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ANALYSIS OF STEROIDAL LACTONES IN *WITHANIA SOMNIFERA* LEAF AND ROOTS

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ABSTRACT

A reversed-phase HPLC method for the simultaneous analysis of two withanolides (withaferin A and withanolide A) from leaves and roots of Indian Ginseng plant *Withania somnifera* has been developed. Thin layer chromatography (TLC) showed the presence of withanolides in the leaves and roots. Quantification through reverse-phase HPLC revealed concentration of withanolides in plant leaves compared to the roots. Accumulation of withaferin A is higher in leaf then roots while withanolide A higher in roots then leaves. withaferin A in leaves and roots are present in 0.162 ± 0.005 and 0.064 ± 0.004mg gfw^{-1} respectively, while Withanolide A in leaves and roots are present in 0.048 ± 0.014 and 1.17 ± 0.0090mg gfw^{-1} respectively.

INTRODUCTION

Withania (Family: Solanaceae) is a highly acclaimed genus in the Indian Ayurvedic system of medicine because of its various pharmaceutical properties. It is a group of herbs distributed from the Canary Islands, the Mediterranean region and Northern Africa to the South-west of Asia [1-2]. Among the twenty-three known species of *Withania*, only two (*Withania somnifera* and *Withaniacoagulans*) are economically significant [3]. *W. somnifera* which is commonly known as 'Ashwagandha', is the most exploited species of the family Solanaceae due to its ethnomedicinal properties [4-5]. The phytochemistry of *W. somnifera* has been studied extensively by several workers and several groups of chemical such as steroidal lactones, alkaloids, flavonoids, tannin etc. have been identified, extracted, characterized and isolated which has been successfully act as an Anti-inflammatory, Anticancer, Hepatoprotective, Hypocholesterolemic, hypolipidemic, anti-oxidant, Antifungal and antibacterial and Immunomodulator[6-9]. The major chemical constituents of this plant, withanolides, are mainly localized in the leaves and roots [10].

During present investigation, leaf and root samples of *W. somnifera* were collected from Manipal University Jaipur campus and subjected to cold extraction method for extraction of secondary metabolites. Further, extracts were analyzed by TLC and HPLC with standard withanolide.

MATERIAL AND METHODS

W. somnifera plant materials (leaf and root) were collected from Manipal University Jaipurregion. All the chemicals and reagents required for extraction of withanolides were procured from Merck, India. Plant samples were washed by distilled water and dried in to shade at 25^0 C to make fine powder. 2g dried plant material (roots and leaves)were extracted three timeswithmethanol: water (1:4) in Erlenmeyer flask (Borosil, India) on a platform shaker at 20-30 rpm for 60 minutes in each extraction. Crude extracts were filtered by Whatman No.1 (GE Healthcare UK Ltd) filter paper. All three filtrates were subjected to liquid-liquid partition chromatography. The extracts were treated with equal volume of n-hexane in a separating funnel to remove the pigments and fatty acids. Hexane layer was discarded and the process was repeated three times. After removal of fats and pigmentsthese extracts were treated with chloroform (equal volume, three times) to recover withanolides in the chloroform layer.Chloroform fractions of each extract were pooled and evaporated to dryness at room temperature.

Qualitative and Quantitative Analysis of Withanolides:

Qualitative withanolide profiling was done through TLC while quantification was carried out through HPLC as described by Sangwan *et.al* [11]. For TLC, 10 µl sample was loaded on precoated silica gel G-10 plates (Merck, India) andrun was performed in a solvent system consisting of chloroform: ethyl acetate:methanol: toluene (70: 4: 8: 34; v/v). Development of the TLC plate was done with anisaldehyde reagent (250 µl anisaldehyde in a mixture of 20 ml acetone, 80 ml water and 10 ml 60% perchloric acid) followed by heating at 110°C. Authentic withanolides including withaferin A, withanone and withanolide A (Chromadex, USA) were used as markers.

Quantification of withanolides in the samples was carried out through HPLC on a reverse-phase (RP) column (Eclipse XDB c-18, particle size 1.8 µm, 4.5 mm x 250 mm, Agilent India). Water (A) and methanol (B) were used as solvent each containing 0.1% (v/v) acetic acid, as solvent. Online UV-Diode Array Detector (DAD) at a reference wavelength of 230 nm was used to detect the withanolides. The solvent gradient was set as A: B, 60: 40 to 25: 75, 0 to 45 min at a flow rate of 0.6 ml min^{-1}. 10 µl sampleswere injected and the column temperature was maintained at 27°C during the run. Authentic withanolides (withaferin A, withanone and withanolide A) were used to determine their distinct resolution from each other. For computation of withanolide concentration in the samples, a calibration curve of concentration*versus* detector response (peak area) and regression equation (Y = mX + C) was developed for withaferin A, withanolide A and withanone separately, using different concentrations (50-1000 ng $µl^{-1}$) of standard solution (1.0 mg ml^{-1}) in HPLC grade methanol.

RESULT AND DISCUSSION

Crude extract of both plant samples were run on TLC plate with standard withanolides(withaferin A and withanolide A).TLC of different extracts revealed that withaferin A and withanolide A were present in leaf and root of *W. somnifera* (Fig. 1).

The study used an analytical reverse phase HPLC providing symmetrical and high resolution peaks of two important withanolides in the plant. Withanolide content was analyzed by HPLC, and standard samples of withaferin A andwithanolide A were used to construct a calibrated graph by plotting peak areas versus the amount of respective withanolide over a range of 50–1,000 ng l^{-1}. The response was linear over the tested concentration range. The identification of withanolides was confirmed on the basis of retention time and absorption spectra on UV-DAD (27.351 min, 215 nmand 32.283 min, 227 nm; for withaferin A and

withanolide A (Fig. 2). The accumulation of withaferin A is higher in leaf then roots while withanolideA higher in roots then leaves.The detection of higher content withaferinA in leaves also points out that the enzymes responsible for biogenesis of withanolides might be optimally active in the leaves then roots of W. somnifera[12]. HPLC analysis of withanolides has also been carried out by researchers which are supporting to our work [13-15].

Table 1:Withanolide content in different plant parts of *W. somnifera*

Sample	Withanolide Content (mg gfw^{-1}) Mean ± SE	
	Withaferin A	Withanolide A
Leaf	0.162 ± 0.005	0.048 ± 0.014
Root	0.064 ± 0.004	1.17 ± 0.0090

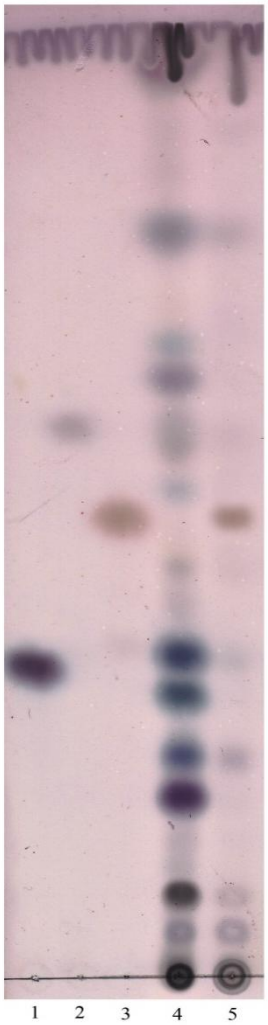

Fig. 1. TLC profile of*Withania somnifera*

1: standard withaferin A, 2: standard withanolide A, 3: standard withanone, 4:samples extracted from leaves, 5: sample extracted from roots

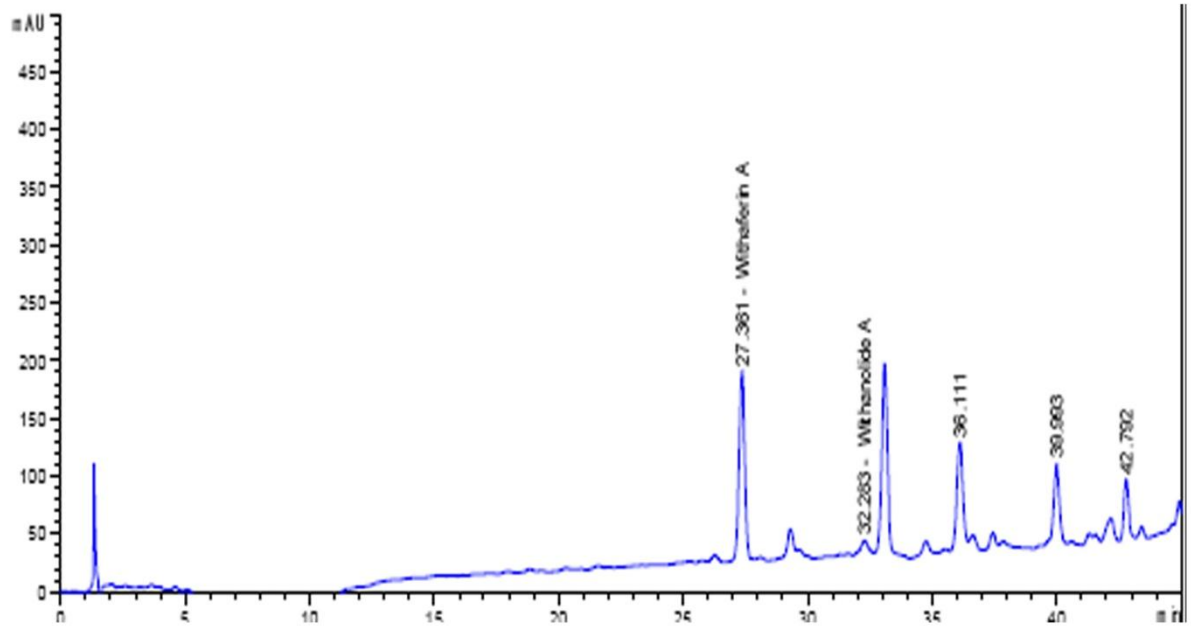

Figure 2: HPLC chromatogram of*Withania somnifera* for withaferin A and withanolide A

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