

Antioxidant, Cytotoxic, and Acetylcholinesterase Inhibitory Activities of Withanolides from *Datura quercifolia*



Authors

Christian Jasso¹, Antonio Nieto-Camacho¹, María Teresa Ramírez-Apan¹, Mahinda Martínez², Emma Maldonado¹

Affiliations

- 1 Instituto de Química, Universidad Nacional Autónoma de México, Circuito Exterior, Ciudad Universitaria, Coyoacán, México
- 2 Facultad de Ciencias Naturales, Universidad Autónoma de Querétaro, Querétaro, México

Key words

Datura quercifolia, Solanaceae, withanolides, acetylcholinesterase inhibitors, antioxidant activity, cytotoxic activity

received 26.11.2019

revised 21.02.2020

accepted 03.04.2020

Bibliography

DOI <https://doi.org/10.1055/a-1156-4229>

Planta Med Int Open 2020; 7: e68–e72

© Georg Thieme Verlag KG Stuttgart · New York

ISSN 2509-9264

Correspondence

Emma Maldonado

Instituto de Química, Universidad Nacional Autónoma de México

Circuito Exterior Ciudad Universitaria

04510 Coyoacán

D. F. México

Tel.: +52 (55) 5622 4412, Fax: +52 (55) 5616 2217

emmaldon@unam.mx

Supporting Information for this article is available online at <http://www.thieme-connect.de/products>.

ABSTRACT

Five withanolides identified as daturalactone (**1**), withanicandrin (**2**), withanolide B (**3**), nicandrin B (**4**), and daturalactone 2 (**5**) were isolated from the aerial parts (flowers, leaves, and stems) of *Datura quercifolia* Kunth. Their structures were determined by analysis of the IR, MS, 1D and 2D NMR spectra. All the isolates were evaluated for their cytotoxic and antioxidant activities, as well as for their capacity to inhibit the activity of the acetylcholinesterase enzyme (AChE). As result, the five withanolides showed weak cytotoxic and pro-oxidant activities, however, they displayed a relevant inhibitory activity against AChE, as indicated by the IC₅₀ values ranging from 1.51 to 12.11 μM. The differences in AChE inhibition seem to be related to the functional group at C-12.

Introduction

Datura is an American genus of the Solanaceae family consisting of 14 species whose natural geographic distribution ranges from southwest of the USA to northern Central America. Most of these species are native to Mexico, which is considered the center of diversity and distribution of the genus. *Datura* species have been classified into three sections, *Datura*, *Dutra*, and *Ceratocaulis*, which contain three, ten, and one species, respectively [1]. *Datura*, like many other genera of the Solanaceae family (*Atropa*, *Hyoscyamus*, *Mandragora*), is characterized by producing tropane-type alkaloids,

mainly hyoscyamine and hyoscyne, although 67 alkaloids have been identified in *Datura stramonium* [2–4]. These compounds are related to the medicinal, magical religious, recreational, and criminal uses of these plants around the world [4, 5]. The most frequent medicinal uses of *Datura* are for the treatment of asthma, rheumatism, inflammation, hemorrhoids, tumors, ulcers, wounds, and a variety of pains; they are also used as a hypnotic, tranquilizer, and sedative [4–7]. However, some of these uses may be related to another type of metabolite whose presence is constant in the genus: the withanolides, a group of C₂₈-steroidal lactones with an ergostane-type

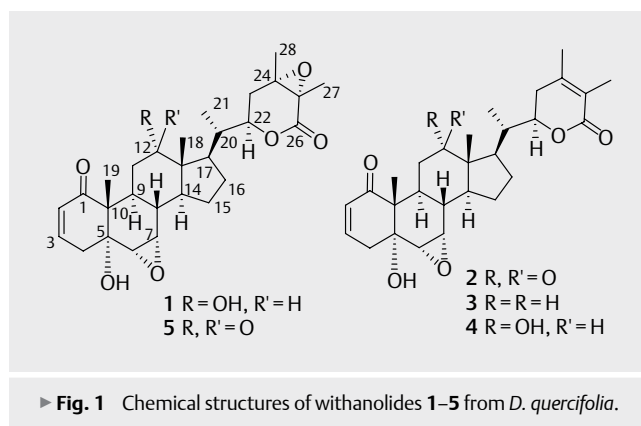
skeleton that can be modified, and that exhibit countless and important biological activities such as anti-inflammatory, cytotoxic, anti-stress, trypanocide, quinone reductase inducer, and cholinesterase inhibitory activities [8, 9]. As a part of our work on Solanaceae plants, we undertook the chemical study of the *Datura* genus with the analysis of *Datura quercifolia*, which together with *Datura ferox* and *D. stramonium* belong to the *Datura* section [1]. These three species biosynthesize closely structurally related withanolides, which in addition to the α,β -unsaturated ketone at ring A and the δ -lactone at the side chain, have an α -hydroxy group at C-5 and a C-6 α , C-7 α epoxy group, which can be open as in the daturalactones 5 and 6, isolated from *D. ferox* [10]. In most of these compounds, a hydroxy or ketone group is present at C-12 and the δ -lactone can be α,β -unsaturated or possess a 24,25-epoxy group [10–17]. In the present work, we report the isolation and structural elucidation of five withanolides from *D. quercifolia*, and the results of the evaluation of their antioxidant and cytotoxic activities. The acetylcholinesterase inhibitory activity of these compounds is also discussed.

Results and Discussion

A series of chromatographic separations of the EtOAc-soluble extract of the plant followed by repeated crystallizations led to the isolation of the ubiquitous mixture of β -sitosterol and stigmasterol and five withanolides, which were identified as daturalactone (**1**) [11, 14–16], withanicandrin (**2**) [11, 18, 19], withanolide B or Lycium substance B (**3**) [11, 20, 21], nicandrin B (**4**) [11, 19, 22], and daturalactone 2 (**5**) [15, 21]. The structural elucidation of these compounds was carried out by analysis of their IR, MS, and 1D and 2D NMR spectra and comparison with literature data (► Fig. 1). Since compounds **1–5** were isolated mostly in the '70s, only the most relevant ^1H NMR signals were reported, and, although their complete ^{13}C NMR data have been published, some assignments, mainly those concerning C-10, C-13, C-14, and C-17, had to be revised. Therefore, the complete and revised NMR data of these compounds are given in ► Tables 15 and 25, Supporting Information.

Withanolides **1–5** were subjected to a series of pharmacological studies that were selected considering the medicinal uses of the plant or the activity exhibited by structurally related compounds. Thus, bearing in mind that some of the medicinal uses of *Datura* species involve diseases that can be related to oxidative stress [23], the antioxidant activity of compounds **1–5** was evaluated in the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay and by the lipid peroxidation indicator TBARS (thiobarbituric acid reactive species) [24–26]. In both assays, concentrations of 1, 10, and 100 μM were used and α -tocopherol was the positive control. The tested compounds were inactive in the DPPH assay; however, contrary to expectations, they exhibited a weak pro-oxidant effect in the TBARS model (► Table 35, Supporting Information). This effect decreased as the concentration was increased. The pro-oxidant effect has been observed for quercetin and some of its derivatives [27].

The cytotoxic activity of withanolides is well documented and it has been mentioned that this activity is relevant when these compounds contain a 5 β ,6 β -epoxy group together with an α,β -unsaturated ketone at ring A [28]. On the contrary, a weak or even no cytotoxic activity has been observed in 5 α -hydroxy, 6 α ,7 α -



► Table 1 *In vitro* AChE inhibitory activity of compounds ► 1–5.

Compound	IC ₅₀ (μM) ^a
1	6.81 ± 1.86
2	1.51 ± 0.22
3	12.11 ± 1.96
4	8.82 ± 0.67
5	2.65 ± 0.45
Physostigmine	0.011 ± 0.001
Galanthamine	0.56 ± 0.09

^aThe IC₅₀ values were calculated from the concentration-response curve of seven concentrations of each tested compound in triplicate (► Table 55, Supporting Information). Values represent the mean ± SD (n = 3)

epoxy-withanolides, despite the presence of the 2-en-1-one functions [29]. In order to confirm this, the cytotoxic activity of compounds **1–5** was evaluated against a panel of six human cancer cell lines [U-251 (glioblastoma), PC-3 (prostatic adenocarcinoma), K-562 (chronic myelogenous leukemia), HCT-15 (colorectal adenocarcinoma), MCF-7 (mammary adenocarcinoma), and SKLU-1 (lung adenocarcinoma)] as previously described [30]. The results (► Table 45, Supporting Information) support that the 5 α -hydroxy, 6 α ,7 α -epoxy groups of these compounds are responsible of their scarce or null cytotoxicity, since at a concentration of 50 μM , the inhibition of the cellular proliferation was in the range of 1.4–21.2%.

Alzheimer's disease (AD) is a serious health problem affecting the elderly population. It is a progressive neurodegenerative disorder characterized by cognitive and memory impairment, whose pathogenesis involves, among others, the deposition of β -amyloid plaques and neurofibrillary tangles in brain. Currently, the therapeutic use of acetylcholinesterase enzyme inhibitors (AChEIs) is one of the more promising strategies for the treatment of AD [31], because they boost the cholinergic neurotransmission by increasing acetylcholine levels in the brain [32]. Taking as a precedent that several withanolides from *Ajuga bracteosa* and *Withania somnifera* [32, 33] have been shown to be AChE inhibitors, compounds **1–5** were screened for this activity as previously described [34, 35]. The results shown in ► Table 1 revealed a potent inhibitory activity of all of them with IC₅₀ values ranging from 1.51 to 12.11 μM (values

of concentration-response curves are available in ► **Table 5S**, Supporting Information) in the order **2** > **5** > **1** > **4** > **3**, which indicate that the C-12-oxowithanolides are more active than those with a C-12-hydroxy group, and these than the one that lacks oxygenated functions at C-12. The combination of poor cytotoxicity and a potent AChE inhibition make compounds **1–5** promissory candidates for the treatment of AD.

Materials and Methods

General experimental procedures

The melting points were determined in a Fisher-Johns apparatus (Fisher Scientific) and are uncorrected. Vacuum-assisted column chromatography (VCC) was performed on silica gel 60 G (Macherey-Nagel). TLC was carried out on precoated plates, Alugram Sil G/UV₂₅₄. Preparative TLC was performed on precoated SIL G 200/UV₂₅₄ plates with a thickness of 2.0 mm. Optical rotations were measured on a JASCO DIP-360 digital polarimeter. IR spectra were determined on a Nicolet FTIR-Magna 750 spectrophotometer. NMR spectra were recorded in CDCl₃ on a Varian Unity Plus 500 spectrometer with TMS as the internal standard. ESI-MS and EI-MS were measured on an ESI Ion Trap Bruker Esquire 6000 and on a JEOL JMS-AX505HA spectrometer, respectively. Quercetin (purity ≥ 95%), AChE from *Electrophorus electricus*, physostigmine (purity ≥ 99%), sulforhodamine B sodium salt (SRB), doxorubicin hydrochloride (purity ≥ 98%), α-tocopherol (purity ≥ 95.5%), 5,5'-dithiobis-2-nitrobenzoic acid (DTNB, purity ≥ 98%), and acetylthiocholine iodide (ATCI, purity ≥ 98%) were purchased from Sigma-Aldrich.

Animals

Adult male Wistar rats (200–250 g) were provided by the Instituto de Fisiología Celular, UNAM. They were kept at 24 ± 2 °C in a 12-h light/dark cycle with free access to food and water. The procedures with animals and their care were conducted in conformity with the protocol approved by the local Animal Ethics Committee (CICUAL-IQ-003–17) in compliance with the Mexican Official Norm for Animal Care and Handling (NOM-062-ZOO-1999).

Plant material

Aerial parts of *D. quercifolia* Kunth were collected in July 30, 2008 in Juriquilla, Querétaro State, México. The vegetal material was identified by Dr. M. Martínez (Universidad Autónoma de Querétaro) and a voucher specimen of *D. quercifolia* (M. Martínez 7244) was deposited at the Herbarium of the Universidad Autónoma de Querétaro.

Extraction and isolation

The dried and ground aerial parts (except fruits) of *D. quercifolia* (1.13 kg) were successively percolated with acetone (~ 10 L) and MeOH (~ 10 L). The resulting extracts were concentrated under reduced pressure to afford 77.25 and 143.65 g of residues, respectively. Both extracts were dissolved in EtOAc (1.0 L), mixed, and extracted with 0.1 N HCl (6 ×, 300 mL). The organic fraction was washed with NaHCO₃ solution and H₂O to give 46.58 g of extract. The aqueous fraction was neutralized with NaHCO₃ to obtain 171 g of residue after solvent evaporation.

The EtOAc fraction was fractionated by silica gel VCC (column A, 10 × 12 cm) eluted with hexane/EtOAc mixtures of increasing polarity (1:0–0:1 v/v) to obtain fractions (1 L each) A1–A9 (1:0), A10–A21 (19:1), A22–A38 (9:1), A39–A46 (17:3), A47–A51 (4:1), A52–A64 (7:3), A65–A78 (6:4), A79–A87 (1:1), A88–A90 (4:6), and A91–A105 (0:1). Fractions A11–A23 (4.03 g) were subjected to a silica gel VCC (5.5 × 9 cm, 250 mL each fraction) eluted with mixtures of hexane-EtOAc 19:1 to 4:1. Crystallization (EtOH) of fractions eluted with hexane-EtOAc 9:1 afforded 431 mg of a mixture of β-sitosterol and stigmasterol. Fractions A59–A76 (4.54 g) were discolored (acetone/activated charcoal) and crystallized from EtOAc-hexane to obtain compound **1**. The mother liquors of **1** (3.44 g) were purified by silica gel VCC (4.5 × 8 cm, 125 mL each fraction) eluted with mixtures of hexane-Me₂CO 4:1 (fractions B1–B34) and 3:1 (fractions B35–B42) to obtain an additional amount of **1** from fractions B7–B11. Mother liquors of **1**, fractions B12–B31, and fractions A77–A82 gave a crystalline mixture. The mother liquors of this mixture were combined (8.16 g) and subjected to VCC (7 × 9 cm, 250 mL each fraction) eluted with a gradient of acetone in CH₂Cl₂ to give fractions C1–C20 (97.5:2.5), C21–C54 (97:3), C55–C62 (96:4), C63–C82 (95:5), and C83–C85 (90:10). Crystallization (acetone-hexane) of fractions C67–C82 gave compound **2**. Fractions C83–C85 (2.81 g) were combined with the previously obtained crystalline mixture (4.04 g) and subjected to VCC (4.5 × 9 cm, 125 mL each fraction, CH₂Cl₂-Me₂CO 92.5:7.5) to give fractions D1–D41. Fractions D2–D5 (395 mg) were purified by VCC (2.7 × 6 cm, 25 mL each fraction) eluted with C₆H₆-EtOAc 9:1 to obtain fractions E1–E38. Compound **3** (32 mg) was isolated after crystallization (EtOAc-hexane) of fractions E25–E37. VCC (4.5 × 8 cm, 125 mL each fraction, CHCl₃-Me₂CO 9:1) of fractions D6–D18 (3.3 g) afforded fractions F1–F41. Fractions F1–F8 (1.44 g) were subjected to VCC (3.5 × 7 cm, 100 mL each fraction) using mixtures of CHCl₃-EtOAc 3:1 as the eluent to obtain fractions G1–G18. A portion (120 mg) of fractions G8–G12 was purified by preparative TLC (CHCl₃-Me₂CO 4:1) to yield compound **4** and an additional amount of **1**. VCC (3.5 × 7 cm, 50 mL each fraction, C₆H₆-EtOAc 4:1) of fractions E3–E13 (1.19 g) gave fractions H1–H39. VCC (1.7 × 6 cm, 25 mL each fraction, C₆H₆-EtOAc 4:1) of fractions H13–H22 (162.3 mg) afforded fractions I1–I25. Crystallization (CHCl₃-methanol-hexane) of fractions H8–H12 and I6–I7 gave 364.4 mg of compound **5**. Crystallization of fractions I9–I25 gave **2** (Total yield 160.7 mg). Fractions G1–G4 and H3–H5 were combined (673.3 mg) and subjected to gel permeation chromatography on Sephadex LH–20 eluted with CHCl₃-MeOH 7:3 to obtain fractions J1–J23. The combined fractions D19–D41 and J4–J11 (4.59 g) were purified by VCC (4.5 × 9 cm, 250 mL each fraction) eluted with toluene-EtOAc 7:3 to give fractions K1–K19. VCC (1.7 × 6 cm, 25 mL each fraction, C₆H₆-EtOAc 4:1) of fractions K14–K19 (840 mg) gave fractions L1–L21. Crystallization of fractions L9–L17 afforded additional amounts of compounds **1** and **4**. The total yields of compounds **1** and **4** were 238.1 and 95 mg, respectively.

Biological activity assays

Antioxidant activity

The antioxidant activity was evaluated by two bioassays: reduction of the DPPH radical and inhibition of lipid peroxidation in rat brain (TBARS), as previously described [24].

Cytotoxic activity

Withanolides **1–5** were assayed for their cytotoxic activity against six human tumor cell lines by the SRB method as previously described [30]. The cell lines used in the assay were U-251 (glioblastoma), PC-3 (prostatic adenocarcinoma), K-562 (chronic myelogenous leukemia), HCT-15 (colorectal adenocarcinoma), MCF-7 (mammary adenocarcinoma), and SKLU-1 (lung adenocarcinoma).

Inhibition of the acetylcholinesterase enzyme

Inhibition of AChE (from *E. electricus*) activity was determined using Ellman's colorimetric method [34] with some modifications.

Statistical analysis

All data are represented as the percentage mean \pm standard error of mean (SEM). Analysis of variance ANOVA followed by Dunnett's test were used to compare several groups with a control. Values of $p \leq 0.05$ and $p \leq 0.01$ were considered significant.

Supporting Information

The ^1H and ^{13}C NMR data of compounds **1–5** as well as the results of their evaluation in the different bioassays are available as Supporting Information.

Acknowledgments

We are grateful to H. Ríos, I. Chávez, B. Quiroz, R. Gaviño, A. Peña, and E. Huerta for the NMR spectra, R. Patiño for the IR and optical rotations, and L. Velasco and J. Pérez for the MS.

Conflict of Interest

The authors declare that they have no conflict of interest.

References

- [1] Luna-Cavazos M, Bye R. Phytogeographic analysis of the genus *Datura* (Solanaceae) in continental Mexico. *Rev Mex Biodivers* 2011; 82: 977–988
- [2] El Bazaoui A, Bellimam MA, Soulaymani A. Nine new tropane alkaloids from *Datura stramonium* L. identified by GC/MS. *Fitoterapia* 2011; 82: 193–197
- [3] Lesiak AD, Cody RB, Dane AJ, Musah RA. Plant seed species identification from chemical fingerprints: A high-throughput application of direct analysis in real time mass spectrometry. *Anal Chem* 2015; 87: 8748–8757
- [4] Benítez G, March-Salas M, Villa-Kamel A, Chaves-Jiménez U, Hernández J, Montes-Osuna N, Moreno-Chocano J, Cariñanos P. The genus *Datura* L. (Solanaceae) in Mexico and Spain – Ethnobotanical perspective at the interface of medical and illicit uses. *J Ethnopharmacol* 2018; 219: 133–151
- [5] Maheshwari NO, Khan A, Chopade BA. Rediscovering the medicinal properties of *Datura* sp.: A review. *J Med Plants Res* 2013; 7: 2885–2897
- [6] Argueta Villamar A. Atlas de las plantas de la medicina tradicional Mexicana. Vol. 3: Cd Mx: Instituto Nacional Indigenista 1994; 1344–1349
- [7] Aguilar A, Camacho JR, Chino S, Jáquez P, López ME. Herbario Medicinal del Instituto Mexicano del Seguro Social. Cd Mx: IMSS. 1994; 65
- [8] Chen LX, He H, Qiu F. Natural withanolides: An overview. *Nat Prod Rep* 2011; 28: 705–740
- [9] Zhang WN, Tong WY. Chemical constituents and biological activities of plants from the genus *Physalis*. *Chem Biodivers* 2016; 13: 48–65
- [10] Veleiro AS, Cirigliano AM, Oberti JC, Burton G. 7-Hydroxywithanolides from *Datura ferox*. *J Nat Prod* 1999; 62: 1010–1012
- [11] Evans WC, Grout RJ, Mensah MLK. Withanolides from *Datura* spp and hybrids. *Phytochemistry* 1984; 23: 1717–1720
- [12] Cirigliano AM, Veleiro AS, Oberti JC, Burton G. A 15 β -hydroxy-withanolide from *Datura ferox*. *Phytochemistry* 1995; 40: 611–613
- [13] Tursunova RN, Maslennikova VA, Abubakirov NK. Withanolides from *Datura stramonium*. II. Withastramonolide. *Chem Nat Comp* 1978; 4: 73–75
- [14] Dhar KL, Raina ML. A novel withanolide from *Datura quercifolia*. *Phytochemistry* 1973; 12: 476–478
- [15] Kalla AK, Raina ML, Dhar KL, Qurishi MA, Snatzke G. Revised structures of daturalactone and 12-oxowithanolide. *Phytochemistry* 1979; 18: 637–640
- [16] Bandhoria P, Gupta VK, Sharma VK, Satti NK, Dutt P, Suri KA. Crystal structure of 6 α ,7 α :24 α ,25 α -diepoxy-5 α ,12 α -dihydroxy-1-oxo-20S,22R-with a-2-enolide isolated from *Datura quercifolia* leaves. *Anal Sci* 2006; 22: x169–x170
- [17] Qurishi MA, Dhar KL, Atal CK. A novel withanolide from *Datura quercifolia*. *Phytochemistry* 1979; 18: 283–284
- [18] Kirson I, Lavie D, Subramanian SS, Sethi PD, Glotter E. Withanicandrin, a ring-C-substituted withanolide from *Nicandra physaloides* (Solanaceae). *J Chem Soc, Perkin I* 1972; 2109–2111
- [19] Bagchi A, Neogi P, Sahai M, Ray AB, Oshima Y, Hikino H. Withaperuvine E and nicandrin B, withanolides from *Physalis peruviana* and *Nicandra physaloides*. *Phytochemistry* 1984; 23: 853–855
- [20] Hansel R, Huang JT, Rosenberg D. Zwei withanolide aus *Lycium chinense*. *Arch Pharm* 1975; 308: 653–654
- [21] Zhang H, Hagan K, Patel O, Tong X, Day VW, Timmermann B. 6 α ,7 α -Epoxy-5 α -hydroxy-1-oxo-22R-witha-2,24-dienolide (withanolide B), 5 β ,6 β -epoxy-4 β ,20-dihydroxy-1-oxo-22R-witha-2,24-dienolide (withanolide D), and 4 β ,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
- [22] Bagchi A, Sahai M, Ray AB. C₂₈-Steroidal lactones of the seeds of *Nicandra physaloides*. *J Ind Chem Soc* 1984; 61: 173–174
- [23] Huang D, Ou B, Prior RL. The chemistry behind antioxidant capacity assays. *J Agric Food Chem* 2005; 53: 1841–1856
- [24] Padierna G, Pérez-Castorena AL, Martínez M, Nieto-Camacho A, Morales-Jiménez J, Maldonado E. Evaluation of the antibacterial, antioxidant and α -glucosidase inhibitory activities of withanolides from *Physalis gracilis*. *Planta Med Int Open* 2017; 4: e1–e4
- [25] Cavin A, Hostettmann K, Dyatmyko W, Potterat O. Antioxidant and lipophilic constituents of *Tinospora crispa*. *Planta Med* 1998; 64: 393–396
- [26] Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal Biochem* 1979; 95: 351–358
- [27] Kessler M, Ubeaud G, Jung L. Anti- and pro-oxidant activity of rutin and quercetin derivatives. *J Pharm Pharmacol* 2003; 55: 131–142
- [28] Damu AG, Quo PC, Su CR, Kuo TH, Chen TH, Bastow KF, Lee KH, Wu TS. Isolation, structures, and structure-cytotoxic activity relationship of withanolides and physalins from *Physalis angulata*. *J Nat Prod* 2007; 74: 1146–1152

- [29] Neogi P, Kawai M, Butsugan Y, Mori Y, Suzuki M. Withacoagin, a new withanolide from *Withania coagulans* roots. *Bull Chem Soc Jpn* 1988; 61: 4479–4481
- [30] Maldonado E, Pérez-Castorena AL, Garcés C, Martínez M. Philadelphicalactones C and D and other cytotoxic constituents from *Physalis philadelphica*. *Steroids* 2011; 76: 724–728
- [31] Dey A, Bhattacharya R, Mukherjee A, Pandey DK. Natural products against Alzheimer's disease: Pharmacotherapeutics and biotechnological interventions. *Biotechnol Adv* 2017; 35: 178–216
- [32] Riaz N, Malik A, Aziz-ur-Rehman Nawaz SA, Muhammad P, Choudary MI. Cholinesterase-inhibiting withanolides from *Ajuga bracteosa*. *Chem Biodivers* 2004; 1: 1289–1295
- [33] Choudary MI, Yousuf S, Nawaz SA, Ahmed S, Atta-ur-Rahman. Cholinesterase-inhibiting withanolides from *Withania somnifera*. *Chem Pharm Bull* 2004; 52: 1358–1361
- [34] Ellman GL, Courtney KD, Andres V, Featherstone RM. A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochem Pharmacol* 1961; 7: 88–95
- [35] Torres F, Pérez-Castorena AL, Arredondo L, Toscano RA, Nieto-Camacho A, Martínez M, Maldonado E. Labdanes, withanolides and other constituents of *Physalis nicandroides*. *J Nat Prod* 2019; 82: 2489–2500