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HPLC ANALYSIS OF WITHANOLIDES IN HEAVY METAL TREATED PLANT DRUG PARTS (LEAVES AND ROOTS) OF *WITHANIA SOMNIFERA* (L) DUNAL.

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ABSTRACT

Plants were grown in pot culture experiments with three treatments in black soil, Treatment No I. a control without any addition to the soil, Treatment No II Cadmium 10ppm, Chromium 20ppm, Nickel 16ppm were introduced into the soil, Treatment No III one % of Calcium hydroxide was also added along with heavy metals to soil. Then plants were grown up to the productivity levels. *Withania somnifera* roots, leaves and preparations of the plant are traditionally used as tonic, sedative and diuretic. It is also having a high medicinal value and possesses potent anti-tumor and antioxidant properties. *Withania somnifera* which mainly contains Withanolides are which biologically active secondary metabolites present in roots and leaves. In the present study the maximum concentration of Withanolide A was observed in the roots and in the leaves of in the control plants (Treatment I) when compared to the other two treatments. Withanolide A concentration is almost same in heavy metal treated plants (Treatment II) and heavy metal +1% Ca $(OH)_2$ treated (Treatment III) plants. The roots showed Withanolides A more concentration when compared with leaves in three treatments. The concentration of Withanolies A of roots and leaves in three treatments there was no significant differences. So leaves can also be used as the raw material for the extraction of Withanolide A from *Withania somnifera.*

KEY WORDS: *Withania somnifera,* Roots and Leaves, Withanolide A, Heavy metal treated plants, High Performance Liquid Chromatography, Crud drugs.

INTRODUTION

Withania somnifera L. Dunal is an important medicinal plant belongs to the family Solanaceae, commonly known in India as Ashwagandha or winter cherry (vernacular: Sanskrit: Ashwagandha; Telugu: Panneru; Trade name: Ashwagandha) is used in more than 100 formulations of Ayurveda, Unani and Siddha and is therapeutically equivalent to ginseng. [24] The genus Withania is systematically placed into the division Magnoliophyta, class magnoliopsida, order solanates and family Solanaceae. [12] It is known classically for its rejuvenating properties, and hence called "Indian Ginseng". [27] The roots and leaves of Ashwagandha contain various alkaloids, Withanolides[2], Withaferins[9] and Withanosides.[34] The plant is said to have a potential property of pacifying 'Vata' in herbal drugs. [1, 28, 8] Withaferin A have been associated with anti-inflammatory [25] and immunosuppressive [26] properties, whereas sitoindosides IX and X are immunostimulatory. [10] Withanolide D has antitumour activity [18] and sitoindosides VII and VIII are antioxidants. [4, 20] Literature scan suggests that there are various new Withanolide glycosides (Withanosides) isolated from Indian ashwagandha roots. [19] The major biochemical compounds of Indian ginseng are steroidal alkaloids and steroidal lactones in a class of compounds named Withanolides. [21] 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. [14, 13]

Indian genetic resources wild as well as cultivated showed many morphological and phytochemical variabilities. [22] Bioactive Withanolides and used in poly herbal preparations. [3 ,30] Phytochemically, the plant is unique in possessing the largest and structurally most diversified set of withanolides (modified steroidal molecules based on an ergostane skeleton), named after the plant. The ethnopharmalogical properties of the plant include adaptogenic, anti-sedative and anti-convulsion activities, and the plant has been employed in the treatment of neurological disorders, geriatric debilities, arthritis and stress- and behaviour-related problems. [21, 15, 8, 4] Several modern molecular pharmacological studies have demonstrated linkage of these therapeutic actions to one or more Withanolides present in the herb. [6,13, 16,17,32,33] The HPLC methods reported earlier[5, 7, 11, 31] are limited to a few markers such as the well-known Withaferin A and withanolide D rather than covering a broad range of differentially functionalized Withanolides.

Indian herbal medicine and Ayurvedic drugs are not being accepted in other developed countries because of heavy metal residue in the drugs. Therefore to find out a solution the following present experiments were taken in *Withania somnifera* in order to reduce heavy metal accumulation in the plant parts.

MATERIALS AND METHODS

(i) Experimental plant material

Withania somnifera seeds were procured from the CIMAP, Hyderabad.

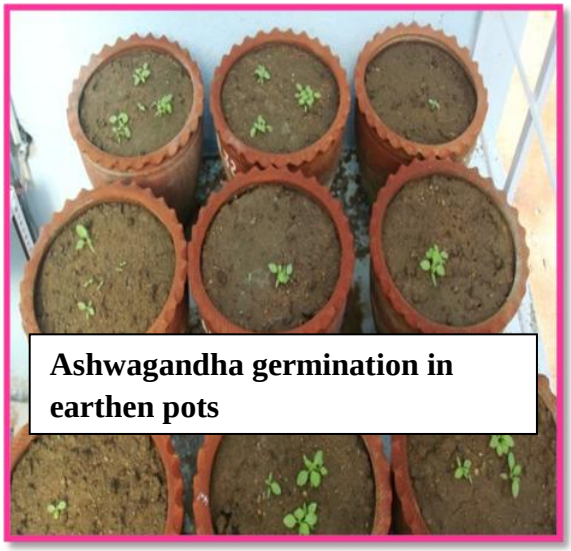
(ii) Plant material and design of experiment

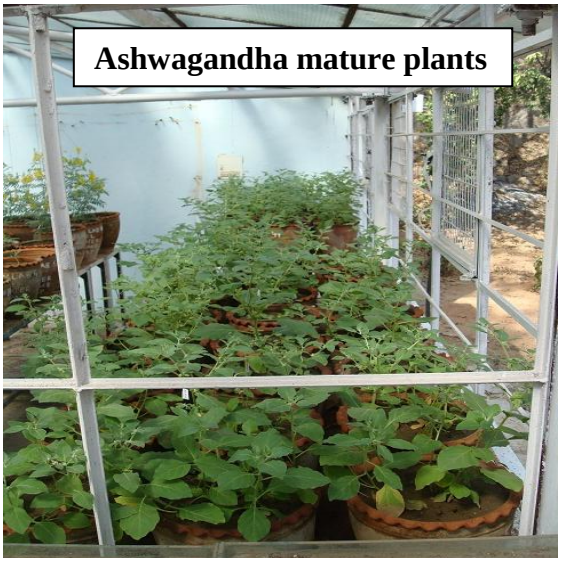
Experimental Plants were grown in pot culture experiments with three treatments in black soil, Treatment No I, a control without any additions to the soil, Treatment No II Cadmium 10ppm, Chromium 20ppm, Nickel 16ppm were introduced into the soil, Treatment No III, one % of Calcium hydroxide was also added along with heavy metals to soil and were grown up to the productivity levels. All the experiments were grown in earthen pots at Green house of Botanical Garden, Department of Botany, Osmania University and Hyderabad. The roots and leaves were air dried at room temperature and powdered mechanically. The powdered roots and leaves were used as the plant material for all analysis.

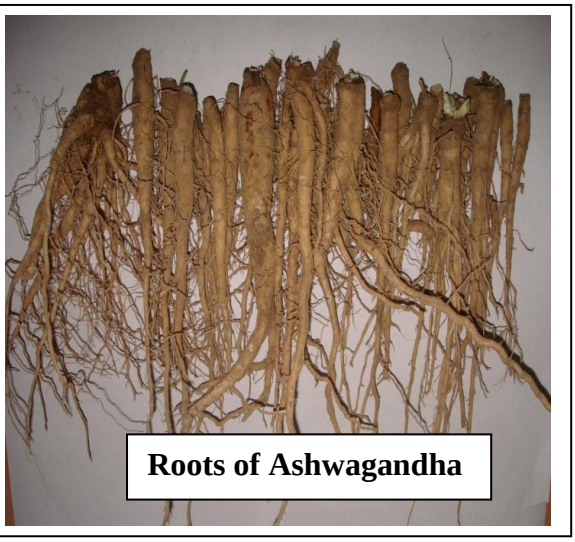
(iii) Extraction and HPLC Analysis

Extraction and HPLC analysis of withanolide A was carried out by following the method.[11] 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 re dissolved in 1 ml of HPLC grade methanol and transferred to a polypropylene micro centrifuge tube, vortexed for 30 s and centrifuged for 5 min at 3000 X g. After centrifugation, the clear supernatant was filtered through 0.45 /m nylon membrane filter (Sigma) 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 were carried out with C18 (5 /m) column with so treat system alcohol: water (80: 20) at a flow rate of 1 ml min^{-1} the column temperature was maintained at 27°C. Withanolide A standard was obtained from Sigma-Aldrich Chemicals. Validation of qualitative method was performed with samples for five injections of 10µL each.

Fig:-Plant material of *Withania somnifera* in three treatments







RESULT AND DISCUSSION

The concentration of Withanolides A in roots in three treatments the Ret. time 2.51, the area % the peak was 99.48 and height %, the peak was 99.54. In leaves the Ret. time 2.51, the area %, the peak was 98.20 and height %, the peak was 96.40 in control plants (Treatment I). Plants grown in heavy metal treated soils (Treatment II) the Withanolides A concentration in roots the Ret. time 2.58, the area %, the peak was 99.34 and height %, the peak was 99.12. In leaves the Ret. time 2.42, the area %, the peak was 96.30 and height %, the peak was 93.92. In plants grown with heavy metal +1% Ca $(OH)_2$ treated soils (Treatment III) in roots the Ret. time 2.53, the area %, the peak was 99.34 and height %, the peak was 99.48. In leaves the Ret. time 2.51, the area %, the peak was 95.98 and height %, the peak was 94.71. The

concentration of Withanolides A was reported maximum in the roots and leaves of the control plants (Treatment I) when compared to the other two treatments. Withanolide A concentration is almost same in heavy metal treated plants (Treatment II) and heavy metal +1% Ca $(OH)_2$ treated (Treatment III) plants. The roots showed Withanolides A more concentration when compared with leaves in three treatments. The concentration of Withanolies A in the roots and leaves in three treatments there was no significant difference in levels were observed.

The roots accumulated highest concentration of Withanolide A and also reported that leaves accumulated higher concentration of Withanolides from two different morphotypes of *Withania somnifera*. [22] Recently, [14] while working on the structureactivity relationships of withasteroids, have concluded that the antiproliferative activity of withanolides is mainly due to the 2-en-1-one in A ring and the hydroxyl groups at C-3, C-4 and C-27. In the similar experiments with *Withania somnifera* the concentration of heavy metals was reported maximum in heavy metal treated plants compared to control plants and heavy metal + 1% Ca $(OH)_2$ treated plants. [23]

Table: 1 HPLC) analysis of withanolides A of *Withania somnifera* roots and leaves in three treatments

Parameters	Treatment No: I (Control plants)			Treatment No: II (Heavy metal plants)			Treatment No: III (Heavy metal + 1%Ca $(OH)_2$ plants)		
	Ret. Time	Area%	Height%	Ret. Time	Area%	Height%	Ret. Time	Area%	Height%
Roots	2.51	99.48	99.54	2.58	99.34	99.12	2.53	99.34	99.48
Leaves	2.51	98.20	96.40	2.42	96.30	93.92	2.51	95.98	94.71

Fig: 1 HPLC (High Performance Liquid Chromatography) analysis of *Withania somnifera* roots in three treatments.

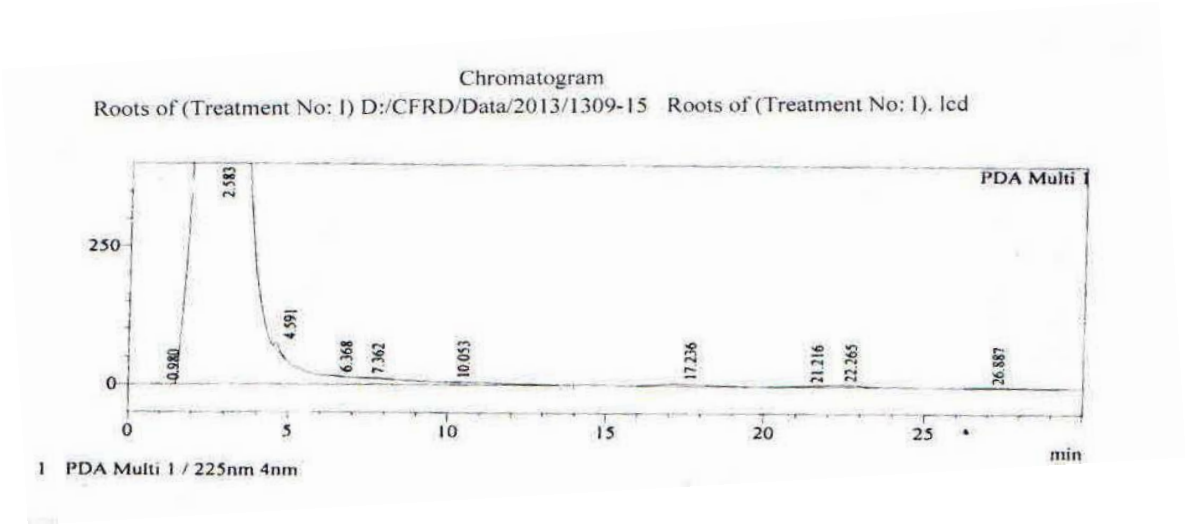


Fig 1: a. Chromatograms obtained from *Withania somnifera* roots (Treatment No. I)

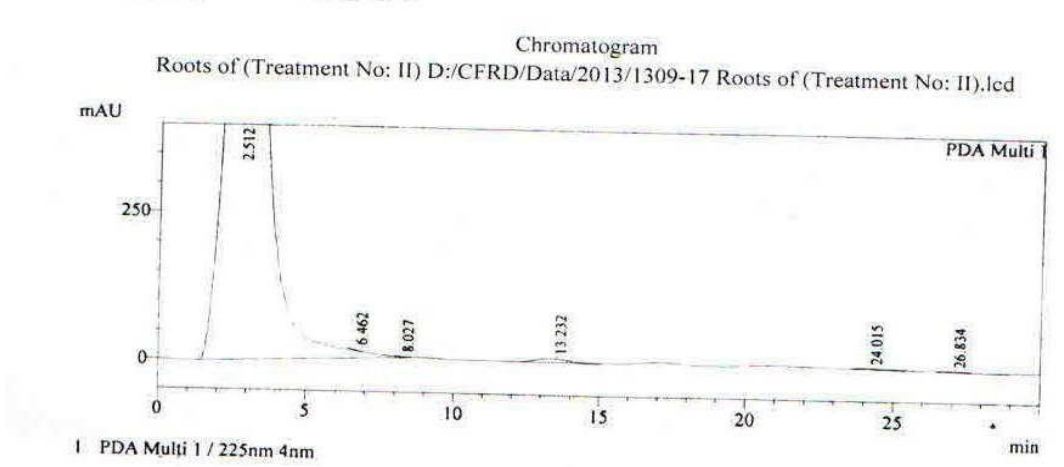


Fig 1: b. Chromatograms obtained from *Withania somnifera* roots (Treatment No. II)

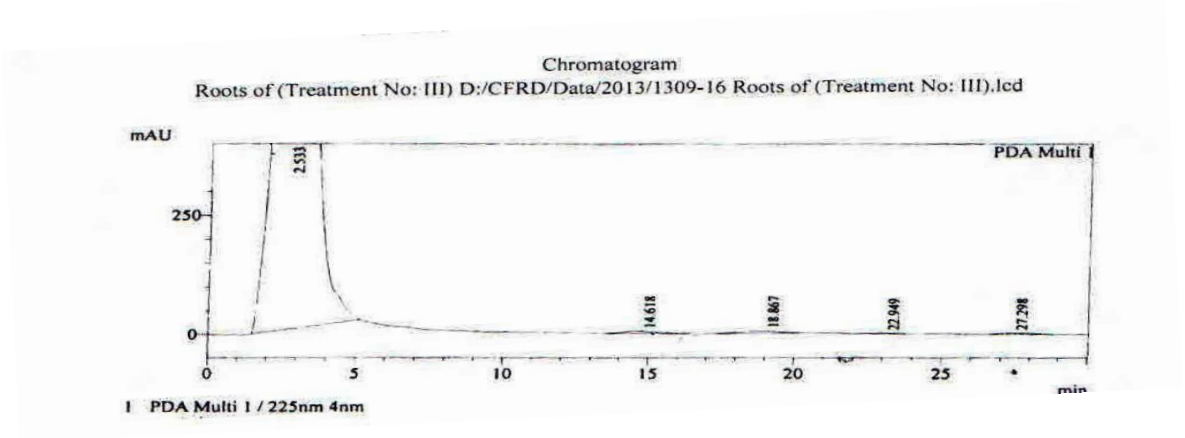


Fig 1: c. Chromatograms obtained from *Withania somnifera* roots (Treatment No. III)

Fig: 2 HPLC (High Performance Liquid Chromatography) analysis of *Withania somnifera* leaves in three treatments.

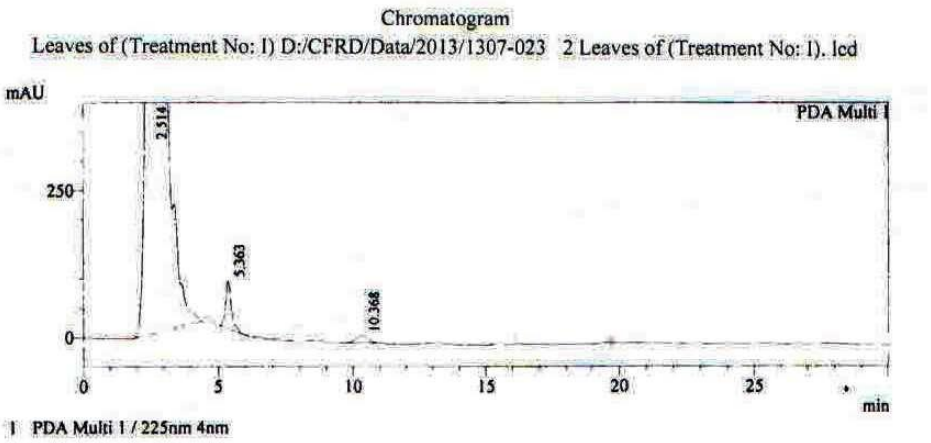


Fig 2: a. Chromatograms obtained from *Withania somnifera* leaves (Treatment No. I)

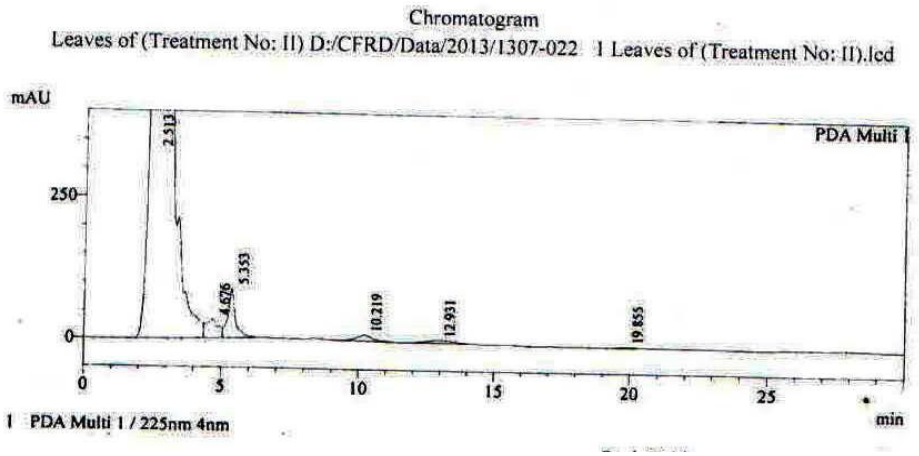


Fig 2: b. Chromatograms obtained from *Withania somnifera* leaves (Treatment No. II)

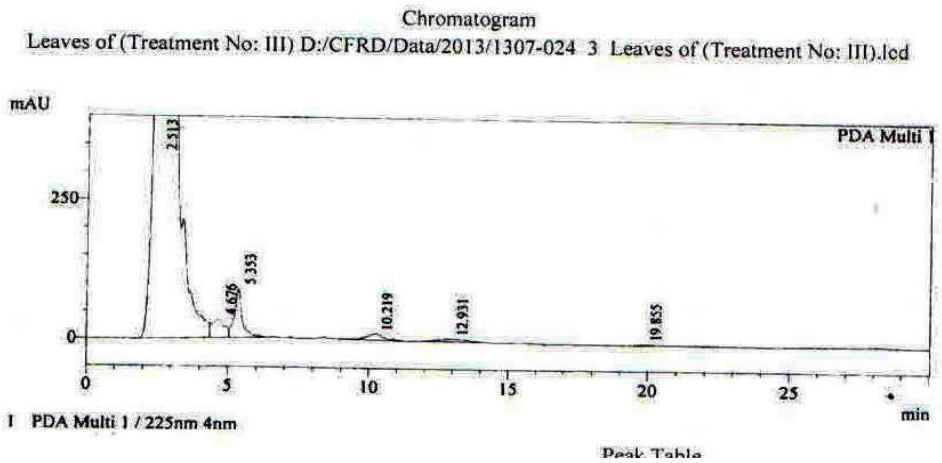


Fig 2: c. Chromatograms obtained from *Withania somnifera* leaves (Treatment No. III).

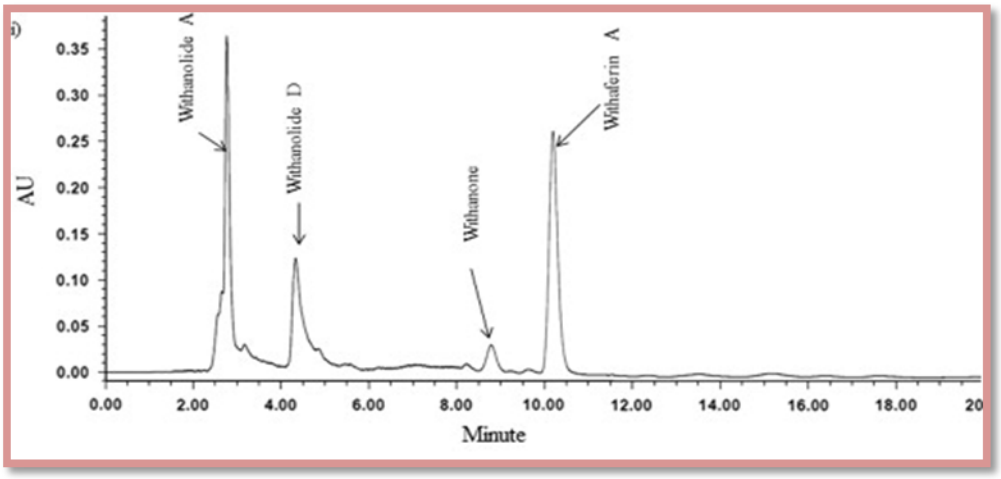


Fig 3: Showing the Withanolide standerds

CONCLUSION

In the present study the maximum concentration of Withanolide A was observed in the roots and in the leaves. So leaves can also be used as the raw material for the extraction of Withanolide A from *Withania somnifera.* The concentration of Withanolides A was reported maximum in the roots and leaves of in the control plants (Treatment I) when compared to the other two treatments. Withanolide A concentration is almost same in heavy metal treated plants (Treatment II) and heavy metal +1% Ca $(OH)_2$ treated (Treatment III) plants. The roots showed Withanolides A more concentration when compared with leaves in three treatments. The concentration of Withanolies A of roots and leaves in three treatments there was no significant differences in levels were observed. *Withania somnifera* plants grown in heavy metal (polluted) contaminated soil should not be used for direct use as crud drug or final product because they accumulate heavy metals in their plants parts. However *Withania somnifera* plants can be treated with 1%Ca $(OH)_2$ can be safely use as crud drug. From this experiment we can produce heavy metal free drugs.

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RERFERENCE

1. Anonymous, Indian Herbal Pharmacopoeia, 1998; 1, 165.
2. Atta UR, Jamal SA, Choudhary MI and Asif E, Two withanolides from *Withania somnifera*. *Phytochem* , 1991; 30:3824–3826.
3. Asthana R and Raina MK, Pharmacology of *Withania somnifera* (L.) Dunal: A Review. Indian drugs, 1989; 26: 199-205.
4. Bhattacharya SK, Satyan KS, Ghosal S., Anti-oxidant activity of glycol withanolides from *Withania somnifera*. *Indian J Exp Biol*, 1997; 35: 236–239.
5. Bala S, Govindrajan R, Rawat AKS, Mahrotra S., HPLC analysis of withaferin A in *Withania somnifera* (L.) Dunal. *Indian J Pharm Sci* , 2004; 66: 236–238.
6. Bargagna-Mohan P, Ravindranath PP, Mohan R., Small molecule anti-angiogenic probes of the ubuquitin proteasome pathway: potential applications to choroidal neovascularization. *Invest Opthal Visual Sci* , 2006;47: 4138–4145.
7. Bessalle R, Lavie D., Semi-quantitative reverse-phase high performance liquid chromatographic analysis of *Withania somnifera* chemotype III. *J Chromatogr*, 1987; 389: 195–210.
8. Dhuley, J.N., Nootropic-like effect of ashwagandha (Withania somnifera L.) in mice. *Phytother. Res*, 2001; 15: 524–528.
9. Devi PU, Kamath R and Rao BSS, Radio sensitization of a mouse melanoma by withaferin A: *in vitro* studies. *Ind J Exp Biol*, 2000; 38:432–437.
10. Ghosal, S., Lal, J., Srivastava, R., Bhattacharya, S.K., Sachidananda, N.U., Jaiswal, A.K., Chattopadhyay, U., Immunomodulatory and CNS effects of sitoindosides IX and X, two new glycosides from Withania somnifera. *Phytother Res*, 1989; 3: 201–206.
11. Ganzera M, Choudhary MI, Khan IA., Quantitative HPLC analysis of withanolides in *Withania somnifera*. *Fitoterapia*, 2003; 74: 68–76.
12. Heiser, C.B and Smith P.C., The cultivated Capsicum. *Econ. Bot*, 1953; 1: 214-227.
13. Ichikawa H, Takada Y, Shishodia S, Jayaprakasam B, Nair, MG and Aggarwal BB, Withanolides potentiate apoptosis, inhibit invasion and abolish osteoclastogenesis through suppression of nuclear factor-kappa B (NF-kappa B) activation and NF-kappa B regulated gene expression. Mol Cancer Ther, 2006; 5: 1434– 1445.
14. Jayaprakasam B, Zhang YJ, Seeram NP and Nair MG, Growth inhibition of human tumor cell lines by withanolides from *Withania somnifera* leaves. *Life Sci*,2003;74: 125–134.

15. Kulkarni SK, George B, Mathur R.,Protective effect of *Withania somnifera* root extract on electrographic activity in a lithiumpilocarpine model of status epilepticus. *Phytotherapy Res*, 1998; 12: 451– 453.
16. Kinghorn AD, Su B-N, Jang DS, Chang LC, Lee D, Gu J-Q, Carcache- Blanco EJ, Pawlus AD, Lee SK, Park EJ, Cuendet M, Gills JJ, Bhat K, Park H-S, Mata-Greenwod E, Song LL, Jang M, Pezzuto JM., Natural inhibitors of carcinogenesis. *Planta Med*, 2004; 70: 691–705.
17. Kaileh M, Berghe WV, Heyerick A, Horion J, Piette J, Libert C, De Keukeleire D, Essawi T, Haegeman G., Withaferin A strongly elicits IκB kinase β hyperphosphoprylation concomitant with potent inhibition of its kinase activity. *J Biol Chem*, 2007; 282: 4253 4264.
18. Leyon, P.V., Kuttan, G., Effect of Withania somnifera on B16F-10 melanoma induced metastasis in mice. *Phytother. Res*, 2004; 18: 118–122.
19. Matsuda, H., Murakami, T., Kishi, A., Yoshikawa, M., Structures of withanosides I, II, III, IV, V, VI and VII, new withanolides glycosides, from the roots of Indian Withania somnifera Dunal. And inhibitory activity for tachyphylaxis to clonidine in isolated guinea-pig ileum. *Bioorg. Med. Chem*., 2001; 9 (6):1499–1507.
20. Panda, S., Kar, A., Evidence for free radical scavenging activity of ashwagandha root powder in mice. *Indian J. Physiol. Pharmacol*, 1997; 41: 424–426.
21. Ray AB and Gupta M, Withasteriods, a growing group of naturally occurring steroidal lactones. In: Herz H**,** Kirby G W**,** Morre E**,** Steglich W**,** Tamm C, (eds.) Progress in the Chemistry of Organic Natural Products. Springer, Berlin, 1994; 63: 1–106.
22. Sharma V, Gupta AP, Bhandari P, Gupta RC and Singh B, A validated and densitometric HPTLC method for the Quantification of Withaferin-A and Withanolide-A in different plant parts of two morphotypes of *Withania somnifera. Chromatographia*, 2007; 66: 801-804.
23. Saidulu Ch,Venkateshwar C and Gangadhar Rao., Heavy metals contamination in ayurvedic plant drug of *withania somnifera* linn. Dunal and their remediation. *Annals of phytomedicine.* An international journal, 2013; 2(2): 110-113.
24. Sangwan, R.S., Chaurasiya, N.D., Misra, L.N., Lal, P., Uniyal, G.C., Sharma, R., Sangwan, N.S., Suri, K.A., Qazi, G.N., Tuli, R., Phytochemical variability in commercial herbal products and preparations of *Withania somnifera* (Ashwagandha). *Curr. Sci*, 2004; 86: 461– 465.

25. Sethi, P.D., Thiagrajan, A.R., Subramanian, S., Studies on the anti-inflammatory and anti-arthritic activity of withaferin-A. *Indian J. Pharmacol*. 1970; 2: 165.
26. Shohat, B., Kirson, L., Lavie, D., Immunosuppressive activity of two plant steroidal lactones withaferin A and withanolide E. *Biomedicine*, 1978; 28: 18–24.
27. Singh, S., Kumar, S.,The Indian Ginseng Ashwagandha. Central Institute of Medicinal and Aromatic Plants Publisher, Lucknow, India, 1998.
28. Singh, B., Saxena, A.K., Chandan, B.K., Gupta, D.K., Bhutani, K.K., Anand, K.K., Adaptogenic activity of a novel, withanolide free aqueous fraction from the roots of *Withania somnifera*. *Phytother. Res*., 2001; 15: 311–318.
29. Sharma V, Gupta AP, Bhandari P, Gupta RC and Singh B, A validated and densitometric HPTLC method for the Quantification of Withaferin-A and Withanolide-A in different plant parts of two morphotypes of *Withania somnifera. Chromatographia,* 2007; 66: 801-804.
30. Sharma K and Dandiya PC, *Withania somnifera* Dunal - present status. Indian drugs, 1992; 29: 247- 253.
31. Shingu K, Furasawa Y, Nohara T.,New withanolides, datutametelins C, D, E, F and G-Ac from Datura metel L. *Chem Pharm Bull*, 1989;37: 2132–2135.
32. Tohda C, Komatsu K, Kuboyama T, Scientific basis for the antidementia drugs of constituents from Ashwagandha (*Withania somnifera*). *J Trad Med*, 2005a; 22: 176–182.
33. Tohda C, Kuboyama T, Komatsu K., Search for natural products related to regeneration of the neuronal network. *Neurosignals* , 2005b;44: 34–45.
34. Zhao J, Nakamura N, Hattori M, Kuboyama T, Tohda C and Komatsu K, Withanolide derivatives from the roots of *Withania somnifera* and their neurite outgrowth activities. *Chem Pharm Bull*, 2002; 50:760–765.