



Research Article

ANTIOXIDANT POTENTIALS OF *PARAMIGNYA TRIMERA* LEAVES

Pham Nguyen Kim Tuyen^{1*}, Do Anh Ky², Nguyen Minh Tien², Vo Minh Tu², Le Van Thanh²,
Nguyen Tuan Dat², Dang Hong Diep², Pham Duc Dung², Duong Thuc Huy^{2*}

¹Saigon University, Vietnam

²Ho Chi Minh City University of Education, Vietnam

*Corresponding author: Pham Nguyen Kim Tuyen – Email: phngktuyen@gmail.com

Received: March 14, 2025; Revised: April 18, 2025; Accepted: April 21, 2025

ABSTRACT

Paramignya trimera or “XAO TAM PHAN,” a traditional medicinal plant native to Vietnam, has been utilized in Asian countries for its therapeutic properties. The primary objective of this research was to isolate bioactive compounds from the leaves of *P. trimera* using a bio-guided routine based on antioxidant properties. The free radical DPPH scavenging activity of various extracts was evaluated, and the most active extract, extract EA, was selected for further chemical investigation. Three compounds, namely luteolin (1), scopoletin (2), and ostruthin (3), were isolated from the extract EA of *Paramignya trimera* leaves. The chemical structures of these compounds were elucidated by comparing their spectroscopic data with previously reported literature data. Upon the free radical DPPH scavenging activity, compound 2 exhibited strong activity with an IC_{50} value of 7.1 μ M.

Keywords: antioxidant; DPPH; luteolin; ostruthin; *Paramignya trimera*; scopoletin

1. Introduction

Paramignya trimera (Oliv.) Burkill (Rutaceae) is especially prevalent in Southern Vietnam, known for its therapeutic properties in treating hepatitis and liver cancer (Phi et al., 2020). It is noted that minor morphological differences between the genera *Paramignya* and *Luvunga*, and suggested that *P. trimera* may be placed within *Luvunga* (Phi et al., 2020). In Vietnam, however, this species is still widely known as *P. trimera* and commonly referred to as “Xao tam phan” (T. H. Tran et al., 2023). Phytochemical studies of *P. trimera* in Vietnam indicated the presence of bioactive phytochemicals. Recent reports of its stems and roots have found 22 coumarin-type compounds, 4r acridone-type alkaloids, and many known compounds (Dang et al., 2017; M. T. T. Nguyen et al., 2018; V. Nguyen & Scarlett, 2019; V. T. Nguyen et al., 2015; Quan et al., 2021; Trinh et al., 2020). Coumarins and alkaloids selectively induced apoptosis in human breast cancer stem cells (Quan et al., 2021; Piao et al., 2021; H. X. Nguyen et al., 2024) and modest alpha-glucosidase inhibition (Dang et al.,

Cite this article as: Pham, N. K. T., Do, A. K., Nguyen, M. T., Vo, M. T., Le, V. T., Nguyen, T. D., Dang, H. D., Pham, D. D., & Duong, T. H. (2026). Antioxidant potentials of *Paramignya trimera* leaves. *Ho Chi Minh City University of Education Journal of Science*, 23(2), 249-255. [https://doi.org/10.54607/hcmue.js.23.2.4804\(2026\)](https://doi.org/10.54607/hcmue.js.23.2.4804(2026))

2017; M. T. T. Nguyen et al., 2018; Quan et al., 2021; Trinh et al., 2020). The discoveries of *P. trimera* leaves are limited. V. T. Nguyen et al. (2017) used microwave drying without isolation to study the antioxidant and anti-proliferative activities of *P. trimera* leaves. They found the total phenolic and flavonoid content, proanthocyanidin and saponin content, and isolated five compounds similar to those in the stems and roots. In addition, the essential oil from the leaves has been reported to contain 43 constituents (H. T. Nguyen et al., 2024; T. Nguyen et al., 2024; Le et al., 2020; Doan et al., 2019). Besides, five flavonoids and three apotirucallanes were isolated from leaves of *P. trimera* (Tran D. et al., 2023; T. H. Tran et al., 2023). Notably, leaf extracts were reported to contain higher total phenolic and flavonoid contents than extracts from other parts of the plant (H. T. Nguyen et al., 2024).

The primary objective of this research was to identify antioxidant compounds (Figure 1) through a bio-guided approach based on TPC, TFC, and DPPH scavenging activity. This systematic investigation examined the antioxidant potentials of *P. trimera* leaves.

2. Experimental

2.1. Plant material

Leaves of *Paramignya trimera* (Oliv.) Burkill were collected in June 2024 from Cu Chi District, Ho Chi Minh City, Vietnam. The plant material was identified by Associate Professor Van Son Dang (Ho Chi Minh City Institute of Tropical Biology). A voucher specimen (UE-P017A) was deposited in the herbarium of the Department of Organic Chemistry, Ho Chi Minh City University of Education.

2.2. Extraction and isolation

Air-dried leaves of *P. trimera* (2.2 kg) were powdered and extracted with MeOH (3 × 12 L) at room temperature. The combined filtrates were concentrated under reduced pressure to afford a crude extract (390 g). This extract was suspended in water and successively partitioned with n-hexane and ethyl acetate to yield the n-hexane extract (H, 110 g), ethyl acetate extract (EA, 88 g), and water extract (W, 170 g). The isolation procedures for compounds 1–3 from the crude MeOH extract are summarized in Scheme 1.

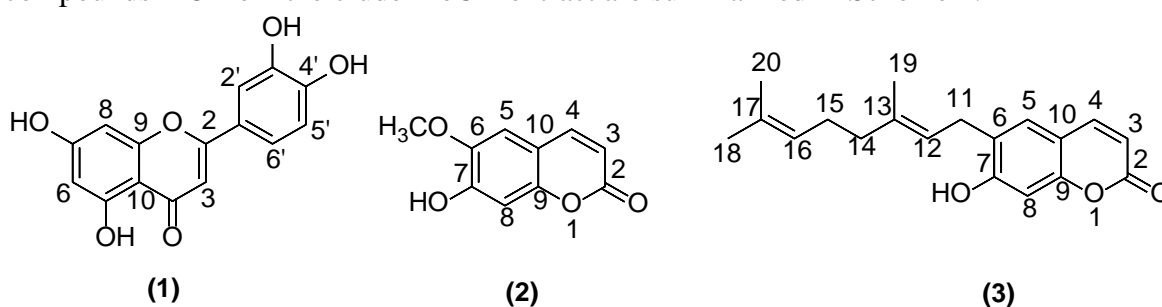
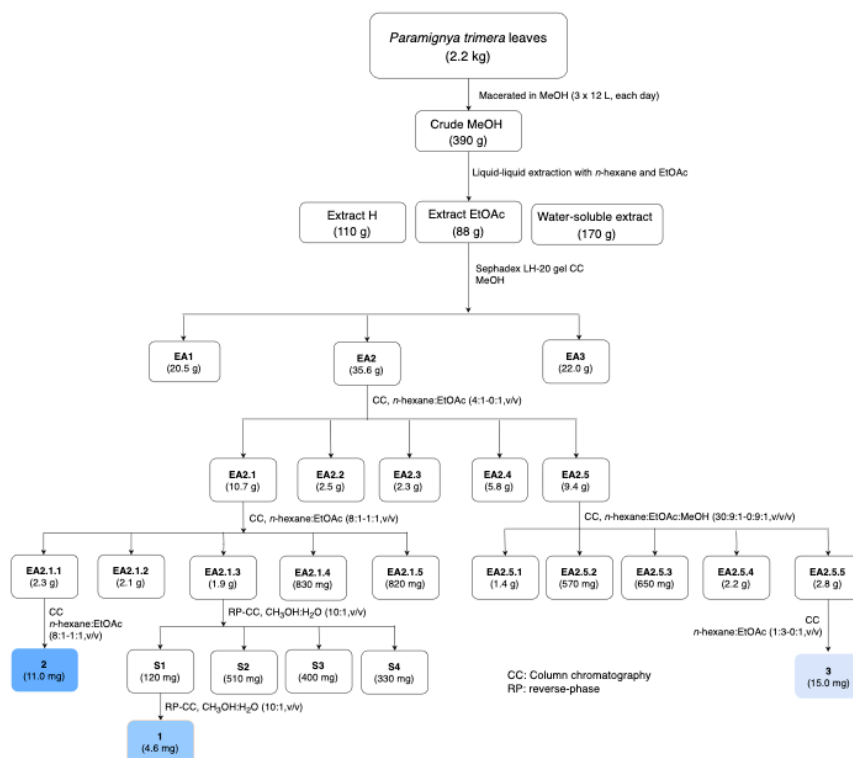


Figure 1. Chemical structures of isolated compounds 1-3

• **Luteolin (1).** Light-yellow amorphous powder. $^1\text{H-NMR}$ data ($^1\text{H-NMR}$ data (500 MHz, Acetone- d_6 , δ_{H} ppm, J in Hertz): 13.02 (1H, s, 5-OH), 6.58 (1H, s, H-3), 6.25 (1H, d, $J = 2.0$ Hz, H-6), 6.52 (1H, d, $J = 2.0$ Hz, H-8), 7.50 (1H, d, $J = 2.5$ Hz, H-2'), 7.47 (1H, dd, $J = 2.5, 8.5$ Hz, H-6'), 7.00 (1H, d, $J = 8.5$ Hz, H-5'). The NMR data of **1** were consistent with the literature (Lin et al., 2015). ESI-MS: $[\text{M}+\text{H}]^+$ 287.05 (calcd. for $\text{C}_{15}\text{H}_{11}\text{O}_6$, 287.06).

• **Scopoletin (2)**. Light-yellow amorphous powder. $^1\text{H-NMR}$ data (500 MHz, Acetone- d_6 , δ_H ppm, J in Hertz): 6.18 (1H, d , $J = 9.5$ Hz, H-3), 7.85 (1H, d , $J = 9.5$ Hz, H-4), 7.20 (1H, s , H-5), 6.80 (1H, s , H-8), 3.90 (3H, s , 6-OMe). $^{13}\text{C-NMR}$ data (125 MHz, Acetone- d_6 , δ_C ppm): 160.3 (C-2), 112.5 (C-3), 143.6 (C-4), 109.1 (C-5), 144.9 (C-6), 55.7 (6-OMe), 154.1 (C-7), 102.8 (C-8), 150.9 (C-9), 111.2 (C-10). The NMR data of **2** were consistent with the literature (Duong et al., 2024).

• **Ostruthin (3)**. Yellow amorphous powder. $^1\text{H-NMR}$ data (500 MHz, Acetone- d_6 , δ_H ppm, J in Hertz): 6.12 (1H, d , $J = 9.5$ Hz, H-3), 7.79 (1H, d , $J = 9.5$ Hz, H-4), 7.30 (1H, s , H-5), 6.76 (1H, s , H-8), 3.34 (2H, d , $J = 7.5$ Hz, H-11), 5.36 (1H, tq , $J = 7.5$, 1.5 Hz, H-12), 2.12 (2H, m , H-14), 2.05 (2H, m , H-15), 5.10 (1H, m , H-16), 1.63 (3H, s , H₃-18), 1.71 (3H, s , H₃-19), 1.56 (3H, s , H₃-20), 9.57 (1H, s , 7-OH). $^{13}\text{C-NMR}$ data (125 MHz, Acetone- d_6 , δ_C ppm): 161.3 (C-2), 112.6 (C-3), 144.8 (C-4), 129.2 (C-5), 126.7 (C-6), 159.6 (C-7), 102.7 (C-8), 155.1 (C-9), 112.6 (C-10), 28.2 (C-11), 122.7 (C-12), 137.1 (C-13), 40.0 (C-14), 27.3 (C-15), 125.0 (C-16), 131.7 (C-17), 25.9 (C-18), 16.2 (C-19), 17.7 (C-20). The NMR data of **3** were consistent with the literature (Duong et al., 2016).



Scheme 1. Isolation procedure of compounds **1-3** from *P. trimera* leaves

2.3. Total Phenolic Contents (TPCs)

The analysis of TPC was conducted using a procedure with some modifications (Duong et al., 2024).

2.4. Total Flavonoid Contents (TFCs)

The TFC in the *P. trimera* extracts were evaluated following AlCl_3 colourimetric method modified with some modifications (Duong et al., 2024).

2.5. DPPH free radical scavenging activity

The free radical 1,1-diphenyl-2-picrylhydrazyl (DPPH) scavenging activity of extracts and compounds was evaluated according to (Duong et al., 2024).

3. Results and discussion

Compound **1** was obtained as a light-yellow amorphous powder. The $^1\text{H-NMR}$ spectrum of **1** showed seven proton signals, including a hydrogen-bonded hydroxy group signal at δ_{H} 13.02 (1H, *s*, 5-OH), two *meta*-coupled protons at δ_{H} 6.25 (1H, *d*, $J = 2.0$ Hz, H-6), 6.52 (1H, *d*, $J = 2.0$ Hz, H-8), and an olefinic proton at δ_{H} 6.58 (1H, *s*, H-3). In addition, the $^1\text{H-NMR}$ spectrum of **1** also showed a presence of a 1,2,4-trisubstituted aromatic ring [7.50 (1H, *d*, $J = 2.5$ Hz, H-2'), 7.47 (1H, *dd*, $J = 2.5, 8.5$ Hz, H-6'), 7.00 (1H, *d*, $J = 8.5$ Hz, H-5')]. A comprehensive comparison of $^1\text{H-}$ and $^{13}\text{C-}$ NMR data obtained from **1** and luteolin demonstrated the consistency of the results. Consequently, **1** was successfully elucidated as luteolin.

Compound **2** was obtained as a light-yellow amorphous powder. The $^1\text{H-NMR}$ spectrum of **2** showed signals of methoxy group [δ_{H} 3.90 (3H, *s*, 6-OMe)]; two *singlet* aromatic protons [δ_{H} 6.80 (1H, *s*, H-8) and 7.20 (1H, *s*, H-5)] and two olefinic protons coupled to each other [δ_{H} 6.18 (1H, *d*, $J = 9.5$ Hz, H-3 and 7.85 (1H, *d*, $J = 9.5$ Hz, H-4)]. The $^{13}\text{C-NMR}$ spectrum of **2** revealed signals for ten carbons, including a carboxyl ester carbon 160.3 (C-2), four methine carbons [112.5 (C-3), 143.6 (C-4), 109.1 (C-5), 102.8 (C-8)], four substituted aromatic carbons [144.9 (C-6), 154.1 (C-7), 150.9 (C-9), 111.2 (C-10)], and one methoxy carbon [δ_{C} 55.7 (6-OMe)]. A comprehensive comparison of $^1\text{H-}$ and $^{13}\text{C-}$ NMR data obtained from **2** and scopletin demonstrated the consistency of the results. Consequently, **2** was successfully elucidated as scopletin.

Compound **3** was obtained as a yellow amorphous powder. The $^1\text{H-NMR}$ spectrum of **3** exhibited the presence of a phenolic group [δ_{H} 9.57 (*s*, 1H, 7-OH)], two *singlet* aromatic protons [δ_{H} 7.30 (1H, *s*, H-5) and 6.76 (1H, *s*, H-8)], and two olefinic protons coupled to each other [δ_{H} 6.12 (1H, *d*, $J = 9.5$ Hz, H-3 and 7.79 (1H, *d*, $J = 9.5$ Hz, H-4)]. These data were highly similar to those of **2**, indicating that **3** was a coumarin. A notable distinction between **2** and **3** was the alteration of substituents at carbon positions C-6 and C-7. A geranyl group was characterized by two triplet olefinic protons [δ_{H} 5.36 (1H, *t*, $J = 7.5, 1.5$ Hz, H-12), 5.10 (*m*, H-16)]; three methyls [δ_{H} 1.63 (3H, *s*, H₃-18), 1.71 (3H, *s*, H₃-19), 1.56 (3H, *s*, H₃-20)]; three methylenes [δ_{H} 3.34 (*d*, $J = 7.5$ Hz, 2H, H₂-11), 2.12 (2H, *m*, H₂-14), δ_{H} 2.05 (2H, *m*, H₂-15)]. The $^{13}\text{C-NMR}$ spectrum of **3** revealed signals for 19 carbons, including nine carbon signals of a coumarin unit [δ_{C} 161.3 (C-2), 112.6 (C-3), 144.8 (C-4), 129.2 (C-5), 126.7 (C-6), 159.6 (C-7), 102.7 (C-8), 155.1 (C-9), 112.6 (C-10)] and ten carbon signals of a geranyl group [δ_{C} 28.2 (C-11), 122.7 (C-12), 137.1 (C-13), 40.0 (C-14), 27.3 (C-15), 125.0 (C-16), 131.7 (C-17), 25.9 (C-18), 16.2 (C-19), 17.7 (C-20)]. A comprehensive comparison of NMR data obtained from **3** and ostruthin demonstrated the consistency of the results. Consequently, **3** was successfully elucidated as ostruthin.

The antioxidant potentials of extracts obtained from *P. trimera* leaves were assessed, including total phenolic content (TPCs), total flavonoid content (TFCs), and DPPH scavenging activity. As shown in Table 1, TPCs and TFCs of extract **EA** from *P. trimera*

are higher than those of extract **H** and the crude extract. The extract **EA** exhibited stronger DPPH scavenging activity, with an IC_{50} value of 34.64 $\mu\text{g/mL}$. Thus, this extract was selected for chemical analysis. The aforementioned biological characteristics were consistent with those previously reported on *P. trimera* leaves. (V. T. Nguyen et al., 2015; Trinh et al., 2020). Compounds **2-3** were evaluated for the free radical DPPH scavenging activity. Compound **2** exhibited moderate activity with an IC_{50} value of 7.1 μM , while compound **3** demonstrated no activity. These findings were consistent with those reported in the literature (Antika et al., 2022; V. T. Nguyen et al., 2015). Compound **1** was previously hypothesized to possess robust antioxidant properties (Gökbulut et al., 2012).

Table 1. Results of total phenolic Content (TPC), total flavonoid content (TFC), and free radical DPPH scavenging activity of *P. trimera* extracts/fractions

Extract	TPCs	TFCs	DPPH
	(mg GAE/g)	(mg QE/g)	IC_{50} ($\mu\text{g/mL}$)
Crude MeOH extract	32.35 \pm 1.04	8.09 \pm 0.10	49.50 \pm 2.15
Extract H	20.70 \pm 2.58	19.35 \pm 0.89	34.72 \pm 14.91
Extract EA	74.99 \pm 2.03	73.95 \pm 5.54	34.64 \pm 5.57
Fraction EA1	52.55 \pm 6.28	50.46 \pm 6.67	5.58 \pm 0.51
Fraction EA2	54.44 \pm 6.72	50.43 \pm 4.81	68.03 \pm 23.37
Fraction EA3	33.09 \pm 4.48	32.06 \pm 1.33	34.58 \pm 3.06
	Ascorbic acid (positive control)		1.13 \pm 0.21

4. Conclusions

From the leaves of *Paramignya trimera* collected in Ho Chi Minh City, three compounds, namely luteolin (**1**), scopoletin (**2**), and ostruthin (**3**), were isolated from the most active EA extract through a bioassay-guided procedure. Among them, scopoletin (**2**) showed notable DPPH radical-scavenging activity, with an IC_{50} value of 7.1 μM . These findings indicate that the leaves of *P. trimera* are a promising source of natural antioxidant constituents and merit further phytochemical and biological investigation.

❖ **Conflict of Interest:** Authors have no conflict of interest to declare.

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HOẠT TÍNH KHÁNG OXY HOÁ CỦA LÁ *Paramignya trimera*

Phạm Nguyễn Kim Tuyền^{1*}, Đỗ Anh Kỳ², Nguyễn Minh Tiến², Võ Minh Tú², Lê Văn Thành²,
Nguyễn Tuấn Đạt², Đặng Hồng Diệp² Phạm Đức Dũng², Dương Thúc Huy²

¹Trường Đại học Sài Gòn, Việt Nam

²Trường Đại học Sư phạm Thành phố Hồ Chí Minh, Việt Nam

*Tác giả liên hệ: Phạm Nguyễn Kim Tuyền – Email: phngktuyen@gmail.com

Ngày nhận bài: 14-3-2025; Ngày nhận bài sửa: 18-4-2025; Ngày duyệt đăng: 21-4-2025

TÓM TẮT

Paramignya trimera hay còn được gọi là “XÁO TAM PHÂN”, một loại cây thuốc cổ truyền có nguồn gốc từ Việt Nam, đã được sử dụng ở các nước châu Á vì đặc tính điều trị của nó. Mục tiêu chính của nghiên cứu này là phân lập các hợp chất hoạt tính sinh học từ lá của *P. trimera* bằng cách sử dụng quy trình có hướng dẫn sinh học dựa trên hoạt tính chống oxy hóa. Hoạt tính ức chế gốc tự do DPPH của các cao chiết đã được đánh giá và cao chiết (cao EA) có hoạt tính tốt nhất được lựa chọn để phân tích về hóa học. Ba hợp chất, cụ thể là luteolin (1), scopoletin (2), và ostruthin (3) được phân lập. Cấu trúc hóa học của các hợp chất này đã được làm sáng tỏ bằng cách so sánh dữ liệu phổ của chúng với dữ liệu tài liệu đã báo cáo trước đó. Hợp chất 2 thể hiện hoạt tính ức chế gốc tự do DPPH mạnh với giá trị IC_{50} là 7.1 μ M

Từ khóa: antioxidant; DPPH; luteolin; ostruthin; *Paramignya trimera*; scopoletin