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HPLC analysis of different parts of *Withania somnifera*

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ABSTRACT

Withania somnifera, popularly known as "Ashwagandha", is one among the top ranking medicinal plants of India and is highly valued for its medicinal and neutraceutical properties. It holds a position of importance similar to ginseng in China. The medicinal properties of *Withania somnifera* were attributed to the presence of steroidal lactones collectively termed as withanolides present in both the leaves and tuberous roots that can be used for treating various diseases. In the present study, an attempt has been made to analyze the variation of withanolide content in methanol extracts prepared from different parts of *Withania somnifera* namely root, rhizome head and stem by HPLC analysis. HPLC chromatogram revealed that similar peaks were found at the retention time of 7.7 and 9.9 minutes for all the samples. Concentration of withanolides calculated using the area count of similar peaks obtained at the retention time of 7.7 and 9.9 minutes revealed that *Withania somnifera* roots has more withanolides compared to the stem and rhizome head portions.

KEYWORDS

Withanolides;
HPLC chromatogram;
Retention time;
Rrea count.

INTRODUCTION

Withania somnifera, also known as Ashwaganda, Indian ginseng and winter cherry forms an ingredient of many formulations prescribed for a variety of musculoskeletal conditions. It can be used as a general health tonic for elderly persons and lactating mothers. It is known for antiseptic properties and can be used as narcotic, anti-epileptic, against female sterility. It is used for the treatment of stomachache, ulcers, colds, rashes, and gonorrhoea[2].

Molecular pharmacological investigations have elucidated the association of these activities with specific secondary metabolites known as withanolides present in it. The roots as well as leaves of the shrub *Withania somnifera* contain C28 steroidal lactones collectively termed as withanolides. Till date around 138 withanolides with both α and β side chain has been reported[3].

The withanolides are classified according to their structural skeleton and the structural variation is responsible for the wide array of pharmacological activities. Much of pharmacological activity of the plant has been attributed to two main withanolides namely withaferin A and withanolide D.

Dhar *et al.*[8] reported the presence of seven isolated withanolides fluctuated both in leaves as well as in roots. And this variability may be due to varying degree of synthesis and utilization of these compounds in the plant.

Ray and Jha[4] quantified the withanolide content of *in vitro* grown shoot cultures of *Withania sominfera*

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from the peak areas of the standard namely the withanolide D and withaferin A obtained at 254nm by HPLC analysis. The mobile phase consisted of HPLC graded methanol and water in the ratio 57:43.Similarly the current study was performed to quantify the withanolide content in different parts of *Withania somnifera* and thereby determining the most suitable part with the maximum withanolide content that can be used for commercial preparation of drugs.

MATERIALS AND METHODS

Materials

The samples namely root, stem and rhizome head of *Withania somnifera* collected from Kallakurichi during the month of January, 2007 was used for the present study. HPLC analysis was performed using degassed solvents by sonication. Acetonitrile (HPLC grade - Ranbaxy, India) and the in house purified Milli-Q water (Millipore Corporation, USA) was used for the present study. All the solvents and samples were filtered through 0.22 μM pore size filters and sonicated (Millipore Corporation, USA).

Sample preparation

Methanol extracts of samples was prepared using the powdered samples of root, stem and rhizome head portions of *Withania sominfera* with constant stirring at 85 rpm for 12 hours[5] in an incubated shaker maintained at 37ºC.This process of extraction was repeated for 4 times and the extracts obtained was then concentrated to 1ml in a water bath maintained at 45°C.

Stem

Root

Rhizome head

Analytical method

HPLC analysis was performed using a Shimadzu HPLC Class *VP* series provided with two LC- 6 AD pumps and a variable wave length programmable photo diode array detector SPD, M20A *VP*. The HPLC system was equipped with Class *VP* series version 6.14SP1 software (Shimadzu). The column was maintained at a constant temperature of 25ºC using the column oven CTO-10AS *VP*. The functioning of the system was controlled by system controller CBM-20A *VP* and phenomenex reverse phase column 18 (250 mm × 4.6 mm) was used.

The mobile phase consists of acetonitrile and water with the initial concentration of water as 50% followed by 0%, 50%, 100% at the retention time of 20, 30 and 32 minutes respectively yielded a column backup pressure of 135 to 145 kgf/cm^2 at the flow rate 1.0 ml/min. The withanolide content in the extracts was quantified with the area count of resolved similar peaks detected at 225 nm obtained within 32 minutes.

RESULTS AND DISCUSSION

HPLC chromatogram of methanol extracts from different parts of *Withania sominfera* showed distinct, baseline-resolved peaks at retention times between 2.0 to 2.5 minutes. Major peaks were eluted between 0 - 20 minutes of 20 minutes run. Peaks that showed an absorbance above 40mAU were considered for analysis. The number of peaks obtained was 5 as observed in figure 1.

Chromatogram obtained from methanol extracts of root, rhizome head and stem of *Withania sominfera* collected from Kallakurichi showed similar pattern of elution peaks at 7.7th and 9.9th minutes as revealed in figure 1. The peak intensity shown in the figure 1 was very low for rhizome head followed by stem when compared to roots indicating that rhizome head contains

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low content of withanolides.

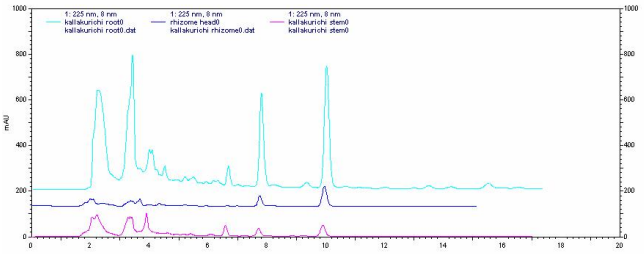

Figure 1 : HPLC analysis of different parts of *Withania somnifera*

The area count of the peaks obtained at various retention time for root, rhizome head and stem portions of *Withania somnifera* was indicated in TABLE 1. Concentration of withanolides calculated from the area count of the similar peaks in percentage are 2.7561×10^{-5} and 4.2667×10^{-5} for stem and 3.9024×10^{-5} and 1.063×10^{-5} for rhizome head at the retention time 7.7 and 9.9 minute respectively which is considerably less compared to same root sample.

TABLE 1 : Retention time and the area count of withanolides from different parts of *Withania somnifera*

S. No.	Name of the sample	Retention time	Area count
1.	Root	2.261	11768315
		3.392	9406314
		6.645	1010232
		7.765	4417133
		9.995	6471421
2.	Stem	3.360	1740051
		3.925	1442669
		6.592	4523385
		7.712	422939
		9.909	654754
3.	Rhizome head	3.680	1925383
		7.744	598860
		9.941	1631397

Similar study of quantifying the withanolides in *Withania somnifera* roots and leaves with the commercially available standard withaferin A was performed by Dalavay *et al.*[6] indicated that High content of withaferin-A in leaf than root was reported from *Withania somnifera.*

Ganzera *et al.*[7] quantified the withanolides in methanolic extracts of different parts of *Withania somnifera* (roots, leaves and stem) with the help of standards namely withaferin A and withanolide D using HPLC and confirmed the presence of withaferin A and withanolide D in all parts of the plant by HPLC but with significant differences in their ratio.

Similarly in the present study the withanolide content in different parts namely stem, rhizome head and root of *Withania sominfera* was quantified by HPLC analysis and the area count of the peaks in HPLC chromatogram revealed that the content of withanolides was found to vary in significant ratio. Higher quantity of withanolide was present in the root portions of *Withania sominfera* followed by stem and rhizome head.

The HPLC results obtained was similar with the results of Dalavay[6] that quantity of withanolides (withaferin-A) was found to vary in different parts of *Withania somnifera* (leaf and roots).

SUMMARY AND CONCLUSION

HPLC chromatograms of methanol extracts obtained from different parts of *Withania sominfera* revealed that *higher* quantity of withanolides was present in root portions when compared to stem and rhizome head. And hence the root from *Withania sominfera* was considered to be mostly preferred for commercial preparation of drugs.

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