PHARMA RESEARCH BULLETIN
Journal Home Page : www.eduspread.com

Research Article

ISSN: 2582-676X

Standardization of Several Commercial Polyherbal Formulations containing *Withania somnifera* Linn.

Suresh Kumar[1]*, Rameshwar Dass[2]

[1]G. D. Goenka University, Sohna, Gurugram, Haryana, India.

[2]Guru Gobind Singh College of Pharmacy, Yamuna Nagar-135001, Haryana, India.

Article History:

Received: 05 July 2019
Revised: 30 August 2019
Accepted: 05 September 2019

How to Cite:

Kumar S, Dass R. Standardization of Several Commercial Polyherbal Formulations Containing *Withania somnifera* Linn. PRB, 2019;1(1):13-22.

Abstract:

India has a rich heritage of traditional medicines which has been flourishing since from very long time. With the ever-increasing use of herbal medicines worldwide and the rapid expansion of the global market for these products, the safety and quality of medicinal plant materials and finished herbal medicinal products have become a major concern for health authorities, pharmaceutical industries and the public. In the present study several pharmaceutical standardization parameters of three commercial available Ayurvedic polyherbal formulations *i.e.* Ashwaganda churna (Aswajith), Ashwaganda lehya (Ashwagandhadi Lehyam) and Ashwaganda arista (Ashwagandharistam) are discussed. In addition, the estimation of active constituents of Ashwagandha particularly withanolide-A and withaferin-A in all three formulations was carried out using HPTLC.

Keywords: *Withania somnifera*, HPTLC, Standardization, Churna, Lehyam, Arista.

Introduction:

Today, herbal remedies derived from medicinal plants represent substantial portion of the global market and in this respect internationally recognized guidelines for their quality assessment and quality control are necessary [1-3]. WHO has also emphasized the need to ensure the quality control of medicinal plant products? Internationally several pharmacopoeias have provided monograph stating quality parameters and standards of many herbs and herbal products. Standardization problem arises from the complex composition of drugs which are used in the form of whole plant, parts of the plants and plant extracts. Directives on the analytical control of herbal drug must take account of the fact that the material to be examined has complex and inconsistent composition [1]. Therefore, the analytical limits can't be as precise as for the pure chemical compound. Quality assurance of traditional remedies relies upon good manufacturing practices with adequate batch analysis and standardized methods of preparation. Various processes used in the manufacture of herbal drugs lack standardized methods.

Corresponding Author: Kumar S, G. D. Goienka University, Sohna, Gurugram, India. E-mail: bhatiwalsuresh@gmail.com

Thus, the same traditional drug prepared by two different manufacturers may vary in its potency and even the physical appearance. The complexity of the multi-herbal dosage forms containing multiple drug constituents is a present day challenge faced during the standardization process. The information given on the standard analytical procedures for herbal drug constituents in polyherbal dosage forms in official books are minimum. Spectrophotometric methods and titrimetric methods may not be suitable for the drugs containing similar type of herbal constituent [4-6]. The literature survey revealed that the several methods related to HPTLC are reliable for the estimation of herbal constituents individually and in combination with other drugs. The fingerprint obtains in HPTLC comprises of scanning UV fluorescence; ultraviolet spectra and photographic images in ultraviolet light (254 and 366 nm).

Withania somnifera is used in Ayurvedic formulations for a variety of health promoting effects. Several phytochemicals of Ashwagandha, particularly withanolide and withaferin are reported for wide variety of health benefits [7-8]. Withaferin-A chemically characterized as 4β, 27-dihydroxy-5β-6β-epoxy-1-oxowitha-2, 24-dienolide, is one of the main withanolide active principles isolated from the plant. Withaferin-A inhibits cyclooxygenase-2(COX-2) but not cyclooxygenase-1 (COX-1), desired for a non-ulcerating anti-inflammatory/ chemotherapeutic drug [9-11] In addition, Withaferin-A has been reported to have immunosuppressive action on B- lymphocyte proliferation. Other withanolides, including glycosylated ones present in medicinal plants are reported to have antioxidant immunomodulatory and other activities some withanolides are known to have quinine reductase induction-mediated protective activity against chemical carcinogenesis [12-14].

Considering the above facts an effort had been taken to develop an analytical methods using HPTLC to explore the finger printing characteristics of the individual plant constituents namely Withanolide-A and Withaferin-A for various polyherbal Ayurvedic formulation (like churna, lehya and arista) containing *Withania somnifera*. The aim of the present study was to standardize commercial products of Ashwagandha by quantify the active constituent namely Withaferin-A and Withanolide-A in polyherbal Ayurvedic formulations.

Materials & Methods

Drugs and chemicals

Standard phyto-constituents namely Withaferin-A and Withanolide-A were obtained as gift sample from M/S Natural Remedies Pvt. Ltd, Bangalore. Three different marketed Ayurvedic formulations (samples) of *Withania somnifera* namely Lehya, Arista, and Churna were obtained from The Arya-Vaidya Pharmacy Pvt. Ltd., Coimbatore. The solvents toluene, ethyl acetate, and methanol were employed during the study are of AR grade.

Instrumention and Chromatographic Conditions:

Quantitative analysis of all polyherbal formulations was carried out at J.S.S. College of Pharmacy, Ooty. The chromatography was performed on (10 cm x 10 cm) aluminum pre coated plates with (0.2 mm) silica gel 60 F254 (E. Merck, Germany). The samples were applied to the plate as bands width 6 mm by using Camag (Muttenz, Switzerland) Linomat 5 applicator fitted with 100 µl syringe (Hamilton, Switzerland). The rate of application was constant at 150µL/s and space between two bands was 15 mm. The mobile phase was used as e toluene: ethyl acetate (7:3), v/v). The Linear ascending development of the plate was carried out in twin–trough glass chamber previously saturated with mobile phase for 20 min at room temperature (250 ± 2) and relative humidity (60 % ± 5). The length of chromatogram run was 80 mm. After development, the plate was removed and the plate was air dried. The densitometric scanning was performed at 560 nm using Camag TLC.

Extraction Process

5g of each polyherbal formulation was extracted with 50 ml of methanol in 100 ml of beaker by UAE method up to one hour at the constant temperature of 40˚C. After extraction, the extract was evaporated by distillation.

Formulation Profile

The formulation profile is given in **Table 1.**

Table 1. Formulation profile

S/n	Properties	Ashwagandhadi Lehyam	Aswajith–Churna	Ashwagandharistam
1	Nature of product	Semisolid	Solid	Liquid
2	Action and use	Adaptogen	Adaptogen	Adaptogen
3	Colour	Chocolate light brown	light brown	light brown
4	Route of administration	Internal	Internal	Internal

Physicochemical Parameters: [3, 15]
Determination of ash values:

Ash values are helpful in determining the quality and purity of crude drugs in powder form. The Controlled incineration of plant drugs result in ash residue, which consist of inorganic mixture of metallic salts and silica. More direct contamination, such as by sand or earth is immediately detected by the ash value. Total ash is produced by incinerating the ground drug at a low temperature, is possible to remove all the carbon. Too higher temperature may result in the conversion of carbonates to oxides.

a). Determination of total ash value

About 2 g of each formulation was accurately weighed and taken in a silica crucible, which was previously ignited and weighed. The crucible was incinerated gradually by increasing temperature by making it red hot until it becomes free from carbon. The procedure was repeated thrice to get constant weight. The percentage of total ash with reference to the air dried drug was calculated and tabulated in **Table 2**.

b). Determination of water soluble ash value

Water soluble ash is that part of the total ash content which is soluble in water. The total ash obtained was boiled for five minute with 25 ml of distilled water, the insoluble matter was collected in an ash less filter paper, incinerated at a temperature not exceeding 450°C, subtracted the weight of the insoluble matter from the weight of the ash. The percentage of water soluble ash with reference to the air dried drug was calculated and tabulated in **Table 2**.

c). Determination of acid insoluble ash value

It is determined by treating the total ash with dilute hydrochloric acid by weighing the residue. The limit particularly indicates the contamination with siliceous materials such as earth and sand. By comparing with the total ash value for the same sample differentiation can be made between contaminating material and the natural ash of the drug. The total ash obtained was boiled for five minute with 25 ml of 2N hydrochloric acid. The insoluble matter was collected in an ash less filter paper, washed with hot water, ignited, cooled in desiccators and weighed. The percentage of acid insoluble ash with reference to the air dried drug was calculated and tabulated in **Table 2**.

Solvent extractive values
This is used to determine the amount of water soluble/alcohol soluble constituents present in a sample. For a drug where the extraction procedure for the constituents commences with water as the solvent any subsequent aqueous extraction of the dried residue will give a very low yield of soluble matter. Hence water soluble extractive is used to indicate the poor quality caused by adulteration with unwanted matter or by incorrect preparation of drug.

a) Water soluble extractive value

Macerated accurately weighed (5 gm) of each formulation and with 100 ml of water in a stopper flask for 24 hrs, shaking frequently during first 6 hrs and allowed to stand for 18 hrs. Filtered rapidly through filter paper taking precaution against excessive loss of water. Then evaporate 25 ml of extract to dryness in tarred flat bottomed shallow dish. Dry at 105˚C and weighed. Calculated the percentage w/w of water soluble extract and the results are tabulated in **Table 3**.

b) Alcohol soluble extractive value

5 gm of each formulation was macerated with 100 ml of 95% alcohol in a stopper flask for 24 hrs, shaking frequently first 6 hrs and allowed to stand for 18 hrs. Thereafter, filtered rapidly taking precautions against loss of alcohol evaporated 25ml of filtrate and dried at 100˚C and weighed. Calculated the percentage w/w of alcohol soluble extract and the results are tabulated in **Table 3**.

Determination of Foaming Index:

About 1gm of each formulation weighed accurately and transferred to a 500ml conical flask containing 100ml of boiling water. Maintained at moderate boiling for 30 minutes. Cool and filtered in 100-ml volumetric flask and added sufficient water through the filter to dilute to volume. Stopper the tubes and shake them in a lengthwise motion for 15sec (two shakes per second). Allow standing for 15 minutes and measured the height of the foam. If the height of the foam less than 1cm, the foaming index is less than 100 and if the height of the foam is more than 1cm, the foaming index is over 1000.

$$\text{Foaming index} = \frac{1000}{A}$$

Where A = the volume in ml of the decoction used for preparing the dilution in the tube where foaming to a height of 1cm is observed. The results are tabulated in **Table 4**.

Moisture content/Loss on drying:

Moisture content is generally determined by subjecting the crude drug material at 105 ˚C to constant weight and calculating the total loss of weight. Some important physical or chemical methods are used to determine the moisture present in drugs. Many pharmacopoeias employ a method of loss on drying for the study of moisture level. The moisture balance is used for checking the loss on drying generally combine both, a process of drying and its simultaneous weight recording up to the point of constant weight. The results of lose of drying are depicted in **Table 5**.

$$\text{Moisture Content (\%w/w)} = \frac{Initial\ weight - Final\ Weight}{Initial\ Weight} * 100$$

Determination of pH:

About 1 gm of the accurately weighed polyherbal formulation was dissolved in distilled water and filtered. pH of filtrate was calculated by employing digital pH meter with a standardized glass electrode and the results are tabulated in **Table 6**.

Chromatographic analysis

i) Thin Layer Chromatographic Analysis:

Dissolved 5g each Poly herbal Ayurvedic formulation (Lehya, Arista, and Churna) in 10 ml of pure methanol and sonicate it for 30-45 minutes at 40˚C and were filtered. TLC profile of each extract was determined using two solvent system *i.e.* Toluene: Ethyl Acetate: Formic acid (5:5:1) (**Figure 1a**) and Chloroform: Methanol (9 : 1)

(**Figure 1b**). Using Iodine chamber for detection of spots (**Figure 1**). The results in terms of Rf Value are given in **Table 7** [16-17].

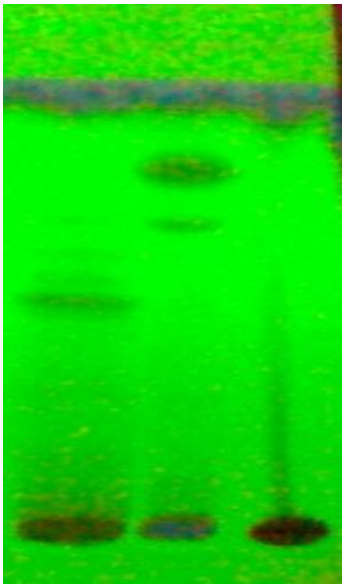

Figure 1a

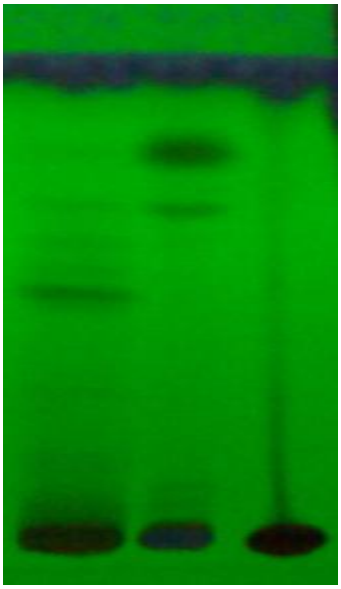

Figure 1b

Figure 1: Thin layer chromatogram of polyherbal formulations

ii). High Performance Thin Layer Chromatography:

HPTLC can almost assess the quality of a drug without elaborate laboratory facilities. Simultaneous multi sample analysis on a single plate that allows a real in system calibration in contrast to other on line chromatographic procedures leads to precise and accurate results with low uncertainly have made HPTLC a versatile, powerful but basically a simple separation technique. It is unenviable and formidable test to set out quality parameters for herbal products usually containing over ten ingredients. It provides the rapid and positive analysis of plant drugs leading to semi quantitative/quantitative information on chief constituents of the formulation. It can also provide fingerprinting for monitoring the identity and purity of drugs and detection of adulteration/substitution [18-19].

Standard preparation

About 1 mg of marker compound Withaferin-A and Withanolide-A were weighed on analytical balance and transferred into 10 ml volumetric flask, added 5 ml of methanol. Dissolved in Sonicator for 10 minutes. Final volume was made up to 10 ml with methanol to get a concentration of 100 mg/ml.

Sample preparation

The sample preparation procedure is to dissolve the sample in a suitable solvent with complete recovery of intact compounds of interest with a suitable concentration of analytes for direct application on the HPTLC plate.
About 1 g of Churna, Lehyam and Aristam formulation was weighed and transferred in 10 ml of volumetric flask, added 5 ml of methanol. Then it was kept in the sonicator for 30 minutes to dissolve. Final volume was made up to 10 ml with methanol and filtered, to get a concentration of 100 mg / ml.

Selection of Mobile Phase:

For the HPTLC method development mobile phase selection different solvent system have been tried on trial and error base on aluminum plate coated with (0.2 mm) silica gel 60 F254 (E. Merck, Germany). Finally from above mention system Toluene: Ethyl acetate: Formic acid in ratio 5: 5: 1 give satisfactory result which gives RF value 0.58.

Preparation of HPTLC Plates

Silica gel pre coated HPTLC plates (20x20 cm) with aluminium support were used for the present study. These plates can be cut to size and shape to suit particular analysis using scissors.

Application of sample

All the sample solutions were applied as a thin band of 6 mm width containing a sample volume 10µl for the lehya formulation (100 mg/ml), 5µl for the churna and Aristam formulation (100 mg/ml), and 2µl for the standard marker compounds Withaferin-A and Withanolide-A (100µg/ml) on six different tracks on the plate by using Camag Linomat IV, which is a microprocessor controlled and programmable applicator.

Chromatographic Development

For the present study, all the plate after drying were developed in previously saturated Camag Twin trough chamber using the above mentioned mobile phase. After proper development, the plates were removed from the chamber and air dried with the help of dryer for faster removal of the mobile phase (**Figure 2**).

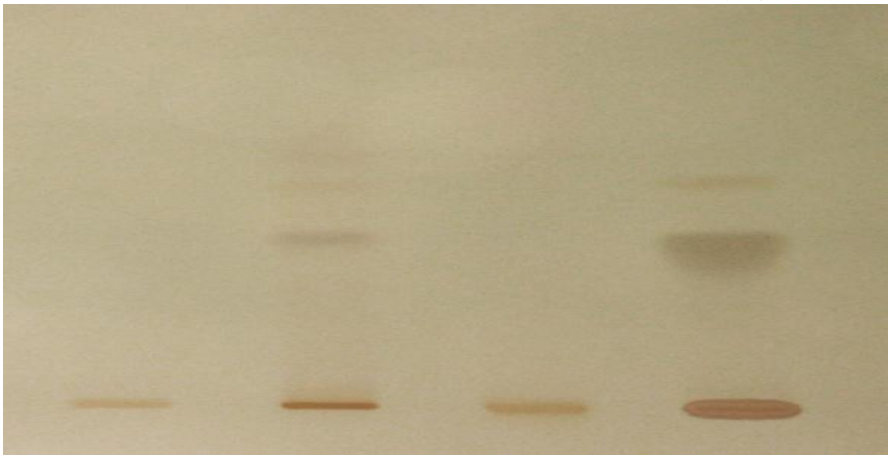

Figure 2: HPTLC- chromatogram of the standard and polyherbal formulations

Densitometric Chromatogram Evaluation (Scanning)

For densitometric measurements of a thin layer chromatogram, its separation tracks are scanned with a light beam in the form of a slit selectable in length and width. Diffusely reflected light is measured by photo sensor. Densitometric measurements can be made by absorbance or by fluorescence. For scanning by fluorescence, the substances excited by UV light, most often at 366 nm. The photo sensor measures the emitted light, which is always of longer wavelength and the measured light intensity is directly proportional to the amount of the fluorescing substance [20].

In order to avoid systematic errors, scanning should always be done in or against the direction of chromatography. The decrease in the light reflectance due to adsorbed compounds gives rise to a signal in the detectors. The signal thus generated is amplified and transmitted to a recorder where the spots by absorbing some of the light cause a signal to lessen and a resultant peak or dip is printed by the detector. The areas included in those peaks measured and related to amount of material in the spot. The scanner is linked to the personal computer, from which all comments are passed to the scanner the scanner transmits all measurement data in digital form to the computer for further processing. For the present study the developed chromatogram where evaluated by using Camag TLC scanner 3 (**Figure 2**). The sequence in the quantitative evaluation of a chromatogram is raw data acquisition-integration- calibration and calculation of result generating the analysis report. For all the samples, integration calibration spectra were recorded and the total area included in the peak was absorbed. The densitograms of

methanolic extracts of polyherbal formulations containing *Withania somnifera* and marker compounds namely Withaferin-A and Withanolide- A have been shown in **Figure 2**. The amount of withaferin-A and withanolide-A was computed from peaks area by using the formula. The results are tabulated in **Table 8** and **9**.

$$\text{Percentage of substance} = \frac{Pspl\ x\ Wt.of\ std.\ x\ D\ x\ std.vol.applied}{Pstd.\ x\ D\ x\ wt.of\ sample\ x\ sample\ vol.applied} * 100$$

Where Pspl - area of the sample peak, Pstd - area of the standard peak, and D - Dilution factor for standard / sample.

Result and Discussion:

The physiochemical evaluation of polyherbal formulations namely Lehya, Arista and Churna were carried out to rationalize their use as a drug of therapeutic importance.

Ash values:

Total ash value, water soluble ash value and acid insoluble ash value were determined and depicted in **Table 2**. The churna showed comparatively higher percentage of ash values.

Table 2: Determination of various ash values reported in %w/w

S/n	Type	Churna Formulation	Lehyam Formulation	Aristam Formulation
1	Total ash	7.26	0.62	0.25
2	Water soluble ash	3.31	0.19	0.06
3	Acid insoluble ash	3.05	0.21	0.07

Extractive values:

Lehya showed a higher percentage of water and alcohol soluble extractives comparatively and the results are tabulated in **Table 3**.

Table 3. Determination of various extractive values in %w/w.

S/n	Types of Extractive	Churna Formulation	Lehyam Formulation	Aristam Formulation
1	Water soluble	72	76.62	21.05
2	Alcohol soluble	8	33.57	20.69

Foaming index:

Results revealed that all the formulation has a foaming index below 100 as depicted in **Table 4**.

Table 4. Determination of foaming Index.

S/n	Type of samples	Height of foam	Foaming index
1	Churna Formulation	0.4	Less than 100
2	Lehyam Formulation	0.6	Less than 100
3	Aristam Formulation	0.5	Less than 100

Moisture content determination:

The moisture content determination is depicted in Table 5.

Table 5. Determination of moisture content in %w/w.

S/n	Type of sample	Moisture content
1	Churna Formulation	13.43
2	Lehyam Formulation	29.23
3	Aristam Formulation	81.23

pH Value:

All the three formulations showed a similar pH, ranging from 4 to 5 as shown in **Table 6**.

Table 6. Determination of pH.

S/n	Types of sample	pH Value
1	Churna Formulation- Aq. Extract	4.6
2	Lehyam Formulation -Aq. Extract	4.8
3	Aristam Formulation -Aq. Extract	4.06

Thin layer chromatographic analysis:

The Rf values of different poly herbal formulations were determined by employing thin layer chromatographic analysis and the results are depicted in **Table 7** (**Figure 1**).

Table 7. Thin layer chromatographic analysis

S/n	Methanolic extract	Toluene: ethyl acetate: formic acid (5 : 5 : 1) R_f values	Chloroform: Methanol (9 : 1) R_f values
1	Churna	0.60, 0.53	0.64, 0.55
2	Arista	0.65	0.64, 0.55, 0.45
3	Lehya	0.53, 0.68	0.45, 0.30

HPTLC Profile:

The determination of withanolide-A content by HPTLC in polyherbal formulations namely lehya and arista was found to be 0.0246%w/w and 0.03727%w/w, respectively. Whereas, Withanlide-A content in churna was not detectable **Table 8**.

Table 8. Determination of withanolide-A ontent in poly herbal formulations

S/n	Methanolic extract	Component present	% of withanolide-A
1	Churna	withanolide-A	Not detected
2	Arista	withanolide-A	0.0373
3	Lehya	withanolide-A	0.0246

The determination of withaferin-A content by HPTLC in polyherbal formulation namely churna was found to be 0.0583 %w/w respectively and Withaferin-A content in arista and lehya was not detectable (**Table 9**).

Table 9. Determination of withaferin-A content in poly herbal formulations

S/n	Methanolic extract	Component present	% of withaferin-A
1	Churna	withaferin-A	0.0583
2	Arista	withaferin-A	Not detected
3	Lehya	withaferin-A	Not detected

Conclusion:

Standardization is an important aspect for maintaining and assessing the quality and safety of the polyherbal formulation as these are combinations of more than one herb to attain the desire therapeutic effect. The polyherbal formulation has been standardized on the basis of organoleptic properties, physical characteristics, and physico-chemical properties. TLC & HPTLC fingerprint profile are used for identification of formulation as well as for deciding the purity and strength and also for fixing standards for the Ayurvedic formulation. Efforts towards narrowing down the phytochemical variations and maintenance of compositional uniformity of Ashwagandha based herbal products are necessitated in view of tightening regulatory frameworks like Dietary Supplements and Health Education Act and the new Natural Health Product Regulations 2003 [21-22].

The objective of the present study was to standardized and estimation of Withanolide-A and Withaferin-A content in three Ayurvedic polyherbal formulations *i.e.* Ashwaganda churna (Aswajith), Ashwaganda lehya (Ashwagandhadi Lehyam) and Ashwaganda arista (Ashwagandharistam) containing *Withania somnifera* (Linn.) Dunal. All The three commercially available polyherbal formulations were evaluated by various physico-chemical characterization such as pH, forming index, methanol extractive value, total Ash value, Acid insoluble Ash value, Water soluble extractive *etc.* and results were depicted from **Table 2** to **5**. Apart from this, the estimation of major herbal ingredients *i.e.* Withanolide-A and Withaferin-A of these polyherbal medicine have been carried out by employing Thin Layer Chromatography and HPTLC. These pharmaceutical standardization parameters can help to study the variation in pharmaceutical effect by daily intake of different poyherbal formulation as they may contains variable concentrations of Withaferin-A and Withanolide–A. The study thus emphasizes the need for stringent phytochemical standardization of herbal products as the results clearly suggest that the commercial products vary by several orders of magnitude with respect to their phytochemical compassion. Standardization of any Ayurvedic formulation is utmost important now-a-days to prove its scientific validation. Hence this attempt was made to make better understanding with scientific approach for several polyherbal formulations containing Ashwagandha.

References:

1. Handa SS, Quality control and standardization of herbal raw materials and traditional remedies. Pharmatimes, 1995;13-21.

2. Indian Pharmacopoeia, Vol.-I, Govt. of India, Ministry of Health & Family Welfare, published by The I.P., Commission, Ghaziabad; 2007, p. 2023-24.

3. The Ayurvedic Pharmacopoeia of India. Part-I, vol.-I, 1st edition, Govt. of India, Ministry of Health & Family Welfare. New Delhi; p. 15-16.

4. Kamalapurkar OS, Mahurkar N, Kamalapurkar VO. Quality assurance for ayurvedic formulations- New Horizons. 2000, p. 17.

5. Kirtikar KR, Basu BD. Indian medicinal plants. 1987; 2nd edition, vol. III, International book distributors, Dehradun, p. 1774-1777.

6. Indian Herbal Pharmacopoeia, Revised New Edition. 2002; p. 467-477.

7. Khajuria RK, Suri KA, Gupta RK, Amina M, et al. Separation, identification, and quantification of selected withanolides in plant extracts of *Withania somnifera* by HPLC-UV (DAD)–positive ion electrospray ionization–Mass Spectrometry. J Sep Sci., 2004;27(7-8):541-6.

8. Budiraja RD, Sudhir S. Review of the biological activity of withanolides. J Sci Indus Res., 1987;46:488.

9. Matsuda H, Murkami T, Kishi A. Structures of withanolides I, II, III, IV, V, VI and VII new 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;96:1499-1507.

10. Elsakka M, Stanescu U. New data referring to chemistry of *Withania somnifera* species. Rev Med Chir Med Nat Iasi., 1990;94(2):385-387.

11. Umadevi P. *Withania somnifera* – a promising drug from cancer chemotherapy and radiosesitization. Ind J Exp Biol., 1996;34(10):927.

12. Duley JN. Effect of Ashwagandha on lipid peroxidation in stress- induced animals. J Ethanopharmacol., 1998;60(2):173-178.

13. Kamath R, Singh UV, Sheena IP, Devi PU, et al. Niosomal Withaferin-A with better antitumor efficacy. Indian J Pharma Sci., 1998;45:48.

14. Fumanowa M. In vitropropagation of *Withania somnifera* and isolation of withanolides with immunosuppressive activity. Planta med. (Germany), 2001;67:146-149.

15. WHO, Geneva, Quality control methods for medicinal plant materials. (2000); A.I.T.B.S. Publishers & Distributors (Regd.) Delhi-51; p. 1-114.

16. Stahl E. Thin Layer Chromatography, A Laboratory Handbook, 2nd ed., Allen and Unwin, London, 1964; p. 311-357.

17. Forni GP, Thin layer chromatography and high pressure liquid chromatography in the analysis of extracts. Fitoterapia, 1980;51:13.

18. Sharma V, Gupta RC. 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;1:4.

19. Kasur RP, Subburaju T, Mahadevan N, Suresh B. HPTLC analysis of Withaferin-A from an herbal extract and polyherbal formulations. J Sep Sci., 2003;26:1707-1709.

20. Gupta AP, Verma RK, Mishra HO. Quantitative determination of Withaferin-A, and alkaloids in *Withania somnifera* by TLC densitometry. J Med Arom Plant Sci., 1996;18(4):788.

21. Namara MSH. Dietary supplements of botanicals and other substances: A new era of regulation. Food Drug Law J., 1995;50,341-348.

22. Cardellina JH. Challenges and opportunities confronting the botanical dietary supplement industry. J Nat Prod., 2002;65:1073-1084.