showed potent antioxidant activity.
Acknowledgment: This study was supported by grants from Nguyen Tat Thanh University, Ho Chi Minh City, Viet Nam.

References
[1] Pham T N, Tran B P, Tran T H, Nguyen D C, Nguyen T N P, Nguyen T Q, Vo D V N, Le X T, Nguyen D T, Bach L G. 2019 IOP Conf. Ser. Mater. Sci. Eng. 479 012012
[2] Nguyen P M, Tran H P T, Nguyen T T T and Bach L G 2019 Research on Crops 20 180-186.
[3] Tran Q T, Le T T T, Pham M Q, Do T L, Vu M H, Nguyen D T, Bach L G, Bui L M and Pham Q L 2019 Molecules 2019 24 895.
[4] Lukhoba C W, Simmonds M S J and Paton A J 2006 J.Ethnopharmacology 103 1–24.
[5] Adel-Mogib M, Albar H A and Batterjee S 2002 Molecules 7 271–301.
[6] Grayer R J, Eckert M R, Veitch N C, Kite G C, Marin P D and Kokubun T 2003 Phytocemistry 64 519–28.
[7] Juch M, Rüedi P. 1997 Helvetica Chimica Acta 80 436–48.
[8] Kumar A, Karunakaran RJ. 2007 Food-Chemistry. 100 356–61.
[9] Nguyen P M, Bach L G, Mac H C, Luu Y L, Vo T B T, Tran V T 2019 J.Pham. Sci. Resear. 11 279-283.
[10] Nguyen P M, Tien M T, Nguyen H T, Bach L G J. Global Pharma Techno. 10 186-192
[11] Dalimunthe A, Hasibuan P A Z ,Silalahi J, Sinaga S F and Satria D 2018 J. Chem., 34 1149-1152.
[12] Vuong Q V, Hirun S, Roach P D, Bowyer M C, Philip PA and Scalett C J 2013 J. Herbal Med. 3 104-111.
[13] Gil M I, Ferreres F, Tomas-Barberan F A 1999 Journal of Agricultural and Food Chemistry 47 2213-2217.
[14] Rice–Evans C. 2004. Free Radic Biol Med 36 827–8.
[15] Sharma S K and Gupta V K 2008 Phcog. Mag. 4 70-74.
[16] Jain N, Goyal S, Ramawat K G 2011 Int. J. Pharm. Pharm. Sci 3 248–53.