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Withanolides from the whole plant of *Physalis angulata* and their anti-inflammatory activities

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Abstract

From the dichloromethane extract of the whole plant of *P. angulata* growing in Vietnam, three withanolides including physagulin L (**1**), physagulin M (**2**), and withaminimin (**3**) were isolated by using various chromatography methods. Their chemical structures were determined by detailed analysis of 1D and 2D-NMR data and comparison with reported data. All isolated compounds were tested for their inhibitory activities against NO production in LPS-stimulated RAW 264.7 macrophages. The results showed that compound **3** had a weak anti-inflammatory activity against NO production in RAW 264.7 macrophages with IC_{50} value of 69.6±4.5 μM.

Keywords. *Physalis angulata*, anti-inflammatory activity, physagulin L, physagulin M, withaminimin.

1. INTRODUCTION

Physalis angulata L. (Solanaceae), a herb known as "tam bop" or "lu lu cai" in Vietnam, widely distributed throughout tropical regions of Asia, Africa, and America.[12] The whole plant of *P. angulata* has been used as a traditional medicine to treat various illnesses in Vietnam, such as fever, diabetes, pharyngintis, furuncle, cough, and mastitis.[1] Previous chemical investigations have revealed withasteroids as the major type of secondary metabolites of this species.[3] Studies on chemical constituents of *P. angulata* have isolated over 70 withasteroids.[4-9] Besides, pharmacological researches on extracts and isolated compounds from the plant showed anti-inflammatory, anti-allergic, anti-nociceptive, anti-parasitic, anti-bacterial, anti-oxidant, diuretic, immunomodulatory, and cytotoxic activities.[9,10-14]. In the present study, we described the isolation and chemical structural elucidation of three withanolides from the dichloromethane extract of the whole plant of *P. angulata* (figure 1). Furthermore, the isolated compounds were examined for their inhibitory activities on LPS-induced NO in LPS-stimulated RAW 264.7 macrophages.

2. MATERIALS AND METHODS

2.1. Plant material

The whole plant of *P. angulata* was collected at Thai Binh, Vietnam in August 2015, and was identified by Dr. Tran Thi Phuong Anh, Vietnam National Museum of Nature, VAST. A voucher specimen (TB14.2015) was deposited at Herbarium of Mientrung Institute for Scientific Research, VAST.

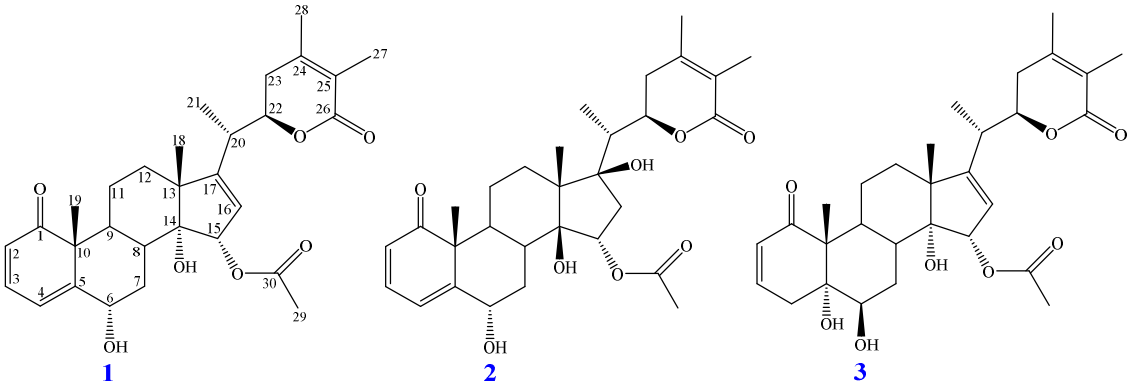
2.2. General experimental procedures

All NMR spectra were recorded on a Bruker AM500 FT-NMR spectrometer (500 MHz for ^{1}H-NMR and 125 MHz for ^{13}C-NMR). NMR measurements, including ^{1}H-NMR, ^{13}C-NMR, HSQC, and HMBC experiments were carried out using 5-mm probe tubes

at 22.2 ºC in CD_3OD solution, with TMS as an internal standard. Optical rotations were determined on a Jasco DIP-370 automatic polarimeter. Column chromatography was performed using a silica-gel (Kieselgel 60, 70-230 mesh and 230-400 mesh, Merck) or RP-18 resins (150 µm, Fuji Silysia Chemical Ltd.), thin layer chromatography (TLC) using a pre-coated silica-gel 60 F_{254} (0.25 mm, Merck) and RP-18 $F_{254}S$ plates (0.25 mm, Merck).

2.3. Extraction and isolation

The whole plant of *P. angulata* was ultrasound extracted with methanol (3 times x 4 L) at 50 ºC. After concentrating under reduced pressure, the methanol extract (PA, 90 g) was suspended in distilled water (1.5 L) and partitioned with dichloromethane and ethyl acetate (3 times, 1.5 L each) to obtain corresponding soluble extracts, dichloromethane (PAD, 24.5 g), ethyl acetate (PAE, 4.6 g), and water-soluble layers (PAW). The PAD fraction was subjected to a silica gel column chromatography eluting with mixtures of dichloromethane/methanol (0 to 100 % methanol in dichloromethane) to provide 5 fractions, PAD1-PAD5. Three subfractions (PAD2A-PAD2C) were obtained from the fraction PAD2 using a silica gel column and eluted with dichloromethane/methanol (20/1, v/v). The PAD2A subfraction was continuously chromatographed on a silica gel column, then eluted with dichloromethane/acetone (6/1, v/v) to yield compounds **1** (10 mg) and **2** (8 mg). The fraction PAD5 was separated on an RP-18 reverse phase column and then eluted with acetone/water (2/1, v/v) to obtain three subfractions, PAD5A-PAD5C. Compound **3** (17 mg) was yielded from the PAD5A subfraction on a silica gel column eluted with dichloromethane/acetone (4/1, v/v).



1 **2** **3**

Figure 1: Chemical structures of compounds **1-3**

Physagulin L (1): White amorphous powder; ESI-MS: *m/z* 511 $[M+H]^+$; $[\alpha]_D^{25}$ +91.4 (*c* 0.1, MeOH); ^{1}H-NMR (CD_3OD, 500 MHz) and ^{13}C-NMR (125 MHz, CD_3OD), see table 1.

Physagulin M (2): White amorphous powder; ESI-MS: m/z 529 $[M+H]^+$; $[\alpha]_D^{25}$ +42.3 (*c* 0.1, MeOH); ^{1}H NMR (CD_3OD, 500 MHz) and ^{13}C-NMR (125 MHz, CD_3OD) see table 1.

Withaminimin (3): White amorphous powder; ESI-MS: m/z 529 $[M+H]^+$; $[\alpha]_D^{25}$ +96.7 (*c* 0.1, MeOH); ^{1}H NMR (CD_3OD, 500 MHz) and ^{13}C-NMR (125 MHz, CD_3OD), see table 1.

2.4. Assay for inhibition of NO production

The effects of isolated compounds on the NO production in LPS-stimulated RAW264.7 macrophages were evaluated as previous description in Ref. [15]. The cells were sowed in a 96-well plate at a density of 2.5×105 cells/well and incubated for 12 h. The plate was pretreated with isolated compounds in various concentrations (from 1 to 100 µM) for 30 min and then incubated for another 24 h with or without 1 µg/ml LPS. 100 µL of the culture supernatant were transferred to other 96-well plate and 100 µL of Griess reagent was added. The absorbance of the reaction solution was read at 570 nm with a microplate reader (XMark microplate reader, Biorad, USA). The nitrite level in each sample was calculated from a standard curve generated with sodium nitrite. Cell viability was measured with a MTT [3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide]-based colorimetric assay. Cardamonin was used as a positive control (IC_{50} = 1.41±0.03 µM).

3. RESULTS AND DISCUSSION

Compound **1** was obtained as a white amorphous powder. The ^{1}H-NMR spectrum of **1** observed signals of four olefinic protons at δ_H 5.66 (1H, d, *J* = 2.5 Hz),

6.01 (1H, d, J = 10.0 Hz), 6.27 (1H, d, J = 6.0 Hz), and 7.12 (1H, dd, J = 6.0, 10.0 Hz); three oxymethine protons at δ_H 4.41 (1H, m), 4.61 (1H, t, J = 2.5 Hz), and 5.24 (1H, d, J = 2.5 Hz); six methyl groups at δ_H 1.18 (3H, s), 1.19 (3H, d, J = 6.5 Hz), 1.47 (3H, s), 1.85 (3H, s), 1.86 (3H, s), and 1.99 (3H, s) suggested the presence of a withanolide skeleton and an acetyl group. The ^{13}C-NMR displayed 30 signals (table 1), which were in accordance with HSQC spectrum corresponded to 10 non-protonated (including three carbonyl carbons at δ_C169.1, 171.3, and 208.0); 10 methine; four methylene; and six methyl carbons at δ_C 12.4, 16.7, 18.0, 18.3, 20.3 and 21.1. The ^{1}H and ^{13}C-NMR data suggest that compound **1** were a withanolide-type steroid with two double bonds at C-2/C-3), C-4/C-5 and an acetyl group at C-15.[16] The HMBC correlations (figure 2) from H-19 (δ_H 1.47) to C-1 (δ_C 208.0)/C-5 (δ_C 160.6)/C-9 (δ_C 46.0)/C-10 (δ_C 55.5), H-2 (δ_H 6.01) to C-4 (δ_C 118.4)/C-10 (δ_C 55.5), H-3 (δ_H 7.12) to C-1 (δ_C 208.0)/C-5 (δ_C 160.6) and H-4 (δ_H 6.27) to C-2 (δ_C 126.5)/C-3 (δ_C 142.7)/C-6 (δ_C 74.5)/C-10 (δ_C 55.5) confirmed the position of the carbonyl carbon (δ_C 208.0) at C-1 and two double bonds at C-2/C-3 and C-4/C-5. The position of an acetyl group at C-15 and a double bond at C-16/C-17 were confirmed by the HMBC correlations from H-18 (δ_H 1.18) to C-12 (δ_C 39.4)/C-13 (δ_C 53.5)/C-14 (δ_C 83.0)/C-17 (δ_C 162.9), H-16 (δ_H 5.66) to C-15 (δ_C 84.6)/C-17 (δ_C 162.9) and H-15 (δ_H 5.24) to C-29 (δ_C 171.3). The HMBC correlations from H-27 (δ_H 1.86) to C-24 (δ_C 152.9)/C-25 (δ_C 122.0)/C-26 (δ_C 169.1) and H-28 (δ_H 1.99) to C-23 (δ_C 33.5)/C-24 (δ_C 152.9)/ C-25 (δ_C 122.0) confirmed the presence of the lactone ring in the side chain. Besides, the HMBC correlations from H-6 (δ_H 4.61) to C-5 (δ_C 160.6)/ C-8 (δ_C 37.7)/ C-10 (δ_C 55.5) and the chemical shifts of C-6 (δ_C 74.5) and C-14 (δ_C 83.0) confirmed the position of two hydroxy groups at C-6 and C-14. Thus, the structure of **1** was elucidated as physagulin L.[16]

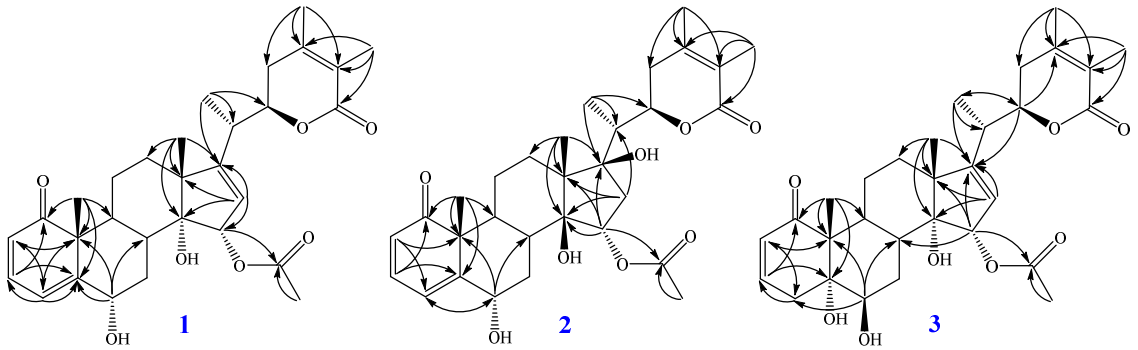


Figure 2: Key HMBC correlations of compounds **1-3**

Compound **2** was also obtained as a white amorphous powder. The ^{1}H-NMR spectrum of **2** exhibited the proton signals of six methyl groups at 1.07 (3H, d, J = 7.0 Hz), 1.24 (3H, s), 1.46 (3H, s), 1.87 (3H, s), 1.88 (3H, s), and 1.99 (3H, s); three oxymethine protons at δ_H 4.61 (1H, t, J = 2.5 Hz), 4.82 (1H, m), and 5.05 (1H, dd, J = 3.5, 8.5 Hz); three olefinic protons at δ_H 6.01 (1H, d, J = 10.0 Hz), 6.27 (1H, d, J = 6.0 Hz), and 7.12 (1H, dd, J = 6.0, 10.0 Hz). The ^{13}C-NMR and HSQC spectra of **2** showed the presence of three carbonyl, seven non-protonated, nine methine, five methylene, and six methyl carbons. The analytical NMR data of **2** indicated that the structure of **2** was close to those of **1** (table 1), except for the presence of a methylene and an oxygenated quaternary carbon in **2** instead of a double bond at C-16/C-17 in **1**. The differences were confirmed by the HMBC correlations from H-15 (δ_H 5.05) and H-18 (δ_H 1.24) to C-13 (δ_C 51.5)/C-14 (δ_C 88.0)/C-17 (δ_C 86.8), and H-16 (δ_H 1.52 and 1.68) and C-13 (δ_C 51.5)/C-14 (δ_C 88.0)/C-20 (δ_C 43.2) (figure 2). Consequently, compound **2** was determined as physagulin M by comparison with spectroscopic data in the literature.[16]

Comparison of the ^{13}C-NMR spectrum of compound **3** with those of physagulin L (**1**) (Table 1.) showed that the double bond at C-4/C-5 (δ_C 118.4, 160.6) in **1** was replaced by a methylene group at δ_C 36.8 and an oxygenated quaternary C-atom at δ_C 77.6 in **3**. The data indicated that **3** exhibited similar signals to **1,** except for the absence of a double bond at C-4/C-5. This was also confirmed by the HMBC correlations from H-2 (δ_H 5.81) to C-4 (δ_C 36.8)/C-10 (δ_C 53.1); H-3 (δ_H 6.68) to C-1 (δ_C 207.7)/C-5 (δ_C 77.6); and H-19 (δ_H 1.29) to C-1 (δ_C 207.7)/C-5 (δ_C 77.6)/C-10 (δ_C 53.1) (figure 2). From all the above evidences, compound **3** was identified as withaminimin.[17]

All isolated compounds were examined for their inhibitory activities on LPS-induced NO in LPS-stimulated RAW 264.7 macrophages. The results showed that compound **3** had a weak effect with IC_{50}

value of 69.6±4.5 μM relative to the positive control,

Table 1: The ^{1}H- and ^{13}C-NMR data for **1-3** and reference compounds

Pos.	$\delta_C^{\#1}$	**1**		$\delta_C^{\#2}$	**2**		$\delta_C^{\#3}$	**3**	
		$\delta_C^{a,b}$	$\delta_H^{a,c}$ mult. (*J* in Hz)		$\delta_C^{a,b}$	$\delta_H^{a,c}$ mult. (*J* in Hz)		$\delta_C^{a,b}$	$\delta_H^{a,c}$ mult. (*J* in Hz)
1	206.2	208.0	-	205.0	207.7	-	204.06	207.7	-
2	125.9	126.5	6.01 (d, *J* = 10.0 Hz)	126.5	126.6	6.01 (d, *J* = 10.0 Hz)	128.71	129.0	5.81 (dd, *J* = 2.5, 10.0 Hz)
3	140.1	142.7	7.12 (dd, *J* = 6.0, 10.0 Hz)	139.7	142.8	7.12 (dd, *J* = 6.0, 10.0 Hz)	141.33	144.1	6.68 (m)
4	117.6	118.4	6.27 (d, *J* = 6.0 Hz)	117.8	118.3	6.27 (d, *J* = 6.0 Hz)	36.03	36.8	2.05 (m) 3.28 (dt, *J* = 2.5, 19.5 Hz)
5	158.1	160.6	-	157.4	160.7	-	77.22	77.6	-
6	72.8	74.5	4.61 (t, *J* = 2.5 Hz)	73.6	74.5	4.61 (t, *J* = 2.5 Hz)	74.26	75.5	3.63 (t, *J* = 3.0 Hz)
7	34.3	36.3	1.46 (m) 2.46 (dt, *J* = 3.0, 13.5 Hz)	34.3	35.9	1.36 (m) 2.36 (m)	26.47	28.2	1.95 (m 2.01 (m)
8	35.4	37.7	2.41 (brd, *J* = 3.0 Hz)	35.4	36.6	2.42 (m)	35.43	37.4	2.07 (m)
9	44.0	46.0	1.94 (m)	43.1	44.9	2.09 (m)	35.43	37.1	2.75 (m)
10	53.6	55.5	-	53.7	55.7	-	52.20	53.1	-
11	21.2	21.8	1.44 (m) 2.00 (1H, m, H_b-11)	20.0	21.1	1.54 (m) 1.90 (m)	23.18	24.6	1.25 (m) 2.18 (m)
12	38.2	39.4	1.43 (m) 1.89 (m)	47.7	48.5	1.84 (m) 2.63 (m)	38.82	39.8	1.69 (m) 1.83 (m)
13	52.0	53.5	-	50.5	51.5	-	52.23	53.7	-
14	82.4	83.0	-	87.0	88.0	-	82.28	83.0	-
15	83.6	84.6	5.24 (d, *J* = 2.5 Hz)	79.4	80.8	5.05 (dd, *J* = 3.5, 8.5 Hz)	83.36	84.2	5.40 (d, *J* = 3.0 Hz)
16	120.4	122.4	5.66 (d, *J* = 2.5 Hz)	30.4	31.6	1.52 (m) 1.68 (dd, *J* = 2.5, 10.0 Hz)	120.37	122.3	5.65 (d, *J* = 3.0 Hz)
17	161.1	162.9	-	86.4	86.8	-	161.30	163.0	-
18	16.6	16.7	1.18 (s)	15.0	15.7	1.24 (s)	16.80	17.1	1.14 (s)
19	19.6	18.3	1.47 (s)	18.3	17.8	1.46 (s)	15.10	15.4	1.29 (s)
20	35.6	36.4	2.58 (m)	41.7	43.2	2.19 (m)	36.11	36.4	2.58 (m)
21	16.8	18.0	1.19 (d, *J* = 6.5 Hz)	9.7	9.7	1.07 (d, *J* = 7.0 Hz)	17.21	18.1	1.22 (d, *J* = 7.0 Hz)
22	78.2	80.6	4.41 (m)	76.9	78.7	4.82 (m)	78.47	80.6	4.42 (m)
23	32.0	33.5	2.29 (dd, *J* = 3.5, 18.5 Hz) 2.54 (m)	32.0	33.0	2.55 (d, *J* = 7.5 Hz)	32.35	33.5	2.32 (dd, *J* = 3.0, 17.5 Hz) 2.55 (m)
24	150.9	152.9	-	150.5	153.3	-	150.25	152.9	-
25	121.1	122.0	-	121.4	122.0	-	121.44	122.0	-
26	167.8	169.1	-	167.1	169.3	-	167.52	169.2	-
27	12.2	12.4	1.86 (s)	12.3	12.4	1.88 (s)	12.37	12.4	1.87 (s)
28	20.4	20.3	1.99 (s)	20.5	20.5	1.99 (s)	20.64	20.3	2.00 (s)
29	21.3	21.1	1.85 (s)	21.1	21.1	1.87 (s)	21.36	21.4	2.04 (s)
30	169.7	171.3	-	169.2	171.0	-	170.63	172.5	-

[a]Measured in CD_3OD, [b]125 MHz, [c]500 MHz, [#1]δ_C of physagulin L in $CDCl_3$[16], [#2]δ_C of physagulin M in $CDCl_3$[16]. [#3]δ_C of withaminimin in $CDCl_3$[17].

4. CONCLUSION

cadamonin with IC_{50} values of 1.41±0.03 μM. The MTT assay showed that this compound had no significant toxicity to RAW 264.7 macrophages up to 100 μM (cell viability in the range of 90-110 %), indicating that the inhibitory effect on NO production was not due to cytotoxicity. Compounds **1**-**2** were considered to be inactive (IC_{50} > 100 μM).

A phytochemical study on the dichloromethane extract of the whole plant of *P. angulata* led to the isolations of three withanolides including physagulin L (**1**), physagulin M (**2**), and withaminimin (**3**). Their chemical structures were elucidated by 1D and 2D-NMR spectra analysis and in comparison with those reported in the literature. Among isolated compounds, withaminimin (**3**) showed inhibitory activity against NO production in LPS-stimulated RAW 264.7 macrophages.

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REFERENCES

1. V. V. Chi. The Dictionary of Medicinal Plants in Vietnam, *Medical Publishing House, Hanoi*, **2012**, *1*, 115-116.
2. Y. S. Lin, H. C. Chiang, W. S. Kan, E. Hone, S. J. Shih, M. H. Won. Immunomodulatory activity of various fractions derived from *Physalis angulata L* extract, *Am. J. Chin. Med.*, **1992**, *20(4)*, 233-243.
3. A. B. Ray, M. Gupta. Withasteroids, a growing group of naturally occurring steroidal lactones, *Fortschritte der Chemie organischer Naturstoffe*, **1994**, *63*, 1-106.
4. F. Abe, S. Nagafuji, M. Okawa, J. Kinjo. Trypanocidal constituents in plants 6. Minor withanolides from the aerial parts of *Physalis angulata*, *Chem. Pharm. Bull.*, **2006**, *54(8)*, 1226-1228.
5. A. G. Damu, P.-C. Kuo, C.-R. Su, T.-H. Kuo, T.-H. Chen, K. F. Bastow, K.-H. Lee, T.-S. Wu. Isolation, structures, and structure-cytotoxic activity relationships of withanolides and physalins from *Physalis angulata*, *J. Nat. Prod.*, **2007**, *70(7)*, 1146-1152.
6. Z. Jin, M. S. Mashuta, N. J. Stolowich, A. J. Vaisberg, N. S. Stivers, P. J. Bates, W. H. Lewis, G. B. Hammond. Physangulidines A, B, and C: Three new antiproliferative withanolides from *Physalis angulata* L, *Org. Lett.*, **2012**, *14(5)*, 1230-1233.
7. S. Nagafuji, H. Okabe, H. Akahane, F. Abe. Trypanocidal constituents in plants 4. Withanolides from the aerial parts of *Physalis angulata*, *Biol. Pharm. Bull.*, **2004**, *27(2)*, 193-197.
8. K. Shingu, S. Yahara, T. Nohara, H. Okabe. Constituents of solanaceous plants. 21. Three new withanolides, physagulins A, B and D from *Physalis angulata* L, *Chem. Pharm. Bull.*, **1992**, *40(8)*, 2088-2091.
9. H. L. Tuan Anh, D. T. Thao, D. T. Dung, P. V. Kiem, T. H. Quang, P. T. Hai Yen, D. T. Tuan, P. V. Cuong, L. C. Viet Cuong, T. M. Hung. Phytochemical constituents and cytotoxic activity of *Physalis angulata* L. growing in Vietnam, *Phytochem. Lett.*, **2018**, *27*, 193-196.
10. G. N. T. Bastos, A. J. A. Silveira, C. G. Salgado, D. L. W. Picanço-Diniz, J. L. M. do Nascimento. *Physalis angulata* extract exerts anti-inflammatory effects in rats by inhibiting different pathways, *J. Ethnopharmacol.*, **2008**, *118(2)*, 246-251.
11. F. Freiburghaus, R. Kaminsky, M. H. H. Nkunya, R. Brun. Evaluation of African medicinal plants for their in vitro trypanocidal activity, *J. Ethnopharmacol.*, **1996**, *55(1)*, 1-11.
12. S. K. Nanumala, K. Gunda, R. Chinna, M. Sriram Chandra. Evaluations of diuretic activity of methanolic extract of *Physalis angulata* L. leaves, *Int. J. Pharm. Sci. Rev. Res.*, **2012**, *16*, 40-42.
13. R. C. Nogueira, V. P. C. Rocha, F. R. Nonato, T. C. B. Tomassini, I. M. Ribeiro, R. R. d. Santos, M. B. P. Soares. Genotoxicity and antileishmanial activity evaluation of *Physalis angulata* concentrated ethanolic extract, *Environ. Toxicol. Pharmacol.*, **2013**, *36(3)*, 1304-1311.
14. R. C. L. R. Pietro, S. Kashima, D. N. Sato, A. H. Januário, S. C. Franca. In vitro antimycobacterial activities of *Physalis angulata* L, *Phytomedicine*, **2000**, *7(4)*, 335-338.
15. N. H. Dang, N. D. Chung, H. M. Tuan, N. Van Thanh, N. T. Hiep, D. Lee, N. T. Dat. 2-Benzyl-benzofurans from the tubers of *Ophiopogon japonicus*, *Chem. Cent. J.*, **2017**, *11*, 15(1)-15(6).
16. Q.-P. He, L. Ma, J.-Y. Luo, F.-Y. He, L.-G. Lou, L. -H. Hu. Cytotoxic withanolides from *Physalis angulata* L, *Chem. Biodivers.*, **2007**, *4(3)*, 443-449.
17. H. E. Gottlieb, M. Cojocaru, S. C. Sinha, M. Saha, A. Bagchi, A. Ali, A. B. Ray. Withaminimin, a withanolide from *Physalis minima*, *Phytochemistry*, **1987**, *26(6)*, 1801-1804.

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