Int J Ayu Pharm Chem

RESEARCH ARTICLE

www.ijapc.com

e-ISSN 2350-0204

Physico-chemical Standization and Heavy Metal Analysis of Ashwagandha Churna

Satya Prakash Chaudhary[1*], Anil Kumar Singh[2] and K.N. Dwivedi[3]

[1, 2, 3]Department of Dravyaguna, IMS, BHU Varanasi, U. P., India

Abstract

Different *samhitas* of Ayurveda have described Ashwagandha as a very important plant in the Ayurveda and herbal medicine. In Ayurvedic medicine Ashwagandha is used in different disease like arthritis, anxiety, tumors, tuberculosis, leukoderma, bronchitis, backache, fibromyalgia, menstrual problems, hiccups, and chronic liver disease etc. Physical and chemical analysis and detection of heavy metals finds an important place in standardization of Ayurvedic drugs in order to make its global acceptability. In present study two market samples and one sample of locally grown plant of Ashwagandha has been taken for physical and chemical analysis in terms of loss on drying, ash values, extractive values, detection of heavy metals like lead (Pb), Cadiume (Cd), Zinc (Zn), and Mercury (Hg) etc.

Keywords Ashwagandha**,** Heavy metal, Churna,Tumor

Greentree Group

Received 12/3/15 Accepted 06/06/15 Published 10/7/15

INTRODUCTION

In the corporate era there is an exponential growth in the field of herbal and Ayurvedic medicine in the last few decades. It is getting popularized in developing as well in developed countries owing to its natural origin and side effects. In ancient times, Ayurvedic physicians used to treat patients on individual basis, and prepare medicines according to the need of the patient. The current perspective of treatment has changed now, Ayurvedic and herbal medicines are manufactured on a large scale in Pharmaceutical units, where manufacturers come across with many problems such as availability of good quality raw material, authentication of raw material, availability of standards, good standardization methodology of single drugs, formulation, quality control parameters[1]etc.

In plant drugs heavy metals such as Lead, Mercury, Cadmium, Arsenic, or Thallium have been reported and published by a number of researchers which is of great concern[2,3,4]. Heavy metals are known to affect biological communities[5,6,7]. When the levels of heavy metals exceed in plants and animals, it can induce a variety of acute and chronic effects in wide range of organisms in various ecosystem[8]. The poor quality control of these products may causes health hazards as some products may contain unusually high concentrations of potent and poisonous amounts of these metals. Last two decades has published research articles reporting presence of excessive toxic heavy metals. The toxicity studies of some commonly used herbs and their chemical constituents exhibited LD_{50} values ranging from the practically nontoxic to super-toxic categories[2]. Research studies on metabolism and toxicity of trace elements revealed interactions between heavy metals (Cd, Pb, and Hg) and some essential trace elements such as Zn, Fe, Se, Cu, Cr, and Mn. This suggests that the presence of the dietary essential trace elements may contribute to the protection of man from the detrimental effect of heavy metal exposure, while their deficiency may increase toxicity[9]. WHO recommends that medicinal plants which form the raw materials for the finished products may be checked for the presence of heavy metals, further it regulates maximum permissible limits of toxic metals like arsenic, cadmium and lead, which amount to 1.0, 0.3 and 10ppm, respectively[10]. *Withania somnifera* (Fam. *Solanaceae*) commonly

known as 'ashwagandha' is one of the most important medicinal plants which is used alone or in combination with other medicinal plants in various ayurvedic formulations. It is used by the local practitioners for the treatment of various disorders. The present study was conducted for the evaluation of three most important heavy metals in the roots of *W. somnifera*, Pd, Zn and Cd by atomic absorption Spectrophotometer.

MATERIALS AND METHODS

2. Identification

The collected samples were identified by Prof. A. K. Singh, Head of Department of Dravyaguna, I.M.S., B. H. U., Varanasi.

3. Physico-Chemical Analysis[11]

Determination of loss and drying

Ten grams of the sample (without preliminary drying) was weighed and placed in a tarred evaporating dish dry at 105º for 5 hours, and weigh. Continue the drying and weighing at one hour interval until difference between two successive weighings corresponds to not more than 0.25 per cent.

Calculation of LOD = $[W_2 – W_3 / W_2\text{-}W_1] \times 100$

W_1-wt of empty tray,W2-wt of empty tray +sample,W_3–after drying wt of sample and tray

Determination of Total Ash

About 2 to 3gm of sample was accurately weighed in tarred silica crucible at a temperature not exceeding 450°C until it was free from carbon. It was then cooled and weighed. The percentage of total ash was calculated with reference to the air- dried drug.

Calculation of total ash = $[w_3\text{-}w_1/w_2\text{-}w_1] \times 100$

W_1-wt of empty crucible,W_2 – wt of sample + crucible,W_3 – weight after dry sample and crucible wt

Determination of acid insoluble ash

The total ash obtained was boiled for 5 minutes with 25 ml of dilute hydrochloric acid; the insoluble matter obtained was collected on an ashless filter paper, washed with hot water and ignited to constant weight. The percentage of acid-insoluble ash was calculated with reference to the air dried drug.

Acid insoluble value = [residue wt/sample wt] x100

Water-soluble ash

The ash obtained in the determination of total ash was boiled for 5 minutes with 25 ml of water. The insoluble matter was

collected on an ash less filter paper and washed with hot water. The insoluble ash was transferred into a tarred silica crucible and ignited for 15 minutes at a temperature not exceeding 450°C. The weight of the insoluble matter was subtracted from the weight of the total ash. The difference in weight was considered as the water- soluble ash was calculated with reference to the air dried drug.

Determination of Water-soluble extractive

Five grams of test sample was weighed and macerated with 100ml of chloroform water in a closed flask for twenty-four hours, shaking frequently during six hours with a standing time of eighteen hours. It was filtered rapidly, taking precautions against the loss of solvent. Twenty five (25 ml) of the filtrate was taken and evaporated to dryness in a tarred flat bottomed shallow dish at 105 ^{0}C, to constant weight and weighed. The percentage of water soluble extractive was calculated with reference to the air dried sample.

Determination of Alcohol-soluble extractive

Procedure for water soluble extractive was followed for the determination of alcohol soluble extractive but 90% ethanol was used instead of chloroform water.

Crude fiber content

Crude fiber contents in the bark samples were estimated according to the method described by Maynard. Two grams of oven dried bark powder was transferred to a 500 ml conical flask and 200 ml 0.255N H_2SO_4 was added to it. The contents were boiled for 30 minutes with bumping chips on hot plate. The flask was cooled and the contents filtered through muslin cloth. The residue was washed several times with hot distilled water. The residue thus obtained boiled with 200ml, 0.313N NaOH (1.25g of NaOH dissolved in 100ml distilled water).

Crude fiber content = [Loss in weight on ignition (W2 –W1) – (W3 –W1)/ Weight of the sample] x 100

Determination of PH range

5gm of the powder sample of Ashwagandha churna was weighed and immersed in 100ml of water in a beaker. The beaker was closed with aluminum foil and left behind for 24 hours in room temperature. Later the supernatant solution was decanted into another beaker and the pH of the formulation was determined using a calibrated pH meter.

4. Qualitative Phytochemical Screening[12]

In this screening Dragendroff's test, Wagner's test, Mayer's test, Hager's tests were applied.

5. TLC[13]

Identification of chemical can be detected by observation of spots of identical R_f value and about equal magnitude obtained, respectively, with an unknown and a reference sample chromatographer on the same plate. A visual comparison of the size and intensity of the spots usually serves for semi-quantitative estimation.

Preparation of plates

Suspension of the coating substance was prepared in accordance with the instructions of the supplier and, using the spreading device designed for the purpose, a uniform layer of the suspension, 0.25 to 0.30mm thick, on a flat glass plate 20cm long was spread. The coated plates were allowed to dry in air, heated at 100ºC to 105ºC for at least 1 hour and allowed cooling, protected from moisture. The plates were protected from moisture and used within 3 days of preparation. At the time of use, the plates were again dried, if necessary, the distance of each spot from the point of its application was measured & recorded the R_f value were calculated by dividing the distance travelled by the spots by the distance travelled by the front of the mobile phase.

6.Determination of physical characteristics of churna[13]

Bulk density

It is the ratio of given mass of powder and its bulk volume. It was determined by transferring an accurately weighed amount of powder sample to the graduated cylinder with the aid of a funnel. The initial volume was noted. The ratio of weight of the volume it occupied was calculated.

Bulk density $=W/V_0$ g/ml

Where, W = mass of the powder,V_0 = untapped volume

Tapped density

It was measured by transferring a known quantity (25gm) of powder into a graduated cylinder and tapping it for a specific number of times. The initial volume was noted. The graduated cylinder was tapped continuously for a period of 10-15 min. The density was determined as the ratio of mass of the powder to the tapped volume.

Tapped volume= W/V_f g/ml

Where, W = mass of the powder, V_f = tapped volume.

Compressibility index

It is the propensity of the powder to be compressed. It is based on the apparent bulk

density and tapped density. The percentage compressibility of the powder was determined using the following formula.

Compressibility index = $[(V_0-V_f)/V_0] \times 100$,

% compressibility = [(tapped density – bulk density)]/ tapped density] x 100

Hausner ratio

It indicates the flow properties of the powder. The ratio of tapped density to the bulk density of the powder is called Hausner ratio. It is calculated as following-

Hausner ratio= Tapped density/bulk density

Angle of repose

The internal angle between the surface of the pile of powder and the horizontal surface was known as the angle of repose. The powder was passed through funnel. The distance between the funnel and table was fixed. The height and the radius of the pile were measured. Angle of repose of the powder was calculated using the formula

Angle of repose= $\tan^{-1}(h/r)$

Where, H=height of the pile, r = radius of the pile

Table 1 Scale of Flow Ability

S. No.	Flow Properties	Angle of Repose	Compressibility Index (%)	Hausner Ratio
1.	Excellent	25-30	<10	1.00-1.11
2.	Good	31-35	11-15	1.12-1.18
3.	Fair	36-40	16-20	1.19-1.25
4.	Possible	41-45	21-25	1.26-1.34
5.	Poor	45-46	26-31	1.35-1.45
6.	Very Poor	55-56	32-37	1.46-1.59
7.	Very very poor	>66	>38	>1.6

Table 1.1 Determination of Physico-Chemical Analysis

S.No.	Parameter	Vindhya Area Sample	Tansukh	Dabur
1.	% Loss on drying	10.56 %	9.43 %	7.53 %
2.	Total Ash value	6.5 %	5.9 %.	5.3%
3.	Acid Insoluble Ash value	2.3%	1.9 %	1.6 %
4.	Water Soluble extract value (%w/w)	2.4 %	2.1 %	1.9%
5.	Alcohol Soluble Extract value (%w/w)	1.5 %	1.2 %	0.9 %
6.	Crude fiber Contents	5.0 %	4.2 %	4.0 %
7.	pH	7.10	7.80	8.40

7. Detection and estimation of heavy metals by AAS material and methods[14]

Samples: Two samples of Ashwagandha powder were procured, one from local market and another from Vindhya area.

Instrument: Atomic absorption spectrophotometer equipped with a deuterium lamp for background correction was used for determination of trace elements and heavy metals. The hollow-cathode lamps for Zn, Cd, and Pb (Photron) were employed as radiation source. The flames used were air/acetylene and N_2O/acetylene. Nitrogen was used as carrier gas.

Chemicals: Nitric acid, hydrochloric acid, sulphuric acid, and hydrogen peroxide were of analytical grade (E. Merck). The water used in all experiment was ultrapure water obtained from Milli-Q-water purification system (Ranken Rion Ltd, India). The standard solutions were prepared in five different concentrations to obtain calibration curve by diluting stock solutions (CPA Ltd) of 1000ppm of each element immediately before use.

Sample preparation: Samples were digested by the wet digestion method. 10ml of nitric acid was added to 2gm of accurately weighed dried sample in a 100ml beaker and was heated on a hot plate at 95°C for 15 min. The digest was cooled and 5ml of concentrated nitric acid was added and heated for additional 30 min at 95°C. The last step was repeated and the solution was reduced to about 5 ml without boiling. The sample was cooled again and 2ml of deionized water and 3 ml of 30% hydrogen peroxide was added. With the beaker covered, the sample was heated gently to start the peroxide reaction. When effervescence became excessively vigorous, sample was removed from the hot plate and 30% hydrogen peroxide was added in 1ml increments, followed by gentle heating until the effervescence was subsided. 5ml of concentrated hydrochloric acid and 10ml of deionized water was added and the sample was heated for additional 15 min without boiling. The sample was cooled and filtered through a Whatman No. 42 filter paper and diluted to 50ml with deionized water.

Sample analysis: Digested samples were analyzed for Pb, Cd, and Zn using flame atomic absorption spectrophotometer technique. The 1000ppm standard solutions of elements were diluted in five different concentrations to obtain calibration curve for quantitative analysis. All the measurements were run in triplicate for the samples and standard solutions. The instrumental conditions during the analysis of trace and heavy metals are listed in table-2 giving details about parameters which are defined for respective metals.

Table 1.2 Determination of Qulitative phytochemical

S.No.	Parameter	Vindhya	Tansukh	Dabur Area

		Sample		
1.	Alkaloid	+	+	+

Table 1.3 TLC

S.No.	Parameter	Vindhya Area Sample	Tansukh	Dabur
1.	Rf value	0.90	0.79	0.89

Table 1.4 Determination of physical characteristics of Churna

S. No.	Parameter	Vindhya Area Sample	Tansukh	Dabur
1.	Bulk density	0.38	0.41	0.47
2.	Tap density	0.46	0.50	0.55
3.	Angle of repose	43	39	35
4.	Compressibility	24	20	14
5.	Hausner	1.45	1.25	1.16

RESULTS AND DISCUSSIONS

From the above method we found that LOD value of the sample of Dabur (Figure A), Tansukh (Figure B) and Vindhya area (Figure C) are 7.53%, 9.43%, and 10.56%, respectively (Table 1.1). These values show the moisture contained in the samples. Dabur had less LOD and Vindhya area sample had maximum LOD, so Dabur sample contained less moisture and Vindhya area sample maximum moisture content. The total ash values of the sample of Dabur, Tansukh and Vindhya area were found to be 5.3%, 5.9% & 6.5% (Table 1.1). The total ash value represents the inorganic salt, naturally occurring in drug and deliberately added to it as a form of adulteration.

Table 1.5 Determination of organoleptic characters of sample

S.No.	Parameter	Vindhya Area Sample	Tansukh	Dabur
1.	Colour	Pinkish brown	Pale brown	Yellowish white
2.	Odor	Characteristics	Characteristics	Characteristics
3.	Taste	Slightly bitter	Slightly bitter	Slightly bitter

Table 1.6 Detection & Estimation of Heavy Metal by AAS

S.No.	Name of heavy metals	Vindhya Area Sample	Tansukh	Dabur
1.	Zn	1.520 ppm	1.987 ppm	1.243 ppm
2.	Cd	.397 ppm	.350 ppm	.305ppm
3.	Pd	11.657 ppm	10.27 ppm	9.12 ppm

Therefore, the sample form Dabur contained less inorganic salt and adulteration as compared to the sample obtained from Vindhya area. Acid insoluble ash values was found to be more for purified sample because it contains siliceous material like sand, clay etc more than unpurified sample, due to addition of some siliceous materials (Table 1.1). Alcohol soluble extractive value and water soluble extractive value indicate the active constituent of drug. According to results Dabur have the lowest amount of

active constituent and Vindhya area sample have maximum active constituents (Tab.1.1). Crude fiber Contents shows the fiber matter of the drug. According to the results Vindhya area sample has maximum amount of fiber (5%) and Dabur has minimum amount of fibers (4%) (Table 1.1). According to results the Vindhya area sample is less basic (pH-7.10) and the Dabur sample is highly basic (pH-8.40) (Tab.1.1). In the alkaloid test all sample shows the presence of alkaloid (Table 1.2). The Rf value of sample indicates the purity of the sample. When the R_f value is higher then compounds are pure and vice-versa. According to the results the Vindhya area samples are pure (0.9) and the Dabur impure (.89) (Tab.1.3). Angle of repose of powder sample shows the flow of powder. So according to results the flowing property of Dabur sample was good (35) and Vindhya area sample flowing property is lesser (43) (Table1.4).Compressibility and Hausner value of powder indicates the compactness of powder. Therefore according to results Vindhya area sample powder has the maximum compactness as compare to sample form Dabur (Tab.1.4). Total of three elements (Zn, Cd and Pb) were determined in the powdered root samples of medicinal plants using atomic absorption spectrophotometer (AAS). The study revealed that all the samples do possess heavy metals but within the prescribed limit of WHO (Table1.6).

Figure A: Sample Dabur

Figure B: Sample Tansukh

Figure C Sample Vindhya area

CONCLUSION

On the basis of the results sample form Dabur (Figure A) and Tansukh (Figure B) were good and the concentration of the heavy metals in them was within limits. Quality of Vindhya area (Figure C) sample was fair with cncetration of heavy metals in limits. Therefore these samples are free from the heavy metal toxicity.

REFERENCES

1. Agarwal, A., Critical issues in Quality Control of Herbal Products, Pharma Times, 37(6), 0911. 2005.
2. Wu J-T, Hsieh M-T, Kow L-C.1998. Role of Proline accumulation in response to toxic copper in chlorella sp. (chlorophyceae) cells. Journal of Phycology 34:113-117.
3. T-H Ueng, J-J Kang, H-W Wang, P-C Lin An overview of toxicology of commonly used traditional Chinese medicine. Yaown Shipin Fenxi 5(4), 241-263.
4. W-L Liang, S-W Lin, K-Y Yen, L-L Yang metal element analysis of commercial Chinese herbal medicines. Yaiwan Kexue, 51(2) (1998), pp.37-56.
5. Hsu, MJ, Selvaraj, K and Agoramoorthy, G. 2006. Taiwans Industrial Heavy Metals Poppulation threatens2.Biol., 28, 685-690. Smith, KA. 1990.
6. Ozdilek, Hasan, G, Paul, P, Mathisen, and Pellegrino, D. 2007. Distribution of heavy metals in vegetation surrounding the Blackstone River, USA Considerations regarding sediment contamination and long term metals transport in freshwater riverine ecosystems. J. Environ. Biol., 28,493-502
7. Sahu, RK, Katiyar, S, Tiwari, J and Kisku, GC. 2007. Assessment of drain water receiving effluent fromvtanneries and its impact on soil and plants with particular emphasis on bioaccumulation of heavy metals. J.Environ.
8. Gawel, JE, Ahner, BA, Friedland AJ and Morel, FMM.1996. Role of heavy metals in forest decline indicated by phytochelatin measurements. Nature. 381, 64-65.
9. Wu, WL. 1992. Occurrence and Physiological activities of trace elements in traditional Chinese drugs. Zhonghu.
10. WHO. Environmental Health Criteria: International Programme on Chemical Safety. World Health Organization, Geneva. Pp 165 (1998).
11. The Ayurvedic Pharmacopoeia of India, Govt. of India, Ministry of Health and Family Welfare, Department of Indian Systems of Medicine and Homeopathy, New Delhi, Published by The Controller

of Publications, Civil Lines, Delhi, 1(1), 143,156 (1989)

12. C.K. Kokate, A.P. Purohit, S.B. Gokhate, Pharmacognosy, Shri D.K. Furia Nirali parkaspublication 14, Budhwar Peth, Jogeshwari Mandir Lane, Pune- 411 002 PP122 (1990).
13. "Indian Pharmacopoeia",2007,Volume I
14. Gomez M.R., Cerutti S., Somb L.L., Silva M.F. andMartınez L.D., Determination of heavy metals for the quality control in Argentinian herbal medicines by ETAASand ICP- OES, Food and Chem. Toxicol., 45, 1060-1064 (2007).