WITHANOLIDES AND FLAVONOIDS FROM THE FRUITS OF *PHYSALIS ANGULATA* AND THEIR ANTIOXIDANT ACTIVITY

Dinh Thi Huong, Nguyen Van Chinh*, Lo Van Xanh**

Title: *Withanolides and flavonoids from the fruits of Physalis angulata and their antioxidant activity*

Keywords: *Physalis angulata, bioactive compounds, withanolides, flavonoids*

Lịch sử bài báo

Ngày nhận bài:

Ngày nhận kết quả bình duyệt:

Ngày chấp nhận đăng bài:

Tác giả: *Faculty of Medicine and Pharmacy, Yersin University of Da Lat*

Email liên hệ: *huongdt@yersin.edu.vn*

Tóm tắt

Hysalis angulata L. (họ Solanaceae) là một dược liệu từ lâu được sử dụng trong điều trị nhiễm khuẩn, bệnh lý gan và các tình trạng viêm ở Việt Nam. Trong nghiên cứu này, có năm hợp chất đã được phân lập từ loài P. angulata và xác định được cấu trúc hóa học , bao gồm hai withanolides và ba flavonoids. Cấu trúc hóa học của các hợp chất được xác định bằng các phương pháp phổ hiện đại, bao gồm NMR 1D and 2D và LC–MS. Hoạt tính chống oxy hóa được đánh giá bằng phương pháp DPPH radical-scavenging assay, cho thấy các flavonoid - đóng vai trò chủ đạo trong khả năng bắt giữ các gốc tự do của loài này. Những phát hiện này cung cấp bằng chứng hóa thực vật và tác dụng dược lý quan trọng, góp phần làm sáng tỏ tiềm năng ứng dụng của P. angulata trong phòng ngừa và hỗ trợ điều trị các bệnh lý liên quan đến stress oxy hóa; Tuy nhiên, cần có thêm các nghiên cứu in vivo và thử nghiệm lâm sàng để xác nhận hiệu quả và độ an toàn trước khi triển khai trong thực hành Dược lâm sàng.

Abstract

*Vietnamese native Physalis angulata L. (Solanaceae) is a medicinal plant that has long been used to treat microbiological infections, liver ailments, and inflammatory conditions. Five chemicals, comprising two withanolides (**1–2**) and three flavonoids (**3–5**), were isolated, and their structures were clarified through phytochemical analysis of P. angulata in this work. The chemical structures of these compounds were determined using spectroscopic techniques, including 1D and 2D NMR analyses and LC–MS data. In addition, the antioxidant activity of the isolated compounds was evaluated using the DPPH radical-scavenging assay. The results indicate that the flavonoids are the main contributors to the antioxidant activity of P. angulata. These findings provide valuable phytochemical and pharmacological evidence contributing to clarifying the potential application of this plant in the prevention and supportive treatment of oxidative stress-related diseases; however, further in vivo studies and clinical trials are needed to confirm efficacy and safety before deployment in clinical pharmacy practice.*

1. Introduction

Natural products have long played a pivotal role in drug discovery and development, serving as an indispensable source of structurally diverse and biologically active compounds (Le Ba Vinh, 2025). A substantial proportion of currently approved drugs, particularly those used to treat inflammation, cancer, and infectious diseases, are derived from natural products or their semi-synthetic derivatives (Nguyen Minh Trang, 2024).

The remarkable chemical diversity and evolutionary optimization of secondary metabolites produced by medicinal plants provide unique molecular scaffolds that are often difficult to access through synthetic approaches alone (Dinh Thi Thanh Thuy, 2022). Consequently, systematic phytochemical and pharmacological investigations of traditionally used medicinal plants remain a highly effective strategy for the identification of novel lead compounds with therapeutic potential (Ngo Viet Duc, 2024).

In tropical and subtropical areas, including Vietnam, *Physalis angulata* L. (Solanaceae) is a common therapeutic plant that has been traditionally used to treat inflammatory disorders, liver diseases, and microbial infections (Ariyani Novitasari, 2024). Owing to its long-standing ethnomedicinal use, P. angulata has attracted increasing scientific attention as a promising source of bioactive natural products (M. Huang, 2020). Phytochemical investigations have revealed that *P. angulata* is particularly rich in structurally diverse secondary metabolites, such as withanolides, flavonoids, and phenolic compounds (Ariyani Novitasari, 2024). These classes of compounds are well recognized for their broad spectrum of biological activities, including antioxidant, anti-inflammatory, anticancer, and immunomodulatory effects (Ariyani Novitasari, 2024). Indeed, previous pharmacological studies have demonstrated that crude extracts and isolated constituents of *P. angulata* exhibit significant bioactivities in both in vitro and in vivo experimental models, supporting its traditional therapeutic applications (E. Carrillo-Perdomo, 2015).

In addition, the wide geographical distribution, rapid growth, and ease of cultivation of *P. angulata* make it an attractive and sustainable natural resource for drug discovery and development. Nevertheless, despite the growing number of phytochemical and pharmacological reports, systematic studies correlating the chemical constituents of *P. angulata* with their specific biological activities remain limited. In particular, detailed investigations focusing on the identification of bioactive flavonoids and withanolides and their roles in antioxidant and anti-inflammatory mechanisms are still insufficient. Therefore, the present study aims to investigate the phytochemical constituents of *P. angulata*, isolate and elucidate the structures of its major secondary metabolites, and evaluate their antioxidant activity. This work is expected to provide further scientific evidence supporting the medicinal value of *P. angulata* and contribute to the rational utilization and standardization of this traditional medicinal plant.

2. Experimental section

2.1. General experimental procedures

Chromatographic isolation of the constituents was achieved by applying open-column techniques with reversed-phase YMC*GEL ODS-A (12 nm, S-150 μm; YMC Co., Ltd., Japan) and normal-phase silica gel (Kieselgel 60, 70–230 and 230–400 mesh; Merck, Darmstadt, Germany) serving as stationary supports. These media were employed in multiple separation steps to generate individual fractions and obtain purified compounds. Thin-layer chromatography (TLC) was routinely conducted to assess separation progress. After examination under UV radiation at 254 and 365 nm, TLC plates were further developed by spraying with 10% (v/v) aqueous H_2SO_4 followed by heating to reveal the chromatographic profiles. Formic acid (FA) and all other HPLC-grade solvents and reagents were purchased from Aldrich Chemical Co. (St.

Louis, MO, USA). Nuclear magnetic resonance (NMR) spectra, including ^{1}H, ^{13}C, and two-dimensional (2D) experiments, were recorded at room temperature on a Bruker Avance III 600 MHz spectrometer.

2.2. Plant Material

The fruits of *Physalis angulata* were collected in Me Linh District, Hanoi, Vietnam, in May 2023. The plant species was taxonomically identified by Dr. Nguyen Cao Cuong. A voucher specimen (TBML 02/2023) was deposited at the herbarium of the Faculty of Medicine and Pharmacy, Yersin University, Da Lat, Vietnam.

2.3. Extraction and isolation

Dried fruits of *Physalis angulata* (4.0 kg) were finely ground and exhaustively extracted by ultrasonic-assisted maceration with ethanol at 35 °C, using 4.0 L of solvent per extraction in three successive cycles. Evaporation of the solvent under reduced pressure afforded a crude methanolic residue (150 g), which was redissolved in distilled water (1.5 L) and subjected to liquid–liquid partitioning. The aqueous suspension was sequentially extracted three times with dichloromethane and ethyl acetate (1.5 L each) to furnish a dichloromethane fraction (QTBD, 35.5 g), an ethyl acetate fraction (QTBE, 8.0 g), and a remaining aqueous fraction (PAW). The QTBD fraction was further resolved by silica gel column chromatography, employing a stepwise gradient of dichloromethane–methanol (0–100% methanol, v/v; 1.5 L per step), yielding five primary fractions (QTBD1–QTBD5). Subsequent chromatographic treatment of QTBD3 (1.8 g) on silica gel with dichloromethane/methanol (15:1, v/v) afforded five subfractions (QTBD3.1–QTBD3.5). Further purification of subfraction QTBD3.3 using silica gel chromatography with dichloromethane/acetone mixtures (15:1 and 15:2, v/v) led to the isolation of substance **1** (8.8 mg) and substance **2** (3.6 mg). The aqueous fraction (QTBW) was processed on a Diaion HP-20 column, initially rinsed with distilled water, followed by elution with aqueous methanol of increasing strength (25%, 50%, 75%, and 100%, v/v), to afford four fractions (QTBW1–QTBW4). Two subfractions, designated QTBW2.1 and QTBW2.2, were obtained by subjecting fraction QTBW2 (3.8 g) to silica gel column chromatography and eluting with dichloromethane/methanol/water (3:1:0.01, v/v/v). Subfraction QTBW2.2 (0.8 g) was further separated on an RP-18 reversed-phase column using methanol/water (1:2, v/v) as the eluent. Fraction PAW4 (3.1 g) was subjected to silica gel column separation, employing a ternary solvent system of dichloromethane–methanol–water (2:1:0.01, v/v/v), which resulted in the formation of two secondary fractions, designated QTBW4.1 and QTBW4.2. The QTBW4.1 fraction (1.1 g) was subsequently purified by passage through a reversed-phase RP-18 column using acetone–water (1:2, v/v) as the mobile phase. Final purification was accomplished by normal-phase silica gel chromatography with ethyl acetate–methanol–water (3:1:0.05, v/v/v), leading to the isolation of substances **3** (6.2 mg) and **4** (7.5 mg).

The remaining QTBW4.2 fraction (0.6 g) was independently processed by silica gel column chromatography, eluting with dichloromethane–methanol–water (2:1:0.01, v/v/v), to afford substance **5** (10.1 mg) in purified form.

2.4. DPPH

The DPPH radical-scavenging activity assay was conducted with minor modifications following our previously reported method [8]. Briefly, 100 µL of each test sample was prepared in distilled water

at final concentrations of 100, 50, 25, 12.5, and 6.25 μM per well. Each sample solution was then mixed with 100 μL of a freshly prepared DPPH solution (100 μM in ethanol). Control wells contained all reagents except the test samples, which were replaced with distilled water. After thorough mixing, the reaction mixtures were incubated at room temperature for 30 min prior to analysis. The decrease in absorbance resulting from the reduction of DPPH from deep violet to pale yellow in the presence of antioxidant compounds was measured at 517 nm using a microplate reader. The ability to neutralize free radicals was quantified in terms of inhibitory efficiency (EC%), calculated using the equation presented below:

The percentage inhibition (EC%) was calculated by subtracting the absorbance of the sample from that of the control, dividing by the control absorbance, and multiplying the result by 100. Where A_{control}represents the absorbance of the control and A_{sample} represents the absorbance of the test sample. Dose–response curves were generated by evaluating a range of sample concentrations, and the EC_{50} values were determined from these curves.

3. Results and discussions

3.1. Extraction and isolation

The EtOH extract of *P. angulata* was successively partitioned according to increasing polarity using dichloromethane and ethyl acetate (EtOAc). A combination of chromatographic separation techniques was employed to isolate five compounds (**1–5**) (Figure 1), including repeated column chromatography (CC) on silica gel, YMC-C18, and Sephadex LH-20.

Compound **1** was obtained as a white amorphous powder. Analysis of the 1H NMR data for compound 1 revealed the presence of five singlet methyl resonances, observed at δ_H 1.11, 1.26, 1.39, 1.87, and 1.98, each integrating for three protons and two olefinic protons at δ_H 6.01 (doublet of doublet, J = 2.5, 10.0 Hz) and 6.99 (1H, ddd, J = 2.5, 6.0, 10.0 Hz), and two oxymethine protons at δ_H 3.25 (1H, d, J = 2.0 Hz) and 4.86 (1H, d, J = 3.5 Hz). Combined evaluation of the ^{13}C NMR and HSQC data established a total of 28 carbon resonances, comprising 10 C, 6 CH, 7 CH_2, and 5 CH_3. The overall NMR spectral features of compound **1** were consistent with a withanolide framework, a metabolite class commonly associated with *P. angulata* (H.E. Gottlied, 1981). Comparison of the ^{1}H and ^{13}C NMR profiles of compound **1** with reported data revealed a close correspondence to those of withanolide E. The existence of a 2,3-unsaturated ketone functionality in ring A was substantiated by diagnostic HMBC interactions, including cross-peaks linking H-2 (δ_H 6.01) with C-4 (δ_C 33.7) and C-10 (δ_C 49.8), H-3 (δ_H 6.99) with C-1 (δ_C 205.4), C-4 (δ_C 33.7), and C-5 (δ_C 63.3), as well as correlations from H-19 (δ_H 1.26) to C-1, C-5, C-9, and C-10. The appearance of the H-6 resonance at δ_H 3.25 (d, J = 2.0 Hz), together with the downfield carbon signals at C-5 (δ_C 63.3) and C-6 (δ_C 65.1), was indicative of a 5β,6β-epoxide moiety located in ring B. Furthermore, long-range HMBC correlations from the methyl protons H-18 (δ_H 1.11) and H-21 (δ_H 1.39) to C-17 (δ_C 88.7), in combination with the pronounced deshielding of this carbon signal, provided compelling evidence for the presence of a hydroxyl substituent at C-17. Based on these spectroscopic analyses and by comparison of the ^{1}H and ^{13}C NMR data with those reported in the literature, compound **1** was identified as withanolide E (H.E. Gottlied, 1981). To the best of our knowledge, this is the first report of the isolation of withanolide E from the fruits of *P. angulata*.

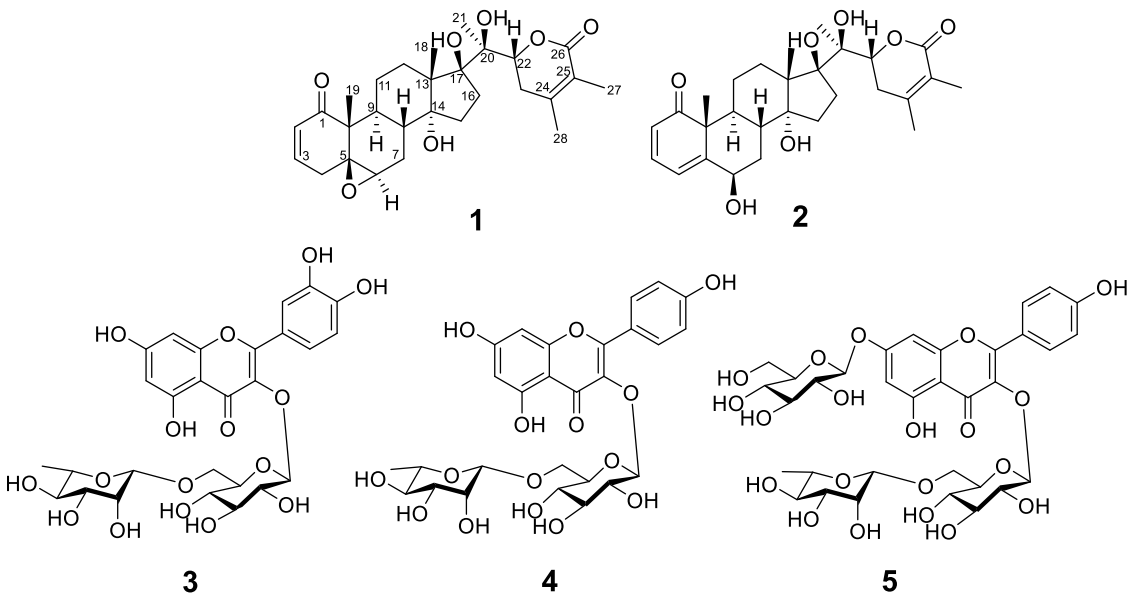

Figure 1: Chemical structures of compounds isolated from the fruits of *Physalis angulata*.

Compound **2** was isolated as a white, amorphous solid. Interpretation of its ^{1}H NMR spectrum revealed characteristic resonances attributable to three olefinic protons, appearing as a doublet at δ_H 6.00 (J = 10.0 Hz), a second doublet at δ_H 6.23 (J = 6.0 Hz), and a doublet of doublets at δ_H 7.08 (J = 6.2, 10.3 Hz). In addition, two oxygenated methine protons were observed at δ_H 4.60 (t, J = 2.6 Hz) and 4.83 (m). The upfield region displayed five singlet methyl signals at δ_H 1.21, 1.39, 1.49, 1.85, and 1.99, accounting for a total of fifteen protons. Detailed inspection of the ^{13}C NMR and HSQC spectra indicated that compound **2** possesses 28 carbon atoms, consisting of two carbonyl carbons, eight quaternary carbons, seven methine carbons, six methylene units, and five methyl carbons. Overall, the NMR spectroscopic profile of **2** closely resembled that of compound **1**, with notable deviations localized in rings A and B. These differences were consistent with the introduction of a C-4/C-5 olefinic linkage and the absence of a C-5/C-6 epoxide moiety in compound **2**. The presence of a 2(3),4(5)-diene system within ring A was substantiated by diagnostic HMBC cross-peaks, including correlations from H-2 to C-4 and C-10 , from H-3 to C-1, C-2, and C-5, as well as from H-4 to C-2, C-3, C-6, and C-10. Further HMBC correlations linking H-6 (δ_H 4.60) with C-4, C-5, C-7, C-8, and C-10, in conjunction with the markedly downfield C-6 resonance at δ_C 75.0, confirmed the substitution of a hydroxyl group at C-6. Comparative analysis of the ^{13}C NMR data of compound **2** with those reported for withaperuvin C demonstrated close agreement across most carbon signals. Minor discrepancies involving the assignments of C-2/C-4 and C-12/C-15/C-23 were clarified through additional HMBC evidence, including correlations from H-4 to C-6, H-18 to C-12, H-15 to C-8, C-13, and C-17, and H-28 to C-23. On the basis of the complete spectroscopic evidence, compound **2** was unequivocally identified as withaperuvin C

(R. Mahrous, 2019). To our knowledge, the occurrence of withaperuvin C in *P. angulata* has not been documented previously, making this the first study to report its isolation from this species.

Using the same analytical methods for NMR spectroscopic data analysis and by comparison with literature data, the other compounds were identified as quercetin 3-O-β-rutinoside (**3**) (M.-A. Beck, 1999), kaempferol 3-O-β-rutinoside (**4**) (C. Clarkson, 2005), and kaempferol 3-O-β-rutinoside-7-O-β-D-glucopyranoside (**5**) (C.A. Elliger, 1992), which have previously been reported as major chemical constituents of *P. angulata*.

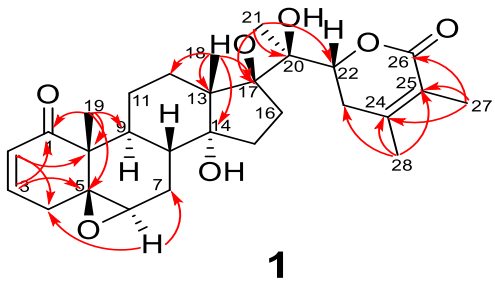

Figure 2. Key HMBC correlations of compound **1**

Table 1. NMR data of two withanolides **1** and **2**

No.	**1**		**2**	
	δ_C (125 MHz)	δ_H (500 MHz, mult., J in Hz)	δ_C (125 MHz)	δ_H (500 MHz, mult., J in Hz)
1	205.4 (C)	–	208.2 (C)	–
2	129.9 (CH)	6.01 (dd, J = 2.3, 10.2)	126.7 (CH)	6.00 (d, J = 10.0)
3	146.7 (CH)	6.99 (ddd, J = 2.6, 6.2, 9.8)	142.9 (CH)	7.08 (dd, J = 6.2, 10.3)
4	33.7 (CH_2)	1.94 (m), 2.97 (dt, J = 2.5, 18.5)	118.4 (CH)	6.23 (d, J = 6.0)
5	63.3 (C)	–	160.4 (C)	–
6	65.1 (CH)	3.25 (d, J = 1.8)	75.0 (CH)	4.60 (t, J = 2.6)
7	27.5 (CH_2)	1.84 (1H, m), 1.94 (1H, m)	37.6 (CH_2)	1.64 (m), 1.95 (m)
8	35.2 (CH)	1.95 (m)	35.8 (CH)	2.47 (m)
9	38.4 (CH)	1.83 (m)	44.2 (CH)	1.80 (m)
10	49.8 (C)	–	55.7 (C)	–
11	24.3 (CH_2)	1.65 (m), 2.01 (1H, m)	22.6 (CH_2)	1.67 (m), 1.72 (m)
12	33.3 (CH_2)	1.55 (m), 1.70 (m)	31.5 (CH_2)	1.37 (m), 2.22 (m)
13	55.6 (C)	–	55.6 (C)	–
14	84.4 (C)	–	84.7 (C)	–
15	31.5 (CH_2)	1.30 (m), 2.28 (m)	33.4 (CH_2)	1.54 (1H, m), 1.83 (1H, m)

16	37.4 (CH_2)	1.56 (dd, J = 8.5, 14.0), 2.58 (m)	37.5 (CH_2)	1.58 (m), 2.56 (m)
17	88.7 (C)	–	88.5 (C)	–
18	21.1 (CH_3)	1.11 (s)	21.3 (CH_3)	1.21 (s)
19	15.2 (CH_3)	1.26 (s)	18.8 (CH_3)	1.49 (s)
20	79.8 (C)	–	79.8 (C)	–
21	19.5 (CH_3)	1.39 (s)	19.5 (CH_3)	1.39 (s)
22	82.9 (CH)	4.86 (d, J = 3.5)	82.8 (CH)	4.83 (m)
23	35.7 (CH_2)	2.53 (m), 2.64 (dd, J = 3.5, 18.5)	35.7 (CH_2)	1.54 (m), 1.69 (m)
24	153.4 (C)	–	153.4 (C)	–
25	122.0 (CH)	–	121.9 (CH)	–
26	169.1 (C)	–	169.0 (C)	–
27	12.4 (CH_3)	1.87 (s)	12.4 (CH_3)	1.85 (s)
28	20.6 (CH_3)	1.98 (s)	20.7 (CH_3)	1.99 (s)

Structural assignments were established through the combined interpretation of one-dimensional NMR spectra (^{1}H and ^{13}C) together with two-dimensional NMR techniques, including ^{1}H-^{13}C HSQC, ^{1}H-^{13}C HMBC, and ^{1}H-^{1}H COSY.

3.2. Antioxidant activity

The isolated compounds were evaluated for their antioxidant activity using the DPPH radical-scavenging assay. Compounds **3–5** exhibited pronounced antioxidant effects, with IC_{50} values of 13.7 ± 0.3, 12.6 ± 0.2, and 21.5 ± 1.2 μM, respectively, demonstrating activities comparable to that of the reference antioxidant ascorbic acid (IC_{50} = 10.8 ± 0.2 μM). Among these compounds, compound **4** displayed the strongest antioxidant activity.

4. Conclusion

In conclusion, the present study provides a comprehensive phytochemical and pharmacological investigation of *P. angulata*, a traditionally used medicinal plant in Vietnam. Five compounds, including three flavonoids and two withanolides, were successfully isolated and structurally elucidated using advanced spectroscopic techniques. Biological evaluation revealed that these constituents exhibited notable antioxidant and anti-inflammatory activities, indicating that flavonoids and withanolides are key contributors to the medicinal properties of *P. angulata*. These findings not only support the traditional use of this plant but also highlight its potential as a valuable natural source of bioactive compounds for drug discovery. Furthermore, this work contributes to the scientific validation and sustainable utilization of Vietnam's medicinal plant biodiversity.

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