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DISTRIBUTION OF WITHANOLIDE A CONTENT IN VARIOUS ORGANS OF *WITHANIA SOMNIFERA* (L.) DUNAL.

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ABSTRACT

Withanolide A is an important secondary metabolite in *Withania somnifera,* which is having a high medicinal value and possesses potent anti-tumor and antioxidant properties. Distribution of withanolide A in various organs of *Withania somnifera* was investigated by High Performance Liquid Chromatography (HPLC) method. The quantitative distribution of withanolide A was different in various organs tested, the accumulation was 386, 342, 272, 206, 102, 56, 35 and 23 µg g^{-1} DW in shoot tips, leaves, nodes, whole plant, internodes, roots, flowers and seeds respectively. The content of withanolide A gradually decreased from aerial parts i.e., from young leaves to the root. In root, the root tip accumulated higher concentration when compared to middle and basal portion. This study provides the data base for the regulation and control of withanolide A, moreover it provides the scientific evidence for the rational development and utilization of the *Withania somnifera* resources.

KEYWORDS

High Performance Liquid Chromatography, Indian ginseng, Secondary metabolite, *Withania somnifera* and Withanolide A.

INTRODUCTION

Withania somnifera, also known as ashwagandha, Indian ginseng and winter cherry, has been an important herb in the ayurvedic and indigenous medical systems. The roots of the plant are categorized as rasayanas, which are reputed to promote health and longevity by augmenting defense against disease, arresting the ageing process, revitalising the body in debilitated conditions, increasing the capability of the individual to resist adverse environmental factors and by creating a sense of mental well being[1]. The plant has been used as an antioxidant, anti-tumor, adaptogenic, aphrodisiac, liver tonic, anti-inflammatory agent, astringent and more recently to treat ulcers, bacterial infections, venom toxins and senile dementia. Clinical trials and animal research support the use of *Withania somnifera* for anxiety, cognitive and neurological disorders, inflammation, hyperlipidemia and Parkinson's disease. Recently *Withania somnifera* is also used to exhibit the development of tolerance and dependence on chronic use of various psychotropic drugs. The major biochemical compounds of Indian ginseng are steroidal alkaloids and steroidal lactones in a class of compounds named withanolides[2]. The biological activities of withanolides, especially of the dominant withanolide A and withaferin A, have been studied extensively and, more recently, have been shown to have anti-cancerous

activity[3,4]. Indian genetic resources wild as well as cultivated showed many morphological and phytochemical variabilities[5]. There are evidences for the presence of more than one chemo-type in India[6]. Thus the systematic morpho-chemical characterization of *Withania somnifera* is of great significance for future programmes on quality enhancement of the crop. Generally, the roots are considered to be enriched with bioactive withanolides and used in polyherbal preparations[7,8]. There are no systematic efforts on quantification of withanolide A in different organs of *Withania somnifera*. However, quantification of such active compounds from various organs is very valuable for the proper standardization of herbs and formulations thereof. In virtue of the important medical effect, it is very important to know the distribution of these compounds in order to choose the right organs and to obtain good resources for extraction.

MATERIALS AND METHODS

(i) Plant Material

The seeds of *Withania somnifera* (L.) Dunal var. Jawahar were collected from the Center for Medicinal and Aromatic Plants, Horticulture Department, University of Agricultural Sciences, Dharwad, India and the plants were grown in the Botanical garden of the Karnatak University, Dharwad, India and voucher specimen has been deposited in the herbarium of the Department of Botany, Karnatak University, Dharwad, India. The whole plant and its parts were separated into shoot tip, leaves (young, middle and old), node (young, middle and old), internode (young, middle and old), roots (basal, middle and tip), flowers and seeds were used. They were washed with distilled water, dried under shade and powdered.

(ii) Extraction and HPLC Analysis

Extraction and HPLC analysis of withanolide A was carried out by following the method of Ganzera et al[9]. The dried, powdered materials (500 mg) were extracted with 2 ml methanol by sonication for 30 mins at room temperature. Methanolic extracts were evaporated to dryness in a vacuum oven. For analysis, the remainder was redissolved in 1 ml of HPLC grade methanol and transferred to a polypropylene microcentrifuge tube, vortexed for 30 s and centrifuged for 5 min at 3000 X g. After centrifugation, the clear supernatant was filtered through 0.45 µm nylon membrane filter (Sigma, USA) and was used for the HPLC analysis.

The analytical HPLC experiments were performed with a Waters High Performance Liquid Chromatography (HPLC) equipped with a variable dual wavelength detector operating at 225 nm (W 2487). Separations was carried out with C18 (5 µm) column with reagent alcohol: water (80 : 20) as an eluent at a flow rate of 1 ml min^{-1} and the column temperature was maintained at 27°C. Withanolide A standard was obtained from Chromadex (Laguna Hills, CA, USA). Validation of quantitative method was performed with samples for five injections of 20 µl each.

RESULTS AND DISCUSSION

The chemical composition of a medicinal plant may vary substantially with the developmental stage of the plants. Therefore, investigations on ontogenetic variation of secondary metabolites from different classes have received considerable interest from plant scientists over several decades. The results of concentration of withanolide A in different organs of *Withania somnifera* are presented in Fig 1. The results demonstrated that the content of withanolide A were significantly different in organs of *Withania somnifera*. The maximum concentration of the withanolide A was obtained in shoot tip (386 µg g^{-1} DW), followed by leaves, nodes, whole plant, internode, roots and flowers (342, 272, 206, 102, 56 and 35 µg g^{-1} DW, respectively). Where as the lowest accumulation of withanolide A was obtained from the seeds (23 µg g^{-1} DW). The content of withanolide A was 1.1, 1.4, 1.8, 3.78, 6.89 and 11.02 times higher in shoot tips than that in leaves, nodes, whole plant, internode, roots and flowers respectively. Generally the roots are considered to be enriched with bioactive withanolides and are used in poly-herbal preparations[8,10,11].

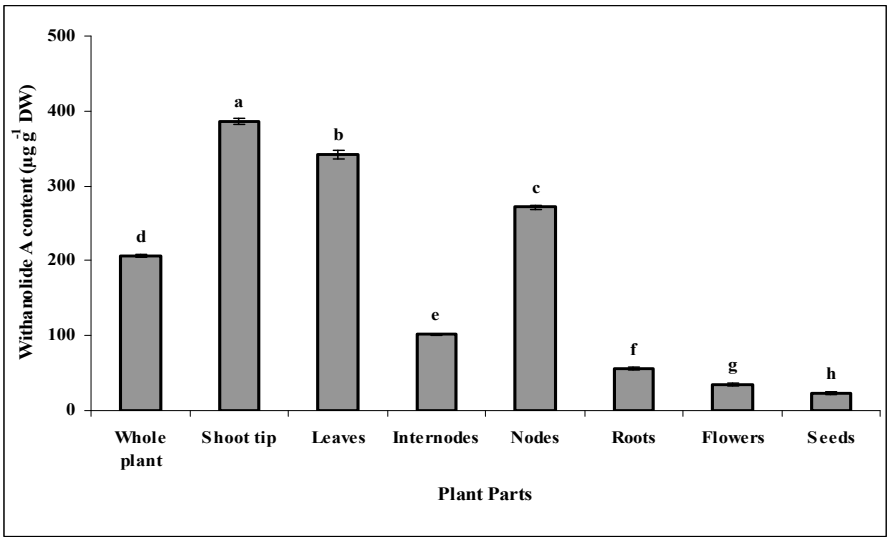

Fig. 1

***Distribution of withanolide A in Withania somnifera*[x].**

[x]*Data represents mean values ± SE of three replicates; each experiment was repeated twice. Means with common letters are not significantly different at P ≤ 0.05 according to Duncan's multiple range test (DMRT).*

In the present study we could observe the maximum content of withanolide A in the shoot tip. The contents of withanolide A in leaves were significantly different, it was 421, 314 and 292 µg g^{-1} DW in young leaf, middle leaf and old leaf respectively. The content of withanolide A in the young, middle and old nodes was 326, 270 and 222 µg g^{-1} DW respectively. The content in the internodal portions also varied and it was 145, 116 and 46 µg g^{-1} DW respectively with the highest being in the young internode followed by middle and old internode. In the roots, the root tip accumulated highest concentration of withanolide followed by middle and basal root (117, 30 and 22 µg g^{-1} DW respectively) (Table 1). Sharma et al.[5] also reported that leaves accumulated higher concentration of withanolides from two different morphotypes of *Withania somnifera*. In the present study we could observe the maximum content of withanolide A in the shoot tip and in the young leaves. Harvesting of the shoot tip yields low amount of the dry mass for the fulfillment of the industrial needs. So leaves can also be used as the raw material for the extraction of withanolide A from *Withania somnifera* as the young leaves accumulated higher concentration of withanolide A than the shoot tips. In general the whole plant can be used for the extraction of withanolide A from *Withania somnifera*.

Table 1

***Contents of withanolide A in various organs and tissues of Withania somnifera*[x].**

Samples	Withanolide A content µg g^{-1} DW ± SE
(a) Whole plant	206 ± 2.30h
(b) Shoot tip	386 ± 3.46b
(c) Leaves	
Young Leaf	421 ± 3.46a
Middle Leaf	314 ± 5.77d
Old Leaf	292 ± 3.46e

(d) Internode	
Young internode	145 ± 2.88i
Middle internode	116 ± 3.46j
Old internode	46 ± 3.46k
(e) Node	
Young node	326 ± 8.08c
Middle node	270 ± 2.88f
Old node	222 ± 2.88g
(f) Roots	
Root tip	117 ± 4.04j
Middle root	22 ± 1.15m
Basal root	30 ± 1.73lm
(g) Flowers	35 ± 2.30l
(h) Seeds	23 ± 1.73m

***Notes**: (c) young leaf - second leaf; middle leaf - fifth leaf; old Leaf - eighth leaf, (d) young internode - between first and second node; middle internode - between fourth and fifth node, old internode – between eighth and ninth node, (e) young node – second node, middle – fifth node, old node – eighth node.*
[x]Data represents means ± SE of three replicates; each experiment was repeated twice. Mean separation within column by Duncan's multiple range test P ≤ 0.05.

CONCLUSION

From the present study conducted, it can be concluded that the various organs of *Withania somnifera* differ in their chemical characteristics. Further, young leaves and shoot tips are enriched in the desired constituents. Leaves and shoot tip can be used as the primary resources and these organs are alternatives for root or whole plant. This species may find important application in medicinal treatment with its high withanolide-A content. Thus the present results might also be useful to obtain enhanced concentration of these compounds.

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