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# Withaferin-A isolated from the cell cultures of *Withania Somnifera*

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## Abstract

Cell suspension culture of *Withania Somnifera* was prepared and was analysed for it's constituents. One Withanolide was isolated from cell suspension culture. The structures of compounds was identified to be Withaferin-A. Based on spectroscopic methods like IR, MASS spectroscopy and $H^1$ NMR spectroscopy all compounds were elucidated.

**Key words:** *Withania Somnifera,* Withaferin-A, Spectroscopy

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## Introduction

As the population expands there has been increasing concern over whether current rates of food productions can support the growing population. In response to the increasing demand, scientists and farmers have been working hard to come up with new ideas to meet these needs. These ideas range from simple procedures including selection of the best seeds to more complex procedures requiring the transplantation of genes into different plant species[1].

*Withania somnifera* syn. Ashwagandha, also known as Indian ginseng is a plant in Solanaceae or nightshade family. It is small or middle-sized shrub, erect, grayish or hoary, branching, perennial about 30 cm to 1.5 m in height. Stem and branches covered with minute star-shaped hairs. One or more fairly long tuberous roots and short stem[2].

Internally, it is used to tone the uterus after miscarriage and treatment of post-partum difficulties. Externally, it is applied as a poultice to boils, swellings and other painful body parts[3].

The literature survey shows that no substantial work has been done by using cell suspension culture of *Withania somnifera.* Hence an effort was made to investigate the cell suspension culture of *Withania somnifera.*

## Results and Discussion

Compound was obtained as white crystals from Chloroform: Methanol (97:3) eluent. It was further purified by preparative TLC on silica gel pre-coated glass plates in n-hexane-acetone (60:40). It's melting point is 246° C[8].

The IR spectrum displayed intense absorption band at 3490, 1718 and 1684 $cm^{-1}$ assignable respectively, to hydroxyl, enone and α, β-unsaturated δ-lactone functionalities. The EI MS spectrum of compound showed the molecular ion at *m/z* 470.2625 corresponding to the molecular formula $C_{28}H_{38}O_6$. The $^1$H-NMR spectrum (400 MHz, $CDCl_3$) of compound exhibited four signals for tertiary methyl groups at δ 0.83 (H-18), 1.28 (H-19), 1.87 (H-27), and 1.92 (H-28) .and a doublet integrating for three protons at δ 1.09 ($J$ = 6.9 Hz) which was assigned to the secondary H-21 methyl group. A doublet of double doublet appeared at δ 5.80 ($J_1$ = 9.8, $J_2$ =3.5, $J_3$ = 1.2 Hz) was due to the H-2 vinylic proton, while another doublet of double doublet appeared at δ 6.57 ($J_1$ = 9.9, $J_2$ = 5.1, $J_3$ = 2.4 Hz) was assigned to H-3 vinylic proton. A doublet resonated at δ 3.17 ($J_{6β,7β}$ = 3.8 Hz) was ascribed to the C-6 methine proton while a double doublet appeared at δ 3.31 ($J_1$ = 3.8, $J_2$ = 2.2, Hz) was due to H-7 methine proton which indicated the presence of an epoxide at this position. A doublet of doublet resonating at δ 4.28 ($J_{22,23}α$ = 13.4, $J_{22,23β}$ 3.5 Hz) was assigned to H-22 methine proton.

## Material and methods

***Chemicals and instruments used***

Optical rotation was recorded on JASCO DIP-360 digital polarimeter in chloroform. Ultraviolet (UV) spectra were recorded in methanol on a Shimadzu, UV-240 spectrophotometers. Infrared (IR) spectra were recorded in chloroform on JASCO IRA-I infrared spectrophotometers. Mass spectra was recorded on electron impact mass spectrometer (EI-MS). $^1$H-NMR spectra were recorded on Bruker AM 300 FT spectrometer. TLC was carried out using Silica-gel G (Merck). Column chromatography was carried out on Neutral Alumina. All the chemicals and reagents used were obtained in high purity either from S.D. fine chemicals Pvt. Ltd., Bombay, India or E – Merck Pvt. Ltd., Mumbai, India.

***Germination of seeds***

Seeds of *W. somnifera* were washed in 1% savlon and then treated with 0.1% Bavestin and rinsed five to six times with sterile double-distilled water (SDDW). Surface sterilization was performed using 70% *v/v* ethanol treatment for 30 s and rinsed thrice with SDDW. This was followed by treatment with 0.01% *w/v* mercuric chloride for 5 min and rinsing with SDDW for four to five times. For aseptic germination, sterilized seeds were then placed on Murashige and skoog (MS)[4] medium with 30 g/L sucrose and 8 g/L agar at 25±2°C in a 16/8-h light/dark cycle with a light intensity of 1,200 lx.

***Initiation of cultures***

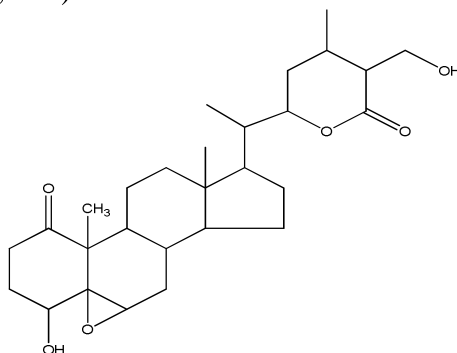
Hypocotyls were used as explants for callus initiation by *agrobacterium*-mediated transformation[5]. Explants from 20-25 days old *in vitro* germinated plants were used for culture initiation studies. Transformation culture was initiated by infecting explants with the *A.tumefacienes* strain (MTCC 2250). The *Agrobacterium* strain used was procured from the

Microbial Type Culture Collection (MTCC), institute of microbial Technology, Chandigarh, India. For this, bacterial colonies were cultured for 2 days on solid yeast mannitol broth medium (YMB) at 25±2°C. The inoculum (2% *v/v*) from this culture was re-inoculated in liquid YMB medium[6] and grown till they achieved an optical density at 600 nm of ~ 1.0. The suspension was then centrifuged at 6,000 rpm for 10 min. the supernatant was discarded, and the pellet was re-suspended in 5mL of fresh liquid YMB media. This concentrated culture was used further for the infection of plant materials.

Forty explants were kept in sterile plate, pricked manually with a 24-gauge metal needle (~5 wound per $cm^2$), dipped in *Agrobacterium* culture, and incubated for 5 min. The liquid YMB medium without bacteria was applied to the explants as a control. The infected explants were pre-incubated for co-cultivation at 25±2°C for 48 h on sterile MS medium, solidified with 10 g/L agar. The infected explants were then transferred to antibiotic (Cefotaxime, 1 g/L) containing MS medium to check the overgrowth of bacteria and were incubated at 25±2°C in 16/8-h light/dark regime. The transformed cultures were transferred to fresh MS medium containing 1 g/L cefotaxime[7]. Axenic cultures were obtained by subsequent subculture to fresh MS medium for every 7 days containing the antibiotic. The transformed cultures were checked for *Agrobacterium* contamination by culturing samples on YMB after every subculture.

***Identification of isolated compound***

Compound I: white crystals; m.p.246° C. UV $\lambda_{max}$ 226nm. IR(KBr) 3490 $^{cm-1}$ (hydroxyl), 1718 $^{cm-1}$ (enone), 3490 $^{cm-1}$ (α, β unsaturated lactone), $^1$H NMR(DMSO), δ 0.83 (3H,m, H-18), δ 1.28 (3H,m, C-19), δ 1.87 (3H,m, C-27), δ 1.92 (3H,m, C-28), δ 1.09 (2H, J=6.9 Hz, H-21), δ 5.80 (vinylic proton, J=9.8, H-2), δ 6.57 (vinylic proton, J=5.1, H-3), δ 3.17 (2H, J=3.8 Hz, H-6), δ 3.31 (2H, J=6.9 Hz, H-7), δ 4.28 (2H, J=13.4 Hz, H-22); HREI MS *m/z* (rel. int. %): 470.2625 ($C_{28}H_{38}O_6$, calcd. 470.266822, 11), 452.2631 ($C_{28}H_{38}O_5$, 14), 311.1852 ($C_{19}H_{25}O_4$, 52), 153.0851 ($C_9H_{13}O_2$, 44), 125.2524 ($C_7H_9O_7$, 100).




**Withaferin A**

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