Acta Pharmaceutica Sciencia
52: 155-157 (2010)

The isolation and structure elucidation of new Withanaoloides from *Withania cogulance* with antidiabetic activity

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Abstract

The hydro alcohol extract of the dried berries parts of *Withania cogulance* gave new withonolides. Their structures were elucidated on the basis of both the chemical and spectroscopic evidence. The isolated compounds showed anti-diabetic activity.

Keywords: *Withania coagulance,* Solanaceae, Withonolides, antidiabetic activity.

Introduction

Withania coagulance belongs to the solanaceae and cultivated throughout central Asia. This family is well known for its medicinal properties like immunomodulatary, mental disability and depression disorders (Choudhary et al. 1995). In the literature survey, it was found that *Withania cogulance* has been found to be relatively rich in Withonolides. Which have attracted considerable interest due to their biological activity and their chemical and physical properties have been investigated extensively (Prsad et al. 1959, Choudhary et al. 1970, Budhiraja et al. 1977, Khan et al. 1993, Choudhary et al. 1995, Rahman et al. 1998, Archna et al. 1999, Andallu et at. 2000, Mohmud et al. 2000, Bhattacharya et al. 2001, Hemlatha et al. 2004, Kharc et al. 2004). In the course of our search for bioactive constituents from herbal plants in India. We have been studying the chemical components of this plant. We described here in the structure elucidation of *Withanaia cogulance* and new Withonolides isolated from this plant which showed antidiabetic activity.

Experimental work

All melting points were measured on a yanagimato melting apparatus and are uncorrected. Spectral data were obtained using the following apparatus: proton and C-13 nuclear magnetic resonance (1H and 13C-NMR). Spectra with a JEOL JNM GSX-400 (1H, 400MHz; ^{13}c, 100MHz) spectrometer with tetra methyl silane (TMS) as internal standard; mass spectra (MS) with a JEOL 5X-102 A mass spectrometer, infrared (IR) spectra with a JASCO FT/IR 8010 infrared spectrometer, ultraviolet (UV) spectra Shimadzu UV-1601 and CHN by using flash EA 1112 CHN analyzer (Themo Finnigen, Italy).

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Plant material: For the present study, the berries of the plant *Withania cogulance* were procured from local market. The berries were identified at, "Department of Botany, Nagpur University, Nagpur" and "Universal Medicament Pvt. Ltd. Nagpur". The authentication number is Acc. No. 8/1.

Extraction and isolation: The hydroalcohol extract obtained from the berries of *Withania cogulance* was subjected to thin layer chromatography by using silica gel G to find out the number of component present in it. HPTLC was carried out using the solvent system methanol: ethylacetate: toluene (2:2:5). The spot were scanned in CAMAG TLC scanners. Then it is separated by column chromatography using silica gel 60-120# and obtained fraction number 1, 2, and 3 gave brown powder.

Evaluation of hypoglycemic activity of separated constituents: The fractions separated were evaluated for the hypoglycemic activity at a dose of 400mg/kg body weight. On 1st day, it was observed from the pattern than Fraction-1 and 3 do not showed decrease in blood glucose level over a period of 5h with respect to corresponding control. The faction no. 2 lowered the blood glucose level at 3rd and 5th days but was not significant.

Table 1. Evaluation of hypoglycemic activity of separated constituents on first day

Treatment	Blood sugar level in (mg/dl)			
	0h	1h	3h	5h
Control (saline)	275.92±3.12	270.64±1.08	269.96±2.21	269.44±3.02
Standard (500 mg/kg) Metformin	262.08±1.16	218.43±2.52	168.68±9.16	135.98±2.57
Constituent-1 (400 mg/Kg)	270.52±1.02	268.49±4.68	260.79±7.12	255.43±5.48
Constituent-2 (400 mg/Kg)	269.42±4.02	265.29±4.09	241.42±3.92	221.49±4.10
Constituent-3 (400 mg/Kg)	284.52±7.12	268.92±5.12	264.42±3.81	260.49±2.92

Result and Discussion

Three fractions obtained through the separation process of the hydroalcohol extract of berries of *Withania cogulance* were subjected to a series of column chromatographic separation to yield new compounds which we given the name withanoloids B. Withanoloides B was obtained in pure crystalline forms of mp 220-222°C. It has molecular formula of $C_{30}H_{44}O_5$ as determined by the high-resolution mass spectrometry. The UV spectrum exhibited an absorption maxima at 214 nm which reveals the presence of only one α β unsaturated carbonyl system in the molecule, it can be therefore calculated that 6 membered ring lactone is saturated.

The IR spectrum showed an absorption band at C=O at 1698 cm^{-1} for a saturated 6 membered ring lactone IR spectrum also exhibited a strong absorption band at 3455cm^{-1} which indicated the presence of OH group might be due to presence of moisture.

The ^{1}H-NMR spectrum revealed signals attributed to the proton at C-22 (22H) δ4.23 ppm which is characteristics of Withanoloids and signals for olifinic protons at C-2, C-3 (2H and 3H) at δ5.28 and 7.46 ppm respectively. Signals were also obtained at δ1.53, δ0.71 and δ1.8 for CH3 group at C-19, C-18, and C-21 (19H, 18H and 21H) respectively and δ2.15, δ4.21, δ2.28, δ0.81 (28H, 27H, 29H, and 30H) respectively. The mass spectra showed prominent peaks (M-1) at m/2 483 and (M-2) at m/2 484, hence molecular weight is 484. The result of spectral studies suggests that the molecular formula is $C_{30}H_{44}O_5$. The attention can be drawn towards signal of the 22H, which is the characteristics and useful for identification of side chain in all Withanoloids.

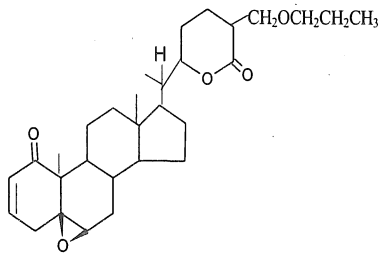

Figure 1. Withanoloids B

References

Ahmad, M., Khan, M.A. and Arshad, M. (2001). Ethanophytotherapeutical Approaches for the treatment of Diabetes by the local inhabitants of Attock. *J. Ethanopharmacol.* 213-214.

Andallu, B. and Radhika, B. (2000). Hypoglycemic, Diuretic and Hyper chlolesterolemic Effect of withania cherry root. *Ind. J. Exp. Biol.* 38: 607-609.

Archna, R. and Namasivayam, A. (1999). Antistressar effect of *Withania somnifera. J. Ethanopharmacol.* 64: 91-93.

Bhattacharya, A., Ghosal, S. and Bhattacharya S.K. (2001). Antioxidant effect of Ashwagandha Glycowithanoloides in chronic Foatshook stress induced perturbations of oxidative free scavenging enzymes and lipid peroxidation rat froxtal cortex and striatum. *J. Ethanopharmacol.* 74: 1-6. P.T.O.

Budhiraja, R.D., Bala and Sand Garg, K.N. (1977). Pharmacological investigation of fruits of withania coagulancs. *Plant Med.* 32: 154-157.

Choudhary, M.I. and Rahman, A.U. (1995). Antifungal steroidal Lactones from *Withania coagulancs. Phytochem.* 40: 1243-1246.

Choudhary, R.R. and Vohra S.B. (1970). Plant with possible hypoglycemic activity in advance in Research in Indian Medicine, Banars Hindu University, Varanasi, 57.

Hemlatha, S., Wahi, A.K., Singh, P.N and Chansoria, J.P.N. (2004). Hypoglycemic activity of *Withania cogulance* Dunal in streptozotocin induced Diabetic Rats. *J. Ethanopharmacol.* 93: 261-264.

Khan, M.T.J., Asharf, M., Tehiyat, M.K., Bukhtair, S., Asharf, S. and Ahmed W. (1993). Antibacterial activity of *Withania cogulance. Fitoterapia* 64: 367-370.

Kharc, C.P. Newyork. (2004). Rational western Therapy, Ayurvedic and other Traditional usages, Springer verlog Berlin Heidelberg, Encylopedia of Indian Medicinal Plants, 479-483.

Mohmud, T.H. (2000). Anti-inflammatory activity of ashwagandha Hamdard Medicus, XLIII, 77.

Prasad, S. and Luthura, S.P. (1959). Pharmacogonastic studies on *Withania coagulance. Dunal* 21: 189-194.

Rahman, A.U., Choudhary, M.I., Qureshi, S., Gul, W. and Youshf, M. (1998). Two ergostane type steroidal lactones from *Withania cogulance, J. Nat Prod.* 61: 812-814.

Received: 13.07.2009
Accepted: 20.08.2009