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Phlogacanthus cornutus: chemical profiles and antioxidant effects

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Abstract

Phlogacanthus cornutus is a rare species and the chemical profiles and the bioactivities of this plant are unknown. In present study, the chemical components of the acetone extract as well as the antioxidant activity of acetone extract and its fractions such as n-hexane, chloroform and ethyl acetate of *P. cornutus* were firstly reported. A total of 33 constituents were identify in the acetone extract of this plant using Gas Chromatography/Mass Spectrometry assay, in which trans-cinnamic acid (21.26%), neophytadiene (6.36%), linolenic acid (5.86%), dihydroagathic acid (5.71%), n-hexadecanoic acid (5.53%), phytol (4.14%) and cis-cinnamic acid (3.23%) were the major compounds. The acetone extract and its fractions such as n-hexane, chloroform and ethyl acetate of *P. cornutus* showed DPPH radical scavenging activity with IC_{50} value of 234.31, 185.95, 758.65 and 458.52 µg/mL respectively.

Keywords

Phlogacanthus cornutus, GC/MS, chemical components, antioxidant activities

Introduction

The genus *Phlogacanthus* is belonging to the family Acanthaceae with around 49 species. This genus is widely found in tropical regions of Asian countries, including Bhutan, Bangladesh, India, China, Myanmar, Indonesia and Vietnam (1-3). Previous studies provided that the various solvent extracts of the different *Phlogacanthus* species possessed many biological effects, including anti-inflammatory, antibacterial, antioxidative and cytotoxic activities (4, 5). About 7 *Phlogacanthus* species have been recorded for the flora of Vietnam such as *P. annamensis, P. geoffrayi, P. colaniae, P. pubiflorus, P. pyramygdalis, P. turgidus* and *P. cornutus* (1-3, 6).

Phlogacanthus cornutus Benoist is an endemic species of Vietnam. It was described for the first time by Benoist in 1927 of which its specimens were collected from Dinh mountain, Ba Ria-Vung Tau province, Vietnam (7). To date, *P. cornutus* is a rare species and the chemical compositions and bioactivities of this species are still unknown. Herein, we firstly provide the chemical profiles of the acetone extract, as well as the antioxidant effects of the acetone extract and its fractions, including-hexane, chloroform and ethyl acetate of *P. cornutus* grown in Binh Chau-Phuoc Buu Nature Reserve, Ba Ria-Vung Tau province, Vietnam.

Materials and Methods

Plant material

The leaves of *P. cornutus* were collected from Binh Chau-Phuoc Buu Nature Reserve, Ba Ria-Vung Tau province, Vietnam, location about 10°37'01.5"N 107°31'19.2"E (Fig. 1).

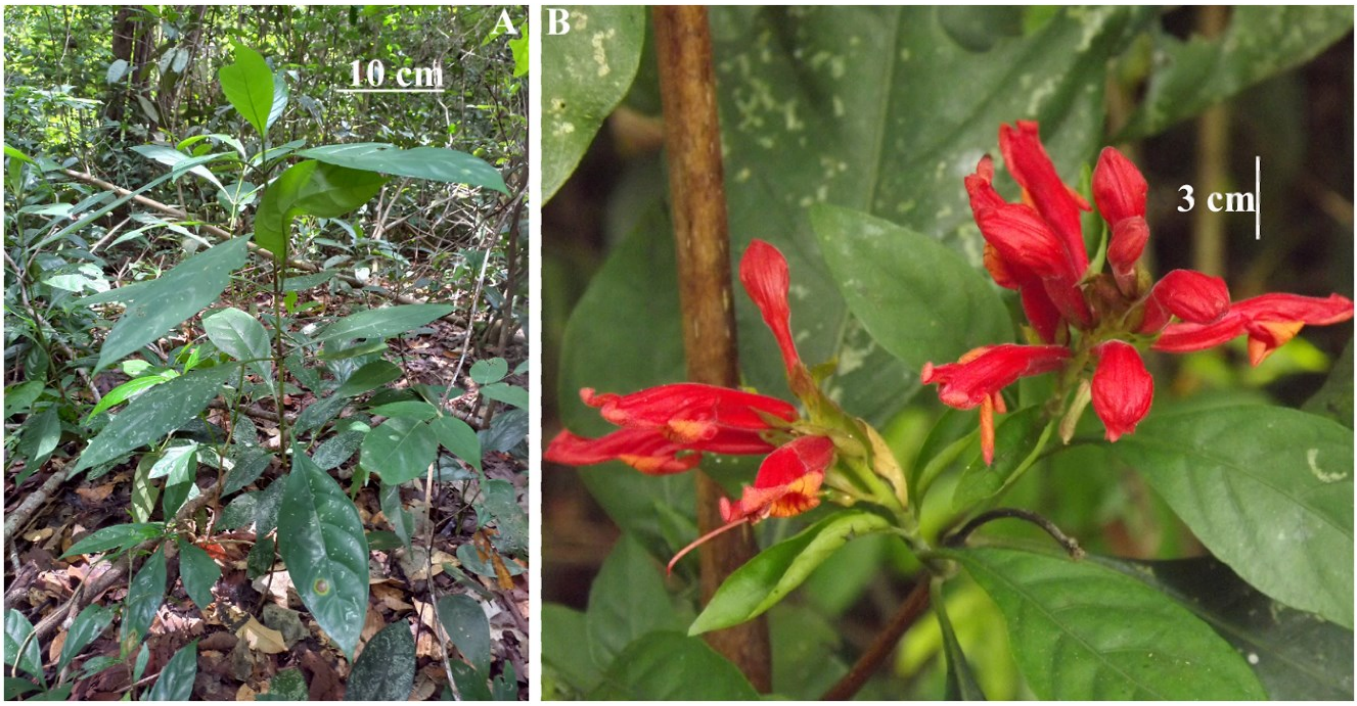

Fig. 1. *Phlogacanthus cornutus*. A. The species in habitat, B. Flower. Photos: Van Son Le.

The scientific name of this species was identified by Van Son Le, a botanist from Binh Chau-Phuoc Buu Nature Reserve. The vouchered specimen (BC 396) are deposited at the Herbarium of Binh Chau-Phuoc Buu Nature Reserve.

Extraction

The plant sample was dried in an oven at 50 °C until constant weight and ground into fine powder using an electric blender. An amount of 100 g of powder was macerated in 500 ml of 99% acetone (Thermo Fisher Scientific, USA) at room temperature for 3 days. The extract was collected and filtered through Whatman filter paper No. 4. The solid material was further soaked in 300 ml and 200 ml sequentially of the same solvent and filtered to collect the extracts. All the three acetone extracts were then combined and condensed on an a rotary evaporator at 40 °C to remove the solvent (8).

Fractionation of acetone extract

30 ml of distilled water was used to dissolve 3 g of the acetone extract. The suspension was subsequently mixed with 30 ml of n-Hexane (Thermo Fisher Scientific, USA), shaken and allowed to stand until layers were formed. The n-hexane layer (upper) was collected. This procedure was repeated 2 more times to obtain 90 ml of hexane extract. The extract was subjected to a rotary evaporator at 40 °C to remove the solvent. The lower layer was then used to collect the ethyl acetate and chloroform (Thermo Fisher Scientific, USA) fractions using the same procedure (8).

GC/MS based chemical profiling of the acetone extract

The acetone extract of the studied species was subjected to chemical composition analysis on TRACETM 1310 Gas Chromatograph (Thermo Fisher Scientific Inc., Waltham, MA, USA) equipped with ISQ 7000 single quadrupole mass spectrometer and DB-5MS column (30 m × 0.25 mm × 0.25 µm). Samples were injected into the GC system thermostated at 250 °C and at a split ratio of 30:1 at the flow rate 36 mL/min with splitless time of 1 min. Helium was used as the carrier gas at a flow rate of 1.2 mL/min. The transfer line temperature was set at 250 °C. The oven temperature was initially set to be hold at 80 °C for 5 min and was then increased to 280 °C at a rate of 20 °C/min and held at this temperature for 10 min. The electron impact ionization was set as 70 eV and the filament source temperature was set at 250 °C. The acquisitions scan mass range of MS was 29-650 m/z with the scanning frequency of 2 scans/sec. The chemical components of the acetone extracts obtained from *P. cornutus* were identified based on the comparison between their mass spectra with NIST 2017 library.

DPPH Free Radical Scavenging Assay

The antioxidant effects of the studied extracts were investigated using DPPH (2,2-Diphenyl-1-picrylhydrazyl) radical scavenging assay (8). First, 2700 µl of DPPH (Thermo Fisher Scientific, USA) solution (0.1 mM) was added to 100 µl of sample. The mixture was then gently shaken and left in the darkness for 30 min at 37 °C. The mixture was measured for absorbance at a wavelength of 517 nm. DPPH Free Radical Scavenging effect of the studied samples was determined using the following formula (I). The radical scavenging activity of samples was expressed as the inhibition concentration (IC_{50}), which is defined as the concentration of sample being able to reduce the initial DPPH concentration by 50%.

$$\% \text{ Antioxidant activity} = [(\text{ODcontrol} - \text{ODsample})/\text{ODcontrol}] \times 100 \quad (I)$$

In which ODcontrol = The optical density of DPPH in acetone and ODsample = The optical density of DPPH mixed with the extract.

Results and Discussion

Chemical compositions of acetone extract of P. cornutus

The chemical profiles of the acetone extract isolated from *P. cornutus* leaves were shown in the Table 1 and Fig. 2. A total of 33 components were identified from the studied extract, of which trans-cinnamic acid (21.26%), neophytadiene (6.36%), linolenic acid (5.86%), dihydroagathic acid (5.71%), n-hexadecanoic acid (5.53%), phytol (4.14%) and cis-cinnamic acid (3.23%) were the major compounds. Notably, there was an unknown compound (at retention time 18.68 min) found in the acetone extract of *P. cornutus* leaves. This component possessed the highest percentage (23.86%) in the studied extract. Several fragments of this compound have been recorded of which the parent mass possessed m/z as 320 (Fig. 3). Furthermore, the mass spectra of this compound did not match with any compound included in NIST 2017 library in spite of the fact that NIST 2017 library, the most innovative library, was used in this study. This compound, thus, should be investigated further to determine whether they are new compounds or not.

Table 1. Chemical profiles of acetone extract obtained from *Phlogacanthus cornutus.*

Retention time (min)	Compounds	% Composition
4.61	Hexanoic acid	0.58
4.49	Glycerin	0.36
5.6	trans-2-Hexenoic acid	0.38
5.91	Benzyl alcohol	0.11
7.26	Phenylethyl Alcohol	0.10
8.11	Cinnamaldehyde	1.05
8.15	Benzoic acid	0.88
8.73	Coumaran	0.95
9.24	7a-Methyl-1,2,3,6,7,7a-hexahydro-5H-inden-5-one	0.29
9.73	4-Hydroxy-2,6-dimethylbenzonitrile	0.79
10.08	*cis*-Cinnamic acid	3.23
10.68	*trans*-Cinnamic acid	21.62
10.75	Bicyclo[4.2.0]octa-1,3,5-triene-7-carboxylic acid	0.57
11.24	2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl-, (R)-	0.22
11.94	2,6-Dimethoxyhydroquinone	0.57
12.26	3-Oxo-7,8-dihydro-α-ionol	0.33
12.71	6-Hydroxy-4,4,7a-trimethyl-5,6,7,7a-tetrahydrobenzofuran-2(4H)-one	0.34
12.92	Neophytadiene	6.36
12.95	Hexahydrofarnesyl acetone	0.41
13.04	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	1.02
13.59	n-Hexadecanoic acid	5.53
14.31	Phytol	4.14
14.44	cis-9,cis-12-Octadecadienoic acid	1.98
14.48	Linolenic acid	5.86
14.56	Stearic acid	0.47
18.38	Dihydroagathic acid	5.71
18.68	Unknown compound	23.86
22.69	dl-α-Tocopherol	0.12
24.66	Campesterol	0.13
25.2	Stigmasterol	1.00
26.2	Sitosterol	1.33
27.07	Lup-20(29)-en-3-one	0.31
27.55	Lup-20(29)-en-3-ol, (3β)-	3.74
	Total	94.32%

The chemical components of other *Phlogacanthus* plants have been reported by previous studies. For instance, turgidol, a new compound, together with 5 known triterpenoids such as lupenone, lupeol, betulin, taraxer and betulinic acid were obtained from the chloroform extract of *Phlogacanthus turgidus* (9). Similarly, 6 chemical components of *P. turgidus* was identified in the ethyl acetate extract, including one derivative of phenylethanoid glycosides (martynoside), one lignan ((+)-syringaresinol), 2 steroids (bsitosterol and daucosterol) and two norisoprenoids ((+)-dehydrovomifoliol and (3S,5R,6R,7E,9S)-megastiman-7-ene-3,5,6,9-tetrol) (10). Studies also demonstrated that *Phlogacanthus pulcherrimus* had the content of nutrition (protein, fat, fiber and moisture) and mineral (calcium, sodium, potassium, magnesium, chromium and iron) (11). Furthermore, *P. pubinervius* and *P. jenkinsii* have been reported to possess some phytochemical contents, including macro nutrients (nuphosphorus, sodium and potassium), micro nutrients (iron, copper, manganese), total free amino acid, total sugar, total free phenol, bound phenol and tannin (12). In addition, the chemical constituents of methanol extracts obtained from 3 *Phlogacanthus* species such as *P. pubinervius, P. thyrsiflorus* and *P. curviflorus* were investigated by using GC/MS assay. Accordingly, the *P. pubinervius* and *P. curviflorus* extracts were found to be rich in squalene, n-hexadecanoic acid and phytol while 2-ethyhexane, n-hexadecanoic acid

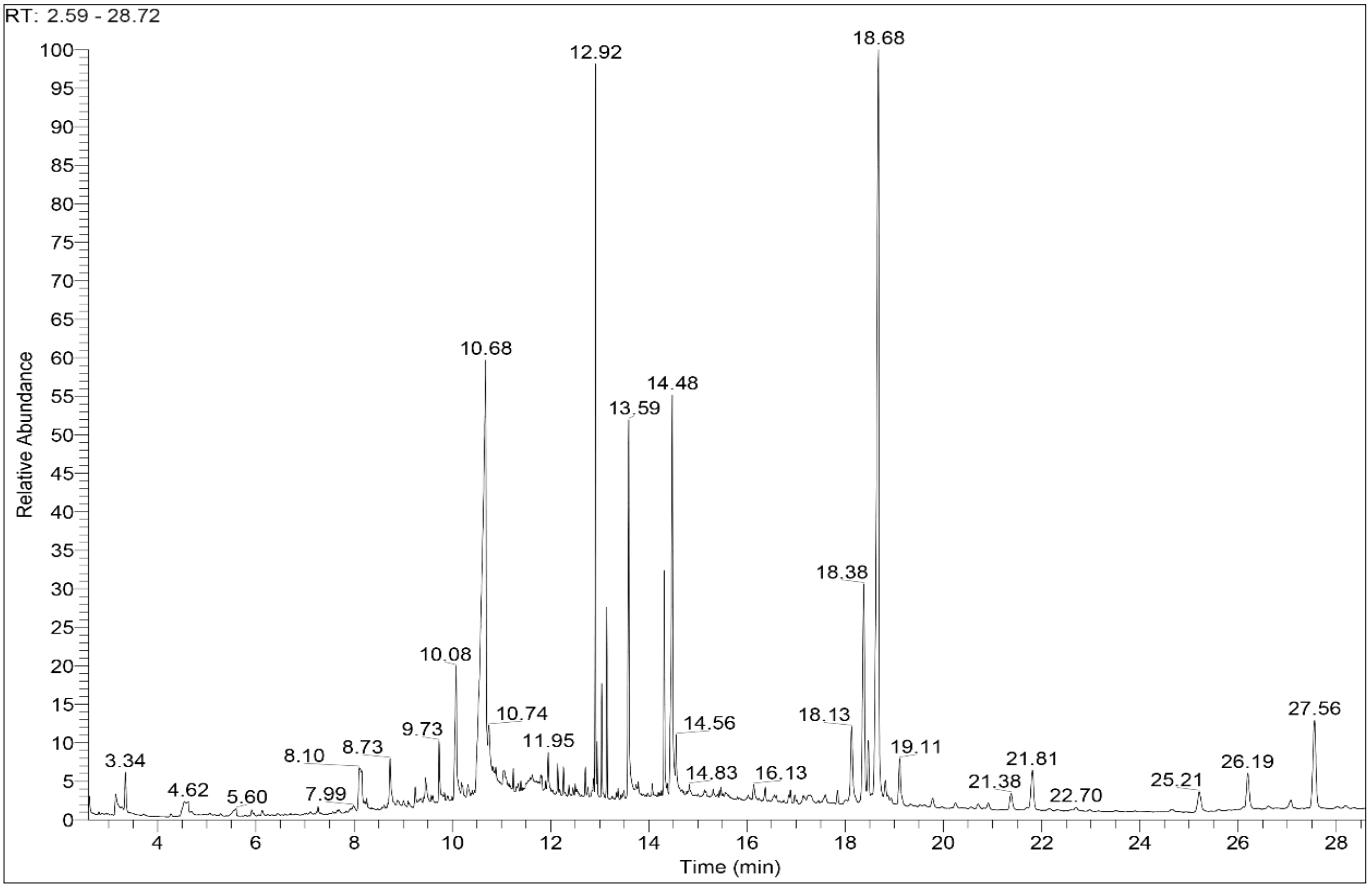

Fig. 2. Gas chromatogram of acetone extract from *Phlogacanthus cornutus.*

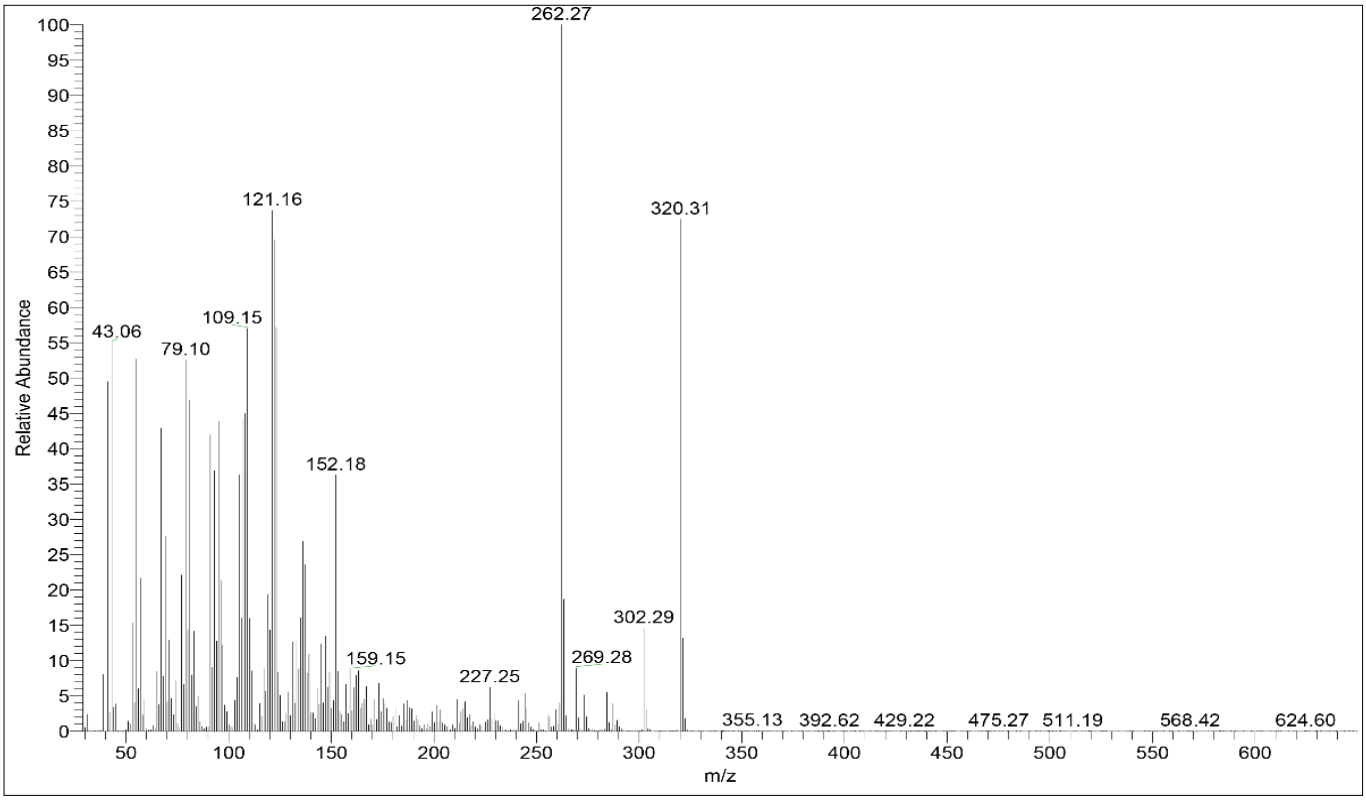

Fig. 3. Chromatogram of unknown compound at retention time 18.68 min.

and octadeca-9,12,15-trienoic acid were the major compounds in the P. thyrsiflorus extract (13).

Antioxidant effects of acetone extract and its fractions of P. cornutus

The antioxidant effects of the acetone extract and its fractions of *P. cornutus*, including ethyl acetate, hexane and chloroform were determined by using 1,1-diphenyl-2-picrylhydrazol (Fig. 4). Accordingly, the hexane fraction showed the highest antioxidant activity with IC_{50} value of 185.95 µg/mL, followed by acetone extract (234.31 µg/mL), ethyl acetate fraction (458.52 µg/mL), and chloroform fraction (758.65 µg/mL). Meanwhile, the positive control

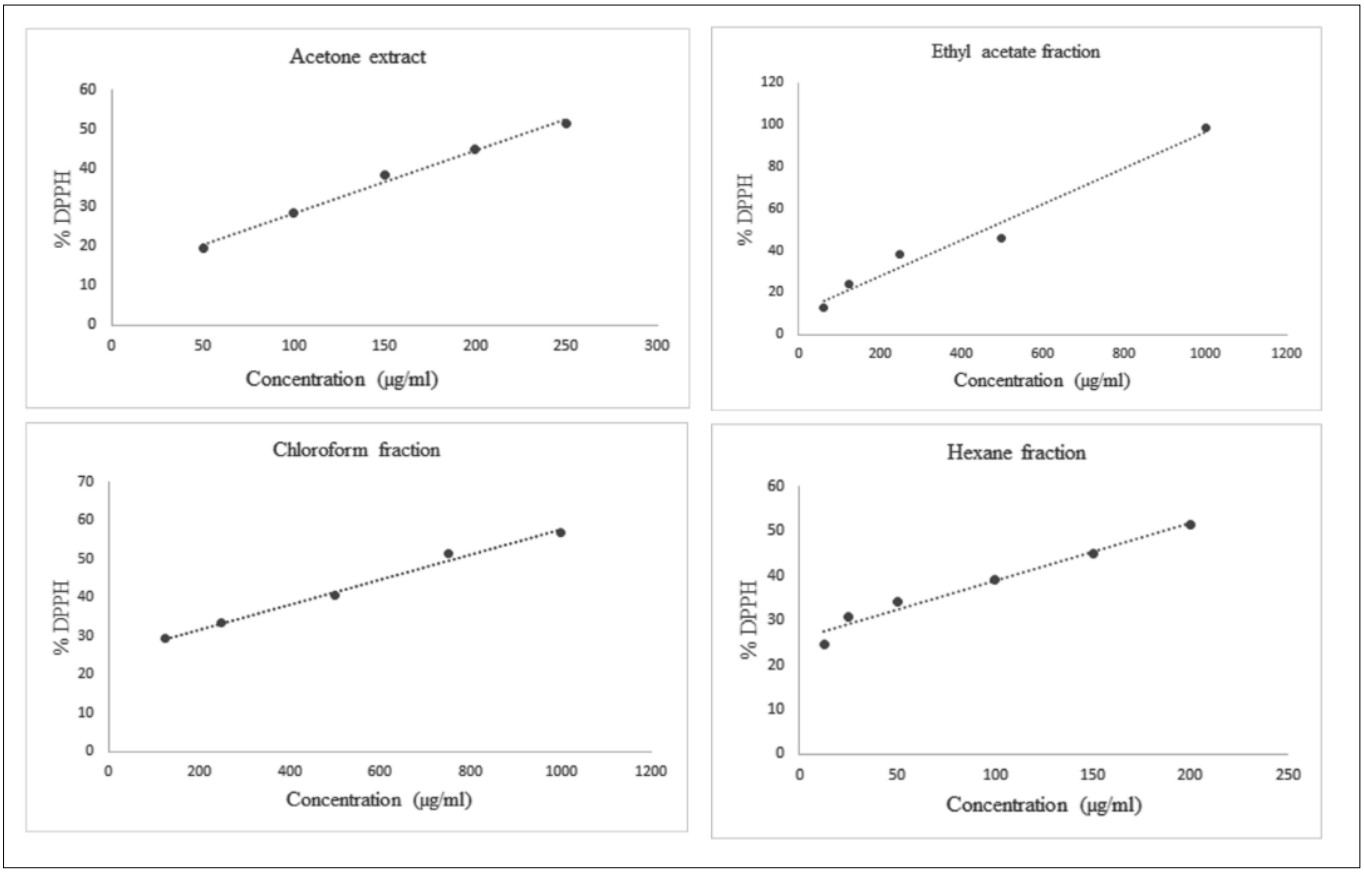

Fig. 4. Radical scavenging activity of acetone extract and its fractions of *P. cornutus.*

(ascorbic acid) displayed activity against DPPH radical-scavenging with IC_{50} value of 1.84 µg/mL.

The antioxidant activities of other *Phlogacanthus* species were also reported by previous studies. For instance, the methanol extract of *P. pubinervius* and *P. jenkinsii* showed the DPPH radical scavenging activity with 93.96% and 357.72% respectively while the percentages of radical scavenging activity of chloroform extract were 20.14% and 32.89% respectively (12). In another report, Kripasana and Xavier provided the DPPH radical scavenging activities of the methanol extracts of three *Phlogacanthus* species such as *P. pubinervius, P. thyrsiflorus, P. curviflorus*. Accordingly, the *P. pubinervius* extract had an inhibitory effect on 57.93% radical DPPH-scavenging at concentration of 150 µg/mL whereas the extracts of *P. thyrsiflorus* and *P. curviflorus* were found to be effective against 92.94% and 94.20% radical DPPH at concentration of 100 µg/mL (13). Previous study also showed the DPPH radical scavenging activities of 4 extracts of *Phlogacanthus pulcherrimus*, including aqueous, dichloromethane, n-butanol, ethyl acetate extracts. As a result, the ethyl acetate extract possessed the highest antioxidant effect with IC_{50} values of 730.28 µg/mL, followed by n-butanol extract (1422.93 µg/mL), aqueous extract (>2000 µg/mL) and dichloromethane extract (>2000 µg/mL) (14).

Conclusion

The present study identified 33 compounds in the acetone extract of *P. cornutus* leaves in which trans-cinnamic acid, neophytadiene, linolenic acid, dihydroagathic acid, n-hexadecanoic acid, phytol and cis-cinnamic acid were the major compounds. The acetone extract and its fractions such as n-hexane, chloroform and ethyl acetate of *P. cornutus* showed DPPH radical scavenging activity with IC_{50} value of 234.31, 185.95, 758.65 and 458.52 µg/mL respectively. The results of this study will provide the evidence for future applications of *P. cornutus* in medicine and other related fields.

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Authors contributions

DHT designed this study. The samples were collected by VSL, a staff of Binh Chau-Phuoc Buu Nature Reserve. All authors performed experiments, handled the research data and data analysis. Duy Hoang Truong drafted the manuscript and resolved all the queries of reviewers.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None

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