Thieme

Evaluation of the Antibacterial, Antioxidant and α-Glucosidase Inhibitory Activities of Withanolides from *Physalis gracilis*

Authors
Gerardo Padierna[1], Ana L. Pérez-Castorena[1], Mahinda Martínez[2], Antonio Nieto-Camacho[1], Jesús Morales-Jiménez[3], Emma Maldonado[1]

Affiliations
1 Instituto de Química, Universidad Nacional Autónoma de México, Cd. Mx., México
2 Facultad de Ciencias Naturales, Universidad Autónoma de Querétaro, Querétaro, México
3 División de Biología Molecular, Instituto Potosino de Investigación Científica y Tecnológica-Centro de Investigación, Innovación y Desarrollo de las Zonas Áridas, San Luis Potosí, México

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Correspondence
Prof. Emma Maldonado
Instituto de Química
Universidad Nacional Autónoma de México
Circuito Exterior
Ciudad Universitaria
Coyoacán 04510, Cd. Mx.
México
Tel.: +52/55/5622 4412, Fax: +52/55/5616 2217
emmaldon@unam.mx

^{1}H and ^{13}C NMR data of compound **1**-**5**, ^{1}H and ^{13}C NMR spectra of compounds **4** and **5**, and a detailed description of the bioassays are available as **Supporting Information**.

ABSTRACT

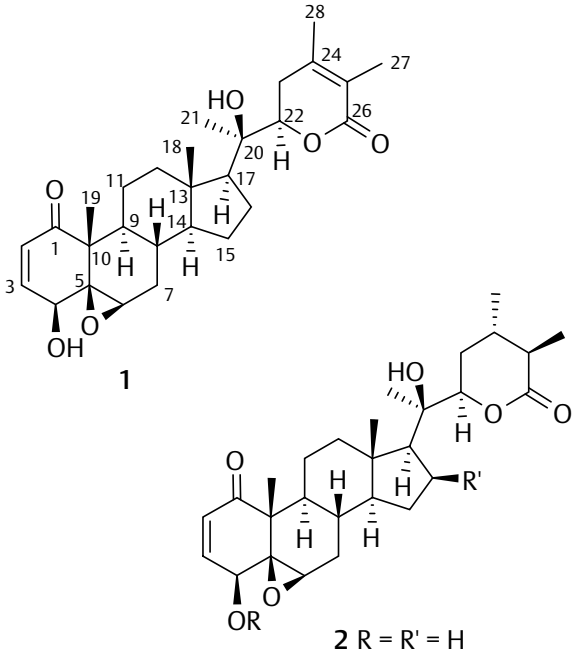
A chemical investigation of the leaves, flowers, and stems of Physalis gracilis led to the isolation of three withanolides identified as withanolide D (**1**), 24,25-dihydrowithanolide D (**2**), and withaphysacarpin (**3**). The structures of these compounds were determined by analyses of their spectroscopic data, including 1D and 2D NMR. The antibacterial, antioxidant, and α-glucosidase inhibitory activities of compounds **1** and **3** and derivatives **4** and **5** were evaluated. None of the compounds showed antioxidant or glucosidase inhibitory activity. Also, they were inactive against gram-negative bacteria. However, compound **3** was found active against Bacillus subtilis (MIC = 65.5 µM) and compound **5** against Staphylococcus aureus (MIC = 27.9 µM).

Physalis is a genus of the Solanaceae family, with about 90 species native to America, and only one species, Physalis alkekengi L., native to the Old World. Mexico is home to the largest number of species (more than 70), most of which are endemic. The most distinctive character of Physalis species is the accrescent fruiting calix that envelops the fruit, which in several species is edible. The most well known and economically important are those of Physalis philadelphica Waterf. ("tomatillo" or "husk tomato") and Physalis peruviana L. ("uchuva") [1, 2]. In traditional medicine, these plants are used to relieve respiratory, digestive, and hepatic problems, and for the treatment of malaria, diabetes, asthma, and cancer [3, 4]. Regarding their chemistry, Physalis species elaborate mainly different types of withanolides, but also labdane diterpenoids, flavonoids, sucrose esters, and ceramides [4–6]. Thus, as a part of our studies on Physalis species, we describe here the results of the first chemical investigation of the aerial parts (except fruits and calixes) of Physalis gracilis Miers, an herb whose fruits, leaves, and stems are eaten in certain regions in Mexico, while the decoction of leaves is used to relieve tonsillitis and gastrointestinal disorders, and the decoction of roots is used to treat stones in the gallbladder [3, 7]. Additionally, and considering the traditional use of some Physalis species in the treatment of several infectious diseases and diabe-

tes, the antibacterial, antioxidant, and α-glucosidase inhibitory activities of compounds **1** and **3**-**5** were also investigated.

Chemical analysis of the EtOAc-soluble fraction, obtained after partition of the acetone and methanol extracts of P. gracilis, led to the isolation of a mixture of the ubiquitous β-sitosterol and stigmasterol together with three withanolides identified as withanolide D (**1**) [8–10], 24,25-dihydrowithanolide D (**2**) [11, 12], and withaphysacarpin (**3**) [13–15], by interpretation of their spectroscopic data. The 4-O-acetyl (**4**) and the 4,16-di-O-acetyl (**5**) derivatives of withaphysacarpin (▶ **Fig. 1**) were obtained. The ^{1}H NMR spectra of **4** and **5** were almost identical to those of **3**, with the exception of the expected low-field shifts of the signals for the protons geminal to the acetoxy groups, H-4 (δ 4.67 d, J = 6.0 Hz) in compound **4**, and H-4 (δ 4.66 d, J = 6.0 Hz) and H-16 (δ 5.41 ddd, J = 7.5, 7.5, 4.5 Hz) in compound **5**. Complete ^{1}H and ^{13}C NMR data of compounds **1**-**5** are presented in **Table S1** and S**2**, Supporting Information.

Previous investigations of withanolides **1**-**3** showed that they exhibit relevant biological activities. Thus, withanolide D (**1**) whose structure has been confirmed recently by X-ray analysis [10], showed potent cytotoxicity against a panel of human cancer cell lines [9, 16, 17], and significant growth-inhibitory activity against Pneumocystis carinii, an atypical fungal microorganism causing lethal pneumonia in immunocompromised patients [17]. 24,25-Dihydrowithanolide D (**2**) and withaphysacarpin (**3**) induced the activity of quinone reductase, which is indicative of a cancer quimiopreventive activity [14].



▶ **Fig. 1** Chemical structures of withanolides **1**-**5**.

In the present work, the antibacterial activity of the plant compounds **1** and **3**, and the derivatives **4** and **5** (purity: 92.1, 92.9, 96.2, and 97.3 %, respectively) was qualitatively tested in vitro against Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Staphylococcus aureus, and Bacillus subtilis by the agar diffusion method [18]. Compounds **3** and **5** showed antibacterial activity against B. subtilis and S. aureus, respectively (▶ **Table 1**). The MIC of both compounds was determined by the broth microdilution method [19]. Neither **3** (MIC = 65.5 μM) nor **5** (MIC = 27.9 μM) were more active than ampicillin, which was used as the positive control. Ampicillin was three orders of magnitude more active than **3** against B. subtilis ATCC® 6673 (MIC = 8.6×10^{-2} μM) and two orders of magnitude more active than **5** versus S. aureus ATCC® 25923™ (MIC = 7.2×10^{-1} μM; ▶ **Table 2**).

Diabetes mellitus is a disease characterized by a deficiency in insulin secretion or by resistance to its action, resulting in high concentrations of glucose in plasma, which, in turn, produces a number of health problems, mainly neuropathy, retinopathy, and cardiovascular disorders. Compounds that can inhibit the activity of α-glucosidase enzymes, and therefore reduce the liberation of glucose from carbohydrates, could be useful therapeutic tools to control postpandrial glucose levels. By these reasons, the α-glucosidase inhibitory activity of withanolide D (**1**) and withaphysacarpin (**3**) was evaluated against yeast α-glucosidase and rat intestine α-glucosidase at three concentrations (1.0, 10.0, and 100.0 μM) [20, 21]. Compounds **1** and **3** exhibited poor activities in both α-glucosidase assays. Even at the highest concentration, the percentages of inhibition were < 25 % in the yeast and < 5 % in the intestine rat enzyme assays (**Table S3**, Supporting information). On the other hand, since many chronic diseases, including diabetes mellitus, are related to oxidative stress, the antioxidant activity of **1** and **3**-**5** was evaluated on the scavenging of the free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) [22], the same as in the thiobarbituric acid reactive substances (TBARS) in the rat brain homogenate assay [23]. None of the compounds were active in these assays (**Table S4**, Supporting information).

The chemical study of P. gracilis revealed that this plant produces withanolides. Labdane diterpenoids present in other Physalis species were not detected in this plant. The isolated withanolides **1** and **3** and the obtained derivatives **4** and **5** did not exhibit antioxidant or α-glucosidase inhibitory activities. In the antibacterial assay, only compounds **3** and **5** showed a moderated activity against B. subtilis and S. aureus, respectively.

Materials and Methods

Melting points are uncorrected. Optical rotations were measured on a Perkin Elmer 343 polarimeter. The IR spectra were recorded on a Bruker Tensor 27 spectrometer. NMR spectra were recorded on a Varian Inova 500 spectrometer (^{1}H at 500 MHz; ^{13}C at 125 MHz) or a Bruker Avance III spectrometer (^{1}H at 400 MHz; ^{13}C at 100 MHz). Chemical shifts are given in ppm with respect to the internal standard (TMS). DART-MS were obtained in a JEOL AccuTOF JMS-T100LC mass spectrometer. Column chromatography operated with vacuum (VCC) was performed on silica gel G (Macherey-Nagel). Preparative TLC was carried out on precoated Macherey-Nagel Sil G-200 UV_{254} plates of 2.0 mm thickness.

▶ **Table 1** Qualitative antimicrobial activities of compounds **1**, **3**-**5**, and standard drugs.

Microorganism	Zone of inhibition (mm)[a]							
	1	**3**	**4**	**5**	**Ampicillin**	**Streptomycin**	**Chloramphenicol**	**Amphotericin B**
Bacteria								
E. coli ATCC® 25922™	0	0	0	0	20	NT	NT	NT
K. pneumoniae ATCC® 700603™	0	0	0	0	0	0	NT	NT
P. aeruginosa ATCC® 27853™	0	0	0	0	0	NT	0	NT
S. aureus ATCC® 25923™	0	0	0	19	28	NT	20	NT
B. subtilis ATCC® 6673™	5	13	0	8	25	18	NT	NT

[a]The zones of inhibition were measured from a disk with 20 µg of compounds or standard drugs. NT = not tested

Aerial parts of P. gracilis were collected in October 2012 from Jalpan (Querétaro, México) and authenticated by Dr. Mahinda Martínez. A voucher specimen (M. Martinez 8768) was deposited at the Herbarium of the Universidad Autónoma de Querétaro.

Dried and milled leaves, flowers, and stems of P. gracilis (303.0 g) were extracted successively with Me_2CO (4 L) and MeOH (5 L). These extracts were combined (50.37 g) and partitioned between EtOAc-H_2O. The EtOAc fraction (11.63 g) was subjected to VCC (8 × 11 cm) using mixtures of hexane-EtOAc (1:0 to 0:1 v/v) as eluents to give fractions (500 mL each) 1A-4A (1:0), 5A-15A (19:1), 16A-19A (9:1), 20A-39A (4:1), 40A-45A (7:3), 46A-65A (3:2), 66A-74A (1:1), 75A-87A (3:7), 88A-89A (1:9), and 90A (0:1). Crystallization (EtOH) of fractions 11A-15A gave a mixture of β-sitosterol-stigmasterol (103.3 mg). Compound **1** (16.7 mg) was obtained from fractions 52A-59A (210 mg) after crystallization from Me_2CO-iPr_2O. The fractions 45A-51A, the mother liquors of **1**, and the fractions 60A-70A were combined (1.33 g) and subjected to VCC (4.5 × 9 cm, 50 mL each fraction) eluted with mixtures of hexane-Me_2CO (17:3 to 1:1 v/v). Fractions eluted with hexane-Me_2CO (4:1 to 7:3 v/v) were combined (244 mg) and purified by VCC (3 × 7 cm, 25 mL each fraction) eluted with hexane-EtOAc (11:9), followed by crystallization to obtain 14.4 mg of compound **2**. Fractions 71A-88A (3.517 g) were decolored with activated charcoal/Me_2CO and crystallized from CH_2Cl_2-hexane to yield 226 mg of compound **3**. The mother liquors of **3** were combined with fractions 89A-90A (3.04 g) and subjected to silica gel VCC (5.5 × 9 cm, 125 mL each fraction) eluted with mixtures of hexane-Me_2CO (17:3 to 0:1 v/v). Fractions eluted with hexane-Me_2CO (7:3) were crystallized from CH_2Cl_2-hexane to obtain an additional amount of compound **3**. The total yield of **3** was 365 mg.

Acetylation of withaphysacarpin (**3**): A solution of compound **3** (147.1 mg) in pyridine (1 mL) and acetic anhydride (1 mL) was left at room temperature by 3 h. The reaction mixture was worked up as usual and separated by VCC (2 × 7 cm; hexane-Me_2CO 4:1 v/v, 20 mL each fraction) to obtain 51.5 mg of 4-O-acetylwithaphysacarpin (**4**) and 83.2 mg of 4,16-di-O-acetylwithaphysacarpin (**5**).

Compound **4**: Colorless crystals; m.p. 264-266 °C (EtOAc-hexane); $[\alpha]_D$ + 101 (c = 0.20, $CHCl_3$); IR ($CHCl_3$): ν_{max} 3569, 3436, 1739, 1682, 1456, 1374, 1189, 1096, 1020, 961, 887 cm^{-1}; HR DARTMS m/z 531.29487 $[M + H]^+$ (calcd. for $C_{30}H_{43}O_8$ 531.29579).

▶ **Table 2** Quantitative antimicrobial activities of compounds **3** and **5** (MIC in µM).

Microorganism	3	5	Ampicillin
B. subtilis ATCC® 6673	65.5	NT	8.6×10^{-2}
S. aureus ATCC® 25923™	NT	27.9	7.2×10^{-1}
NT = not tested			

Compound **5**: Colorless crystals; m.p. 250-252 °C (EtOAc-hexane) (m.p. 245-248 °C from EtOH [13]); $[\alpha]_D$ + 123 (c = 0.19, $CHCl_3$); IR ($CHCl_3$): ν_{max} 3573, 1740, 1682, 1456, 1375, 1194, 1097, 1021, 961, 887 cm^{-1}; HR DARTMS m/z 573.30610 $[M + H]^+$ (calcd. for $C_{32}H_{45}O_9$ 573.30636).

Qualitative and quantitative antimicrobial assays were performed by the agar diffusion [18] and broth microdilution methods [19]. The inhibition of the activity of yeast and mammalian α-glucosidases was determined using modified methods previously described [20, 21]. The DPPH [22] and TBARS [23, 24] assays were used to evaluate the antioxidant activity.

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Conflict of Interest

The authors declare no conflict of interest.

References

[1] Martínez M. Revision of Physalis section epeteiorhiza (Solanaceae). Ann Inst Biol Bot UNAM 1998; 69: 71–117

[2] Whitson M, Manos PS. Untangling Physalis (Solanaceae) from the physaloids: A two-gene phylogeny of the physalinae. Syst Bot 2005; 30: 216–230

[3] Argueta VA.coordinator Atlas of the plants of the traditional Mexican medicine, Vol. I. México, D. F.: Instituto Nacional Indigenista; 1994: 344, 431

[4] Zhang WN, Tong WY. Chemical constituents and biological activities of plants from the genus Physalis. Chem Biodivers 2016; 13: 48–65

[5] Pérez-Castorena AL, Oropeza RF, Vázquez AR, Martínez M, Maldonado E. Labdanes and withanolides from Physalis coztomatl. J Nat Prod 2006; 69: 1029–1033

[6] Maldonado E, Pérez-Castorena AL, Romero Y, Martínez M. Absolute configuration of labdane diterpenoids from Physalis nicandroides. J Nat Prod 2015; 78: 202–207

[7] Santiaguillo HJF, Blas YS. Aprovechamiento tradicional de las especies de Physalis en Mexico. Rev Geogr Agr 2009; 43: 81–86

[8] Lavie D, Kirson I, Glotter E. Constituents of Withania somnifera Dun. Part X. The structure of withanolide D. Isr J Chem 1968; 6: 671–678

[9] Cordero CP, Morantes SJ, Páez A, Rincón J, Aristizábal FA. Cytotoxicity of withanolides isolated from Acnistus arborescens. Fitoterapia 2009; 80: 364–368

[10] Zhang H, Hagan K, Patel O, Tong X, Day VW, Timmermann BN. 6α,7α-Epoxy-5α-hydroxy-1-oxo-22R-witha-2,24-dienolide (withanolide B), 5β,6β-epoxy-4β,20-dihydroxy-1-oxo-22R-witha-2,24-dienolide (withanolide D), and 4β,27-dihydroxy-1-oxo-22R-witha-2,5,24-trienolide (5,6-deoxywithaferin A) in roots of Withania somnifera: Isolation and their crystal structures. J Chem Crystallogr 2014; 44: 169–176

[11] Kirson I, Glotter E, Abraham A, Lavie D. Constituents of Withania somnifera Dun-XI: The structure of three new withanolides. Tetrahedron 1970; 26: 2209–2219

[12] Eastwood FW, Kirson I, Lavie D, Abraham A. New withanolides from a cross of a South African chemotype by chemotype II (Israel) in Withania somnifera. Phytochemistry 1980; 19: 1503–1507

[13] Subramanian SS, Sethi PD. Steroidal lactones of Physalis ixocarpa leaves. Indian J Pharm 1973; 35: 36–38

[14] Kennelly EJ, Gerhäuser C, Song LL, Graham JG, Beecher CWW, Pezzuto JM, Kinghorn AD. Induction of quinone reductase by withanolides isolated from Physalis philadelphica (Tomatillos). J Agric Food Chem 1997; 45: 3771–3777

[15] Maldonado E, Pérez-Castorena AL, Garcés C, Martínez M. Philadelphicalactones C and D and other cytotoxic compounds from Physalis philadelphica. Steroids 2011; 76: 724–728

[16] Hsieh PW, Huang ZY, Chen JH, Chang FR, Wu CC, Yang YL, Chiang MY, Yen MH, Chen SL, Yen HF, Lübken T, Hung WC, Wu YC. Cytotoxic withanolides from Tubocapsicum anomalum. J Nat Prod 2007; 70: 747–753

[17] Roumy V, Biabiany M, Hennebelle T, Aliouat EM, Pottier M, Joseph H, Joha S, Quesnel B, Alkhatib R, Sahpaz S, Bailleu F. Antifungal and cytotoxic activity of withanolides from Acnistus arborescens. J Nat Prod 2010; 73: 1313–1317

[18] Clinical and Laboratory Standards Institute. Performance standards for antimicrobial disk susceptibility tests, approved standard. 7th ed. (CLSI document M02-A11)Wayne, Pennsylvania: Clinical and Laboratory Standards Institute; 2012

[19] Clinical and Laboratory Standards Institute. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. approved standard (CLSI document M07-A9)Wayne, Pennsylvania: Clinical and Laboratory Standards Institute; 2012

[20] Ye XP, Song CQ, Yuan P, Mao RG. α-Glucosidase and α-amylase inhibitory activity of common constituents from traditional Chinese medicine used for diabetes mellitus. Chin J Nat Med 2010; 8: 349–352

[21] Jo SH, Ka EH, Lee HS, Apostolidis E, Jang HD, Kwon YI. Comparison of antioxidant potential and rat intestinal α-glucosidases inhibitory activities of quercetin, rutin, and isoquercetin. Int J Appl Res Nat Prod 2009; 2: 52–60

[22] Cavin A, Hostettmann K, Dyatmyko W, Potterat O. Antioxidant and lipophilic constituents of Tinospora crispa. Planta Med 1998; 64: 393–396

[23] Chans GM, Nieto-Camacho A, Ramírez-Apan T, Hernández-Ortega S, Álvarez-Toledano C, Gómez E. Synthetic, spectroscopic, crystallographic, and biological studies of seven-coordinated diorganotin (IV) complexes derived from Schiff bases and pyridinic carboxylic acids. Aust J Chem 2016; 69: 279–290

[24] Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem 1979; 95: 351–358