PROTEIN AND ALKALOID PROFILING FROM SEEDS AND ROOT OF INDIAN GINSENG (WITHANIA SOMNIFERA DUNAL)

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ABSTRACT

Withania somnifera is a pantropic native medicinal plant growing all over India belonging to family Solanaceae, and is popularly known as “Ashwagandha” due to specific odour (smell of horses). It holds a place in the ayurvedic traditional medicine similar to “Ginseng” in Chinese therapies. During present study SDS-PAGE divides the crude protein extract of seeds and root in 6-10 bands. Three active components were clearly identified by TLC in crude extract of seeds and root of Ashwagandha.

Key words : Withania somnifera, Alkaloid, Withaferin , Withanolide, Ashwagandha.

INTRODUCTION

Ashwagandha is held in high repute in traditional Indian medicine and is an important medicinal plant. It is well known for its medicinal properties since 1000 B.C. in ayurvedic texts including Charka samhita, Sushrut samhita, and Bhara prakasha, mentioned it to be a general tonic as well as a cure for morbidity arising from diseases such as pain, arthritis and inflammation (Dash and Junius, 1983). Ashwagandha is reported to have anti-carcinogenic effects in animal and cell cultures by decreasing the expression of nuclear factor-kappa B, suppressing intercellular tumor necrosis factor, and potentiating apoptotic signalling in cancerous cell lines (Ichikawa, 2006).

The distinctive earthy odour and flavour of ashwagandha is due to the presence of certain steroidal lactones collectively known as ‘Withanolides’ (Bhatnagar et al., 1976). Withanolides possess antibacterial, antifungal, antioxidant, anti tumour, antiarthritis, anti-inflammatory and immunosuppressive activities (Singh and Kumar, 1998). Today W. somnifera is widely cultivated in the drier parts of India (more than 4,000 ha) i.e. Manasa, Neemuch and Jawad tehsils of the Mandsaur District of Madhya Pradesh, Punjab, Sind and Rajastan (Sharma, 2004 and Anonymous, 1976). It is possible to formulate ashwagandha into pills, capsules and alcoholic extracts to create greater public acceptance. On the basis of above wide spectrum applications of the steroidal lactones and alkaloids from seed and root extracts, there is a need to conserve their active ingredients for different formulations.

MATERIALS AND METHODS

Preparation of plant extracts for protein estimation : 5.0 gm seeds / root power were weighted and macerated in mortar and pestle in the presence of 10 ml of Tris HCl buffer. The mixture was transferred in the 15 ml centrifuge tube and spinned at 10000 rpm for 20 minutes. The supernatant was used to estimate protein content

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(Lowry et al., 1951) using BSA as standard followed by SDS-PAGE (Sodium Dodecyl Sulphate Poyacrylamide Gel Electrophoresis) profiling.

**Alkaloids Isolation and TLC analysis:**

- 2 g dried seed/root powder
- Extraction with methanol (20 ml×3)
- Filtration and evaporation
- Extract was defatted with Hexane (10 ml×3)
- Extract with 1% H₂SO₄ (5 ml×3) basified with ammonia
- H₂SO₄ insoluble fraction extracted with Di-ethyl ether (10 ml×3)
- Dried it by anhy Na₂SO₄
- Filtration and evaporation
- Conformation by TLC

The pre coated TLC plates were used for fractionation of active ingredients. Chloroform: Ethyl acetate: Methanol: Benzene in the ratio 72:4:18:16 were used as solvent system (Sawhney and Singh, 2006). Plates were taken off, dried and spots were developed by spraying with Liberman-Burchard reagent. The major components were identified and confirmed with standard Rₜ values of withaferin and withanolides.

**RESULTS AND DISCUSSION**

Soluble protein content in the seeds and root were isolated and its Con. was estimated according to Lowry’s method. Seeds contains 820.62µg/mL protein, whereas, root contains 280.58µg/mL protein. Proteins from seeds and root of ashwagandha were subjected for fractionation with the help of SDS-PAGE. The electrophoresed gel is shown in Fig 1. According to banding pattern root protein were fractionated in 6 to 7 bands, whereas, seed protein in 8 to 10 bands. Molecular weight of different bands ranges from 14.2 KDa to 52 KDa in seeds as well as in roots. Banding intensity is higher in seed lanes as compared to root lanes, shows higher amount of proteins in seeds as in roots. Similar types of inter and intra genetic variability was reported in ashwagandha by Negi, (2000).

The seeds and root extract of *Withania somnifera* were subjected on pre coated silica plate for the qualitative analysis of alkaloids (Anderson et al., 1985 and Jaswinder et al., 2001). Rₜ of major visualized spots were 0.32, 0.50 and 0.86.
respectively (Fig 2), which coincide the standard Rf values for withaferin, withanolide D and withanolide A (0.34, 0.51 and 0.86). It confirmed the presence of above alkaloids in crude extract of seed and root (Slight variations in Rf may be due to experimental conditions).

Similar findings were reported by Ray (2001) and Rahman et al. (1991).

The above qualitative analysis reveals that seeds contain higher amount of protein as compared to root, whereas, root contain higher amount of alkaloids as compared to seeds.

REFERENCES