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Distribution Of Withaferin A, an Anticancer Potential Agent, In Different Parts of Two Varieties of *Withania somnifera* (L.) Dunal. Grown in Sri Lanka

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Abstract: *Withania somnifera* (L.) Dunal. (Family: Solanaceae) is a therapeutically important medicinal plant in traditional and Ayurveda systems of medicine in Sri Lanka. Withaferin A, is a potential anticancer compound found in *W. somnifera*. In the present study, attempts have been made to compare withaferin A content, in different parts of (root, stem, bark, leaf) two varieties of (LC1 and FR1) *W. somnifera* grown in same soil and climatic conditions. Ground sample (1g) of leaves, bark, stem and roots of two *W. somnifera* varieties were extracted with CHCl₃ three times. Thin Layer Chromatographic analysis (TLC) of withaferin A in both plant extracts were performed on pre-coated Silica gel 60 GF₂₅₄ plates in hexane: ethyl acetate: methanol (2: 14: 1) mobile phase. Densitometer scanning was performed at λ_{max} = 215 nm. HPLC of *W. somnifera* extracts was performed using Kromasil C₁₈ reverse phase column. Both varieties of *W. somnifera* differed in withaferin A. After visualizing TLC plates with vanillin-sulphuric acid leaf and bark extracts of both varieties showed high intensity purple colour spots (R_f 0.14) than in stem and roots. The highest amount of withaferin A (3812 ppm) was observed in leaves of variety LC1 while the lowest amount was observed in roots of variety FR1 (5 ppm). According to the results it could be concluded that content of Withaferin A was vary leaf > bark > stem > roots in both varieties. Therefore, there is a high potential of incorporation of leaves and bark of *W. somnifera* for the preparation of Ayurveda drug leading to anticancer activity instead of roots.

Key words: *Withania somnifera*, withaferin A, ashwagandha

INTRODUCTION

Withania somnifera (L.) Dunal. (Family: Solanaceae) is a shrubby perennial herb which is traditionally known as Ashwagandha and has been widely used in traditional and Ayurveda systems of medicine for over 3,000 years to treat an array of physiological disorders (Anonymous, 1979; Jayaweera, 1982). Due to its diversified therapeutic potential, it has also been given considerable focused in modern scientific studies. Withanoloides, the major chemical constituent of *W. somnifera*, is a group of naturally occurring C-28 steroidal lactone triterpenoids (Mirjalili *et al.*, 2009). Withaferin A, an anti-cancer potential steroidal lactone (4β,5β,6β,22R)-4,27-dihydroxy-5,6:22, 26-diepoxyergosta-2, 24-diene-1,26-dione) is one of the therapeutically important withanoloides occurs in different parts of the *W. somnifera*. Several previous studies indicated that Withaferin A possess antitumor, antiangiogenesis and radiosensitizing activity (Shohat *et al.*, 1976; Bargagna-Mohan *et al.*, 2007).

Further, withaferin A showed promising anti-cancer activity against several cell lines such as leukemia cells (Malik *et al.*, 2007), breast cancer cells (Stan *et al.*, 2008), prostate cancer cells (Srinivasan *et al.*, 2007; Yang *et al.*, 2007) and melanoma cells (Devi and Kamath, 2003). *In vitro* antiproliferative activity of withaferin A against pancreatic cancer cells has also been reported (with IC₅₀ values of 1.24, 2.93 and 2.78 mM) in pancreatic cancer cell lines Panc-1, MiaPaCa2 and BxPc3, respectively (Yu *et al.*, 2010). Moreover, (Choudhary *et al.*, 2010) isolated 3 chlorinated steroidal lactone from aerial parts of the *W. somnifera* and found that withaferin A being the most potent compound against human lung cancer cell line (NCI-H460), among three compounds tested. Further it has been associated with anti-inflammatory (Sethi *et al.*, 1970) and immunosuppressive properties (Shohat *et al.*, 1967). Meanwhile, 15 accessions of *W. somnifera* have been evaluated for their major withanoloides groups such as withanolide A, withanone, withaferin A and withastramonolide etc. and found that almost all accessions contained withaferin A as the major constituent (Dhar *et al.*, 2006).

However, in Sri Lanka two varieties of *W. somnifera* are found in small scale cultivations, one of which possesses starchy roots while other contains fibrous roots. Moreover, these two varieties have not yet been evaluated for their chemical constituents. The aim of this study was to examine the distribution of withaferin A in different parts of the two varieties of *W. somnifera* in order to diversify the use of other plant parts such as leaves stem and bark for different therapeutic purposes instead of conventional use of roots and to establish the most suitable variety in commercial scale cultivations.

MATERIALS AND METHODS

Collection of plant material: Fresh leaves, bark, stem and roots were obtained from same aged, two *W. somnifera* varieties (LC1 and FR1) which have been maintained in similar soil and climatic conditions in institutional research plots. Herbarium specimens for both varieties were prepared and deposited in institutional herbarium (HTSLC1 and HTSFR1). Collected samples were spreaded in blotting paper and dried in shade until constant weight. Then these were ground to powder using grinder. All the solvents used for the extraction of phytochemicals were from plant tissues were purchased from Sigma Chemical Company (USA).

Preparation of crude extracts: One gram of the finely powdered plant material (roots, leaves, stem and bark) was extracted into 10 mL of water: MeOH (1:1, v/v) overnight. Extract was separated by centrifugation (4,800 rpm for 5 min), of which 3 mL was partitioned with equal volume of CHCl₃ three times. The CHCl₃ fractions were combined and solvent was completely evaporated under reduced pressure. The residue was dissolved in 1 mL of MeOH and the sample was filtered through 0.45 µm (Millipore) prior to use. The entire experiment was done in triplicate.

TLC analysis of withaferin A: Thin layer chromatographic analysis of withaferin A in both plant extracts were performed on pre-coated Silica gel 60 GF₂₅₄ plates. Approximately 10 µL of the samples were spotted on the TLC plate, air-dried and placed in the chromatographic chamber previously saturated with the solvent system. TLC plate was developed using hexane: ethyl acetate: methanol (2:14:1) as the mobile phase. Densitometer scanning was performed at λ max = 215 nm (Shimadzu, CS-9301PC, Japan) before spraying. Each analysis was repeated three times and baseline correction (lowest slope) was used. Spots were observed after spraying with vanillin-sulphuric acid followed by heating at 105 °C for 3-5 min.

HPLC analysis of withaferin A: HPLC (Shimadzu, 10AVP, U.S.A.) of *W. somnifera* extracts was performed

using Kromasil C₁₈ reverse phase column (4.6 mm×25 cm, 5µm) and the mobile phase consisted of MeOH: 1% aqueous NH₄OH (1:1, v/v) at a flow rate of 1 mL min⁻¹ for a run time of 30 min. The Photo Diode Array (PDA) detector was set to detect at 214 nm. Pure withaferin A (Sigma) was used as the standard to quantify withaferin A present in different extracts. Every sample solution was injected in triplicate. Each run was followed by 5 min. wash with 100% MeOH.

Statistical analysis: Results of the HPLC analysis were analyzed by General Linear Model (GLM) of ANOVA test, and mean comparison followed by Duncan's Multiple Range Test (DMRT).

RESULTS

TLC and densitometric analysis: Thin Layer Chromatographic (TLC) profiles of leaf, bark, stem and root of *W. somnifera* showed that sharp spot at R_f value = 0.14 indicated the presence of withaferin A in almost all the parts of *W. somnifera*.

After spraying Vanillin sulphate leaf extract of variety LC1 resulted 5 spots with R_f values 0.05, 0.14, 0.2, 0.28 and 0.32 while withaferin A appeared at R_f = 0.14. Leaf extract of variety FR1 showed 4 spots with R_f values 0.14, 0.2, 0.28 and 0.32. Bark extract of variety LC1 showed 4 compounds with R_f values 0.05, 0.14, 0.24 and 0.32 while bark extract of variety FR1 resulted 8 spots with R_f values 0.14, 0.19, 0.24, 0.3, 0.42, 0.49, 0.56 and 0.67. Six compounds corresponding to the R_f values of 0.2, 0.3, 0.42, 0.49, 0.56 and 0.67 were found in stem extracts of both varieties. However, the intensity of the spot at R_f value 0.14 in variety LC1 stem extract was less than that of variety FR1. Nine discernible spots were observed in root extract of variety LC1 with a R_f values of 0.14, 0.24, 0.3, 0.32, 0.43, 0.49, 0.53, 0.56 and 0.67. Similarly, variety FR1 resulted 7 spots at R_f values 0.14, 0.19, 0.3, 0.32, 0.42, 0.53 and 0.67.

However, in densitometric analysis the corresponding peak for R_f value 0.14 was observed only in leaf and bark extracts of *W. somnifera*, while no peak was observed at R_f value = 0.14 for root and stem extracts both varieties. Even after visualizing TLC plates with vanillin-sulphuric acid no spot was observed at R_f 0.14 for root and stem extracts while purple colour spots were appeared for leaf and bark extracts of both varieties indicating that withaferin A is accumulated in leaves and bark at detectable levels compared to stem and roots in these varieties.

HPLC analysis: As shown in Fig. 1 and 2, the content of withaferin A in two varieties varied from 5 ppm to 3800 ppm. The highest amount of withaferin A (3812 ppm) was

observed in leaves of variety LC1 while the lowest amount was observed in roots of variety FR1 (5 ppm).

The difference of withaferin A content between leaves of both varieties were not significant ($p>0.05$) However, difference of withaferin A content between barks of two varieties was highly significant ($p<0.005$). The amount of withaferin A in bark of variety LC1 was almost 5 times higher than that of variety FR1. Both root withaferin A (Fig. 2) and there were no significant difference ($p>0.05$) between varieties. Even though the stem extract of variety LC1 contained almost double the extracts contained comparatively low amount of amount of withaferin A than that of variety FR1, the difference was not significant ($p>0.05$). According to the results it could be concluded that content of Withaferin A was

vary leaf > bark > stem > roots in both varieties. It was also found out that LC1 has higher content of Withaferin A compare to FR1 in all parts tested.

DISCUSSION

Composition and content of secondary metabolite profile differs from variety to variety even in the same species. Even in the same variety it may vary with the organ. Therefore, investigations on distribution and variation of secondary metabolites from different varieties have received considerable interest from plant scientists over several decades. The amounts of withaferin A exist in two different varieties of *W. somnifera* and variation of withaferin A among parts of the plant is presented in Fig. 2 and 3.

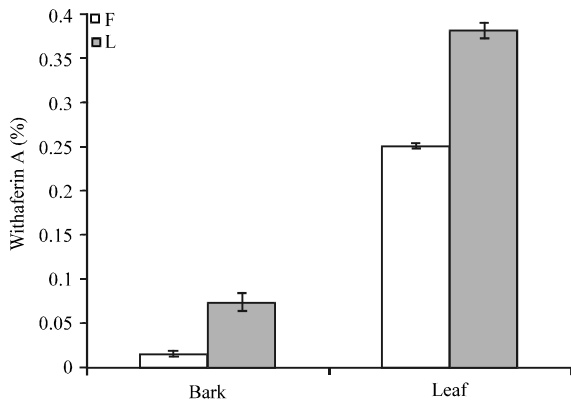


Fig. 1: Two varieties of *Withania somnifera* (A-variety LC₁; B-variety FR₁; A₁ Leaf of variety LC₁; B₁ Leaf of variety FR₁; A₂-Seed pod of variety LC₁; B₂-Leaf of variety FR₁; A₃-Root of variety LC₁; B₃-Root of variety FR₁)

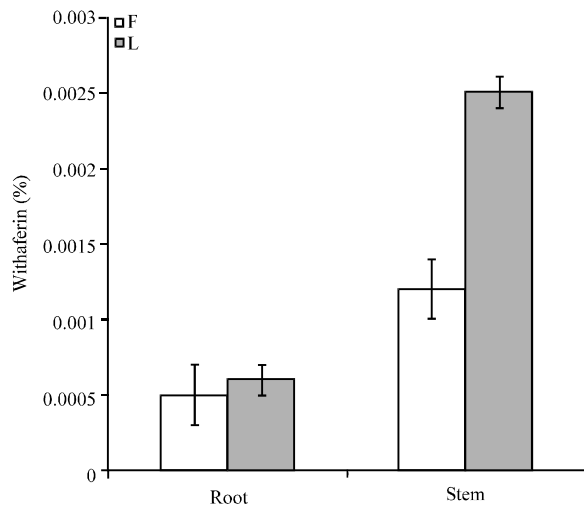


Fig. 2: Amount of withaferin A in bark and leaves of *W. somnifera* (F = variety FR1, L = variety LC1)



Fig. 3: Amount of withaferin A in root and stem of *W. somnifera* (F = variety FR1, L = variety LC1)

The content of withaferin A in two varieties varied from 5 ppm to 3800 ppm. As described by (Abraham *et al.*, 1968), these results also demonstrated that the amounts of withaferin A was significantly different ($p < 0.05$) in different parts of *W. somnifera*. Gupta *et al.* (1996) also reported that leaves accumulated higher concentration of withaferin A in *W. somnifera*.

CONCLUSION

Since leaves and bark contain the higher content of withaferin A, there is a huge potential of incorporation of leaves and bark of *W. somnifera* for the preparation of ayurvedic drug leading to anticancer activity instead of roots.

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