

Comparative Quality Evaluation of Three Different Marketed Brands of Ashwagandha Churna (Powder)

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6

Abstract

Ashwagandha has been a crucial herb in the traditional medical systems for more than 3000 years. The plant roots are categorized as Rasayana (tonic) for its wide-ranging health benefits. Objective: As the standardization of herbal formulation is of great concern for its safety and efficacy for that reason this work is aimed at comparative evaluation of various quality parameters of three marketed brands of Ashwagandha churna (powder). Methods: Three different and popular marketed formulations of AshwagandhaChurna (powder) were assessed comparatively for their organoleptic, physicochemical and phytochemical properties as per the methods prescribed in Pharmacopoeias. Results: The data analysis revealed that all the parameters of three brands of AshwagandhaChurna (powder) had approximately similar values with some significant variations in a few. The value of water soluble and alcohol soluble extractives of Brand B was lesser than the standard values, and the pH was higher than the other two brands. There was also a considerable difference between the flow properties of the powder of all three brands. All the three brands were found to contain Cadmium concentration slightly more than the prescribed values.

22

Index terms— quality evaluation, ashwagandha churna (powder), pharmaceutical, physico-chemical, phyto-chemical, heavy metal analysis.

1 I. Introduction

ithanasomnifera, also known as Ashwagandha has been a crucial herb in the Ayurvedic and indigenous medical systems for more than 3000 years. The roots of the plant are classified as Rasayana, which are renowned for promoting health and longevity by increasing the defense against diseases, stopping the aging process, revitalizing the body in conditions of weakness, increasing the Individual's ability to resist environmental factors adverse effects and creating a sense of mental wellbeing. It has been in use for a long time for all age groups and for both sexes and also during pregnancy without side effects. ??1] The biologically active chemical constituents are alkaloids (Isopelletierine, Ana ferine), steroidal lactones (Withanolides, withaferins), saponins containing an additional acyl group (Sitoindoside VII and VIII), and withanolides with a glucose at carbon 27 (Sitoindoside XI and X). It is also rich in iron. Much of Ashwagandha's pharmacological activity has been attributed to two main withanolides, withaferin A and Withanolide D. Other constituents include: Anaferine, Anahydriene, Beta-Sisterol, Chlorogenic acid (in leaf only), Cysteine (in fruit), Cuscohygrine, Iron, Pseudo tropine, Scopoletin, Somniferinine, Somniferiene, withanine and withanolides A-Y. [2].

It boosts the function of the brain and nervous system and also improves the memory. It also acts as a reproductive enhancer by promoting a healthy sexual and reproductive balance. Being a powerful adaptogen, it improves the body's resistance to stress. Ashwagandha improves the body's defense against diseases by improving the cell-mediated immunity. It also has powerful antioxidant properties that help protect against cell damage caused by free radicals. It also possesses antioxidant, anxiolytic, adaptogenic, anti-Parkinson,

7 B) DETERMINATION OF MOISTURE CONTENT/ LOSS ON DRYING (LOD)

43 anti-venom, anti-inflammatory, anti-tumor, immunomodulation, hypolipidemic, antibacterial, cardiovascular
44 protection properties. [3] II. Need for Evaluation

45 In the traditional medicine system, plants in raw form, both fresh and dried, are used for their healing effects
46 against a variety of human disorders. The quality control of medicinal herbs and their biological components
47 is critical to justify their acceptability in the modern medical system. The fundamental problem faced by
48 the user industry is the lack of availability of rigid quality control profiles for herbal raw materials and their
49 formulations. With the emergence of revolutionary analytical tools and instrumental technologies, it is possible
50 to suggest a practical quality assurance profile for a raw drug or its bioactive component. [4,5] W This paper
51 reports the comparative determination of pharmaceutical, physicochemical and phytochemical parameters like
52 bulk density, tapped density, the angle of repose, ash values, extractive values, loss on drying, etc. of three
53 marketed preparations of Ashwagandha Churna (powder).

54 2 a) Procurement of Samples

55 The following marketed Ashwagandha Churna (powder) preparations were used in the present study. Brand A
56 (Batch No. AL 0207), Brand B (Batch No. F-1701), Brand C (Batch No. #A-A G C015). All brands of the
57 Ashwagandha Churna (powder) were procured from the local market from the registered Ayurvedic Pharmacy.

58 3 b) Organoleptic Evaluation

59 All the organoleptic properties viz. color, odor, taste, and texture of the drug to touch were performed as per
60 standard procedure and noted down.

61 4 c) Pharmaceutical Evaluation

62 Pharmaceutical parameters like Bulk density, Tapped density, Carr's Index, Hausner's Ratio and Angle of repose
63 were determined as per standard protocols.

64 i. Determination of Bulk Density and Tapped Density Bulk density is defined as the mass of many particles of
65 the material divided by the total volume they occupy. The total volume includes particle volume, interparticle
66 void volume, and internal pore volume. Tapped density is the term used to describe the bulk density of powder (or
67 granular solid) after consolidation/ compression prescribed regarding "tapping" the container of powder measured
68 number of times, usually from a predetermined height.

69 The term bulk density refers to a measure used to describe a packing of particles or granules and the term
70 Tapped density refers to the true density of the particles or granules. ????????? ? ? ? ?????????? (%) =
71 ?????????????? ?????????????? ? ????????? ?????????????? ?????????????? ?????????????? × 100

72 iii. Determination of Hausner's Ratio Hausner's ratio is related to inter-particle friction and as such can be
73 used to predict the powder flow properties. The Formula for calculation:????????????????? ? ? ? ?????????? =
74 ?????????????? ?????????????? ? ?????????? ?????????????? × 100

75 5 iv. Determination of Angle of Repose

76 The angle of repose is a parameter used to estimate the flow ability of a powder. It is defined as the maximum
77 angle possible between the surface of the pile of powder and the horizontal plane. Powders with low angles of
78 repose will flow freely, and powders with high angles of repose will flow poorly. The Formula for calculation: tan
79 ?? = ? ?? Where, ? = Angle of repose h = Height of pile r = radius of the base of the pile

80 6 IV. Physico-Chemical Evaluation

81 Physicochemical parameters like Foreign matter, Moisture content (Loss on Drying), pH, Total ash, Acid-Insoluble
82 ash, Water-soluble extractive, Alcohol-soluble extractive values of all three samples were determined as per
83 standard protocols. All the procedures are described as follows: a) Determination of Foreign Matter 100 g of
84 sample was taken and spread in a thin layer on a suitable platform and was examined in daylight with the unaided
85 eye (or using 6x or 10x magnifying glass), and the foreign matter was separated and weighed. The percentage
86 of foreign matter was calculated with reference to the drug sample. Standard: The sample should not contain
87 more than 2% of foreign matter unless otherwise specified in the individual monograph.

88 7 b) Determination of Moisture Content/ Loss on Drying (LOD)

90 An accurately weighed 5g of polyherbal formulation powder was taken in a tared evaporating dish. The crude
91 drug was then heated at 105°C in an oven for 3 hours. The drying and weighing were continued at half an
92 hour interval until the difference between two successive weighing corresponded to, not more than 0.25 percent.
93 Percentage moisture content of the sample was calculated with reference to the air-dried powdered drug material.
94 The Formula for Calculation: % ?????? = ?? 2 ? ?? 3 ?? 3 ? ?? 1 × 100 %

95 Where, W 1 = weight of container (g) W 2 = weight of container + wet sample (g) W 3 = weight of container
96 + dried sample (g) W 2 - W 3 = weight of moisture W 3 - W 1 = weight of dried sample

97 8 c) Determination of Loss on Ignition (LOI)

98 An accurately weighed 5g of polyherbal formulation powder was taken in a previously ignited and tared silica
99 crucible and was heated in the oven at 105°C overnight (or the previously dried sample can also be used). The
100 crucible was cooled and reweighed. The crucible was then placed into the furnace tray and was ignited in the
101 Muffle-Furnace at 500°C for about 4 hrs. The sample was then cooled in a dessicator for 30 min., and reweighed
102 with the ash in it (W A). The observations were noted. The Formula for calculation: % ?????? = ?? ?? ? ?? ??
103 ?? ?? ? ?? ?? × 100 %

104 Where, W C = weight of crucible (g) W S = weight of the sample (g) W A = weight of ash (g)

105 9 d) Determination of Total ash

106 An accurately weighed 3 g of the sample was taken in a previously ignited and tared silica dish/crucible. The
107 material was evenly spread and ignited in a Muffle-Furnace by gradually increasing the temperature to not more
108 than 450°C -600°C till the carbon-free ash was not obtained. The total ash value was calculated with reference
109 to the air-dried powdered drug material. The Formula for calculation: % ?????????? ????? = ?????????? ?????
110 ????? ?????????? ????? ????? ?????????? ????? × 100 %

111 e) Determination of Acid Insoluble ash Ash above obtained, was boiled for 5 min with 25ml of 1M Hydrochloric
112 acid and filtered using an ash less filter paper. Insoluble matter retained on filter paper was washed with hot
113 water, and filter paper was burnt to a constant weight in a Muffle-Furnace. The percentage of acid insoluble ash
114 was calculated with reference to the air-dried powdered drug material. The Formula for calculation: % ??????????
115 ? ?????????????????? ????? = ?????????? ????? ?????????? ?????????????????? ?????????????? ??????????????
116 ????? ????? ?????????? ????? ????? × 100 %

117 f) Determination of Water-Soluble ash 1g of ash obtained in Total ash experiment was boiled for 5 min with
118 25ml water and insoluble matter collected on an ashless filter paper which was then washed with hot water
119 and ignited for 15 min at a temperature not exceeding 450°C in a Muffle-Furnace. The difference in weight
120 of ash and weight of insoluble matter was determined as difference represents the value. The percentage of
121 water-insoluble ash was calculated with reference to the air-dried powdered drug material. The Formula for
122 calculation: % ?????????? ?????????????? ????? = ?????????? ????? ?????????????????? ?????????????? ?????
123 ?????????? ????? ????? ?????????? ????? × 100 % g) Determination of Extractive Values i.

124 Determination of Alcohol Soluble Extractives 5 gm of churna (powder) was accurately weighed and placed
125 inside a glass-stoppered conical flask. It was then macerated with 100ml of ethanol. The flask was shaken
126 frequently during the first 6 hours and was kept aside without disturbing for 18 hours. It was then filtered,
127 and about 25ml of the filtrate was transferred into a tared flat-bottomed shallow dish and was evaporated to
128 dryness on a water bath. It was then dried to 105° C for 6 hours, cooled and finally weighed. The percentage of
129 Alcohol Soluble extractives was calculated with reference to the air-dried powdered drug material. The Formula
130 for calculation: % ?????????? ?????????????? ????? = ?????????? ????? ?????????????????? ?????????????? ×
131 100 × 100 25 × ?????????? ????? ????? ?????????? ????? %

132 ii. Determination of Water-Soluble Extractives Proceed as directed for determination of Alcohol-Soluble
133 Extractive, using chloroform-water (2.5 ml chloroform in purified water to produce 1000 ml) instead of ethanol.

134 10 h) Determination of pH Value

135 The powder sample of Ashwagandhachurna (powder) was weighed to about 5g and immersed in 100 ml of water
136 in a beaker. The beaker was closed with aluminum foil and left behind for 24-hours at room temperature. Later
137 the supernatant solution was decanted into another beaker, and the pH of the formulation was determined using
138 a calibrated digital pH meter.

139 11 V. Phytochemical Evaluation

140 The aqueous and alcoholic extracts of the respective formulations were prepared and were subjected to preliminary
141 phytochemical screening. These tests reveal the presence of various bioactive secondary metabolites which
142 might be responsible for their medicinal attributes. Methods for preliminary qualitative phytochemical tests
143 of the plant extracts are given below in Table 2. Three reference solutions of the element being examined
144 having different concentrations were prepared to cover the range recommended by the instrument manufacturer.
145 Separately the corresponding reagents were added to the test solution, and the blank solution was prepared with
146 the corresponding reagents. The absorbance of the blank solution and each reference solution were measured
147 separately, and the readings were recorded. A calibration curve was prepared with the average value of 3 readings
148 of each concentration on the ordinate and the corresponding concentration on the abscissa. A test solution of
149 the substance being examined was prepared as specified in the monograph. The concentration was adjusted such
150 that it falls within the concentration range of the reference solution. The absorbance was measured three times,
151 and the readings were recorded, and the average value was calculated. The mean value was interpolated on the
152 calibration curve to determine the concentration of the element.

21 VIII. DISCUSSION

153 12 b) Preparation of Lead standard solution

154 Lead standard solutions were prepared from Stock solution (1000 ppm Sisco Research Laboratories Pvt. Ltd.
155 stock solution). Standard solutions of concentrations, 2, 4, 6, 8 and 10 ppm were prepared. The absorption of
156 the standard solution measured at 217 nm using hollow cathode lamp as a light source & air acetylene blue flame
157 on Atomic absorption Spectrophotometer.

158 13 c) Preparation of Cadmium standard solution

159 Cadmium standard solutions were prepared from Stock solution (1000 ppm Sisco Research Laboratories Pvt.
160 Ltd. stock solution). Standard solutions of concentrations 0.2, 0.4, 0.6, 0.8 and 1.0 ppm was prepared. The
161 absorption of the standard solution measured at 228.8 nm using hollow cathode lamp as a light source & air
162 acetylene blue flame on Atomic absorption Spectrophotometer.

163 14 d) Preparation of Test solution

164 About 0.5 g of the coarse powder of the substance being examined was accurately weighed, transferred into a
165 caspian flask, 5-10 ml of the mixture of nitric acid (HNO₃) and perchloric acid (HClO₄) in the ratio of 4:1
166 was added. A small hopper was placed on the flask-top, macerated overnight, heated to slake on the electric hot
167 plate, till white smoke dispersed, and the slaked solution becomes colorless and transparent. It was then cooled,
168 and transferred into a 50 ml volumetric flask. The container was washed with 2% nitric acid solution (HNO₃),
169 and the washing solution was added into the same volumetric flask and diluted with the same solvent to make-up
170 the volume. Synchronously the blank reagent solution was also prepared according to the above procedure.

171 15 e) Determination

172 An accurate of 1 ml of the test solution and its corresponding reagent blank solution respectively were measured,
173 and to it 1 ml of the solution containing 1% NH₄H₂PO₄ and 0.2% Mg(NO₃)₂ was added. The mixture
174 was shaken well, and an accurate of 10-20 all solution was pipetted out to determine the absorbance.

175 16 f) Sample analysis

176 The analysis of the digested samples was carried out using an Atomic Absorption Spectrophotometer (EC
177 Electronics Corporation of India limited AAS Element AS AAS4141) for Lead and Cadmium. The instrumental
178 conditions for Lead analysis are depicted in Table 3.

179 17 VII. Results

180 18 a) Organoleptic Evaluation

181 The observations for the organoleptic evaluation of three brands of Ashwagandha Churna (powder) are reported
182 in Table-4.

183 19 c) Physico-Chemical Evaluation

184 The observations for the physicochemical evaluation of three brands of Ashwagandha Churna (powder) are
185 reported in Table-6. Water Soluble Ash 0.85% 0.79% 1.60% -

186 20 d) Phytochemical Evaluation

187 The observations for the phytochemical evaluation of three brands of Ashwagandha Churna (powder) are reported
188 in Table-7.

189 21 VIII. Discussion

190 Ashwagandha churna (powder) of Brand A was of the powder form of Creamish color with a characteristic odor
191 and bitter taste. This preparation had pH value of 5.0, and Loss on drying value of 4.17% w/w. Preparation
192 has Alcohol-soluble extractives and Watersoluble extractives values of 11.2% w/w and 25.6% w/w respectively.
193 The bulk density and tapped density of the powder were 0.478 and 0.641 respectively. The powder flow was
194 fair-passable as it had the Carr's Index of 25.43% (Passable), Hausner's ratio of 1.34 (Passable) and Angle of
195 repose of 37.715° (Fair). It had Total Ash value of 6.06% w/w, and Acid-insoluble ash and Water-soluble ash
196 value of 0.97% w/w and 0.85% w/w respectively. Loss on ignition was found 94.54% w/w. The concentration for
197 heavy metals Lead and Cadmium were found to be 4.825 and 0.227 respectively of which Lead concentrations
198 were within the prescribed limits and of Cadmium was a little more than the standard value. Phytochemical
199 screening revealed the presence of Glycosides, Carbohydrates, Steroids, and Terpenoids in both the extracts;
200 Alkaloids in alcoholic extract only and Saponins in aqueous extract only.

201 Ashwagandha churna (powder) of Brand B was of the powder form of Yellowish brown color with a
202 characteristic odor and very bitter taste. This preparation had pH value of 5.6, and Loss on drying value of
203 5.37% w/w. Preparation had Alcohol-soluble extractives and Water-soluble extractives values of 6.4% w/w and

204 10.4% w/w respectively. The bulk density and tapped density of the powder were 0.381 and 0.612 respectively.
205 The powder flow was poor-good as it had the Carr's Index of 25.43% (Poor), Hausner's ratio of 1.37 (Poor)
206 and Angle of repose of 32.619? (Good). It had Total Ash value of 5.52% w/w, and Acid-insoluble ash and
207 Water-soluble ash value of 0.84% w/w and 0.79% w/w respectively. Loss on ignition was found 94.79% w/w.
208 The concentration for heavy metals Lead and Cadmium were found to be 5.786 and 0.363 respectively of which
209 Lead concentrations were within the prescribed limits and of Cadmium was a little more than the standard value.
210 Phytochemical screening revealed the presence of Glycosides, Carbohydrates, Steroids, and Terpenoids in both
211 the extracts; Alkaloids in alcoholic extract only and Saponins in aqueous extract only.

212 Ashwagandha churna (powder) of Brand C was of the powder form of off-white color with a characteristic
213 odor and very bitter taste. This preparation had pH value of 5.0, and Loss on drying value of 7.07% w/w.
214 Preparation had Alcohol-soluble extractives and Water-soluble extractives values of 8.8% w/w and 20.8% w/w
215 respectively. The bulk density and tapped density of the powder were 0.584 and 0.751 respectively. The powder
216 flow was passable-excellent as it had the Carr's Index of 22.24% (Passable), Hausner's ratio of 1.29 (Passable)
217 and Angle of repose of 29.052? (Excellent). It had Total Ash value of 5.10% w/w, and Acid-insoluble ash and
218 Water-soluble ash value of 0.6% w/w and 1.60% w/w respectively. Loss on ignition was found 95.09% w/w. The
219 concentration for heavy metals Lead and Cadmium were found to be 4.253 and 0.334 respectively of which Lead
220 concentrations were within the prescribed limits and of Cadmium was a little more than the standard value.
221 Phytochemical screening revealed the presence of Glycosides, Carbohydrates, Steroids, and Terpenoids in both
222 the extracts; Alkaloids in alcoholic extract only and Saponins in aqueous extract only.

223 22 IX. Conclusion

224 Thus, all the parameters of three brands of Ashwagandha Churna (powder) had approximately similar values and
225 were compatible with the standard values mentioned in the Pharmacopoeias except the value of Water-soluble
226 and Alcohol-soluble extractives of Brand B i.e. 10.4% and 6.4% which was lesser than the standard values of
227 15% and 10% respectively. The pH of the formulation was also higher than the other two with the value of 5.6.
228 There was also a considerable difference between the flow properties of the powder of all three brands. All the
229 three brands were found to contain Cadmium concentration slightly more than the prescribed values.

230 Hence, it can be concluded that the present study on the pharmaceutical, physicochemical and phytochemical
231 characters can serve as a vital source of information and provide suitable reference standards for the quality
232 control of these formulations for future investigations. It is also emphasized to perform quality checks on every
233 batch to optimize the final product according to the Pharmacopoeial standards. Abbreviations API -Ayurvedic
Pharmacopoeia of India IP -Indian Pharmacopoeia Pb -Lead Cd -Cadmium

1

Angle of Repose	Carr's Index	Hausner's Ratio	Flow Properties
25-30	<10	1.00-1.11	Excellent
31-35	11-15	1.12-1.18	Good
36-40	16-20	1.19-1.25	Fair
41-45	21-25	1.26-1.34	Passable
46-55	26-31	1.35-1.45	Poor
56-65	32-37	1.46-1.59	Very Poor
>66	>38	>1.60	Very Very Poor

Figure 1: Table 1 :

2

S. No.	Phyto- Constituents	Name of Tests	Procedure	Observation
1.	Alkaloids	Mayer's test	2 ml extract + few drops of HCl + Mayer's reagent	Cream Precipitation
2.	Carbohydrates	Hager's test	2 ml extract + few drops of HCl + Hager's reagent	Yellow Precipitation
		Wagner's test	2 ml extract + few drops of HCl + Wagner's reagent	Reddish brown color
3.	Reducing sugars	Molisch test	2 ml extract + 2 Drops of Molisch reagent + few drops of Conc. H ₂ SO ₄	Violet or Reddish color
4.	Flavonoids	test	1 ml extract + 1 ml Fehling Solution (A and B)	First a Yellow and then Brick Red Precipitation
		Alkaline	2 ml extract + few drops of 40% NaOH solution	Intense yellow color forms which become
5.	Saponins	Lead acetate test	2 ml extract + few drops of the Lead Acetate solution	colorless on the addition of dilute acid
6.	Tannins	Foam test	2 ml extract + 4 ml distilled H ₂ O	Yellow precipitation
		Braymer's test	Mix well and shake vigorously	
7.	Steroids	Salkowski's test	2 ml extract + 2 ml H ₂ O + 2-3 drops of 5% FeCl ₃	Black green or bluish color
8.	Proteins	Millon's test	2 ml extract + 2 ml Chloroform + 2 ml Conc. H ₂ SO ₄	Chloroform layer appears red, and acid layer shows greenish-yellow fluorescence
			3 ml extract + 5 ml Millon's reagent	White precipitate which turns brick red on warming
9.	Glycosides	Keller Kil-liani's test	2 ml extract + Glacial Acetic Acid + 1 drop of 5% FeCl ₃ + Conc. H ₂ SO ₄	Reddish brown color appears at the junction of 2 layers, and upper layer
10.	Phenols	-	2-3 ml of extract + few drops of 5% FeCl ₃ solution	appears bluish green
			2-3 ml of extract + few drops of the Lead Acetate solution	Deep blue-black color
				White precipitate

Figure 2: Table 2 :

3

Parameters	Pb	Cd
Wavelength (nm)	217	228.8
Slit width (nm)	1.0	0.5
Light Source	Hollow Cathode Lamp	Hollow Cathode Lamp
Flame type	Air/C 2 H 2	Air/C 2 H 2
Current	10	3.5
AAS Technique	Flame	Flame

Figure 3: Table 3 :

4

S. No.	Properties	Brand A	Brand B	Brand C	Standard(IP)
1.	Appearance	Powder	Powder	Powder	Powder
2.	Color	Creamish	Yellowish	Off-White	Buff to Greyish Yellow
3.	Odor	Characteristic	Characteristic	Characteristic	
4.	Taste	Bitter	Very Bitter	Very Bitter	Slightly mucilaginous/Bitter/Acid
5.	Texture	Fine Powder	Fine Powder	Very Fine Powder	

b) Pharmaceutical Evaluation

The observations for the pharmaceutical evaluation of three brands of Ashwagandha Churna (powder) are reported in Table-5.

Figure 4: Table 4 :

5

S. No.	Properties	Brand A	Brand B	Brand C
1.	Bulk Density	0.478	0.381	0.584
2.	Tapped Density	0.641	0.612	0.751
3.	Hausner's Ratio	1.34	1.37	1.29
4.	Carr's Index	25.43%	27.17%	22.24%
5.	Angle of Repose	37.715?	32.619?	29.052?

Figure 5: Table 5 :

22 IX. CONCLUSION

6

S. No.	Properties	Brand A	Brand B	Brand C	Standard (IP)
1.	Foreign Matter	Nil	Nil	0.4%	NMT 2.0%
2.	pH	5.0	5.6	5.0	-
3.	Loss on Drying/ Moisture Content	4.17%	5.37%	7.07%	NMT 12.0%
4.	Water Soluble Extractive	25.6%	10.4%	20.8%	NLT 15.0%
5.	Alcohol Soluble Extractive	11.2%	6.4%	8.8%	NLT 10.0%
6.	Loss on Ignition	94.54%	94.79%	95.09%	-
7.	Total Ash Value	6.06%	5.52%	5.1%	NMT 7.0%
8.	Acid Insoluble Ash	0.97%	0.84%	0.60%	NMT 1.2%
9.					

Figure 6: Table 6 :

7

S. No.	Phyto-Constituent	Name of Tests	Brand A				Brand B				Brand C			
			Aq.	Alco.	Aq.	Alco.	Aq.	Alco.	Aq.	Alco.	Aq.	Alco.	Aq.	Alco.
1.	Alkaloids	Hager's test	-	+	-	+	-	-	-	+	-	-	+	-
		Wagner's test	-	+	-	+	-	-	-	-	-	-	+	-
		Mayer's test	-	+	-	+	-	-	-	-	-	-	+	-
2.	Glycosides	Keller Killani's test	+	+	+	+	+	+	+	+	+	+	+	+
3.	Carbohydrates	Molisch's test	+	+	+	+	+	+	+	+	+	+	+	+
4.	Proteins	Biuret's test	-	-	-	-	-	-	-	-	-	-	-	-
		Millon's test	-	-	-	-	-	-	-	-	-	-	-	-
		Ninhydrin's test	-	-	-	-	-	-	-	-	-	-	-	-
5.	Amino Acids	Salkowski's test	+	+	+	+	+	+	+	+	+	+	+	+
6.	Steroids	Alkaline Reagent test	-	-	-	-	-	-	-	-	-	-	-	-
7.	Flavonoids	Lead acetate test	-	-	-	-	-	-	-	-	-	-	-	-
		Copper Acetate test	+	+	+	+	+	+	+	+	+	+	+	+
		Ferric Chloride test	-	-	-	-	-	-	-	-	-	-	-	-
8.	Terpenoids	Foam test	+	-	+	-	-	-	-	+	-	-	-	-
		Ferric Chloride test	-	-	-	-	-	-	-	-	-	-	-	-
		Lead Acetate test	-	-	-	-	-	-	-	-	-	-	-	-
9.	Tannins													
10.	Saponins													
11.	Phenols													

e) Determination of Heavy Metals (Lead And Cadmium)

Figure 7: Table 7 :

8

S. No.	Properties	Brand A	Brand B	Brand C	Standard (API)
a.	Lead	4.825	5.786	4.253	10 ppm
b.	Cadmium	0.363	0.325	0.334	0.3 ppm

Figure 8: Table 8 :

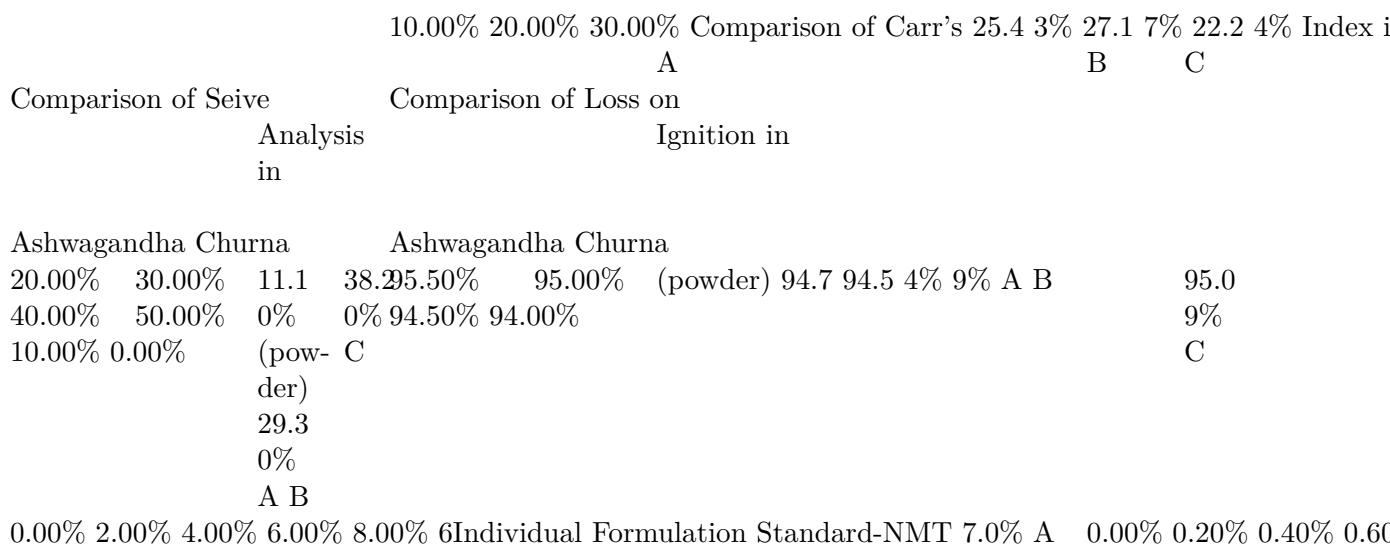


Figure 9: Comparison of Total Ash Value in Ashwagandha Churna (powder)

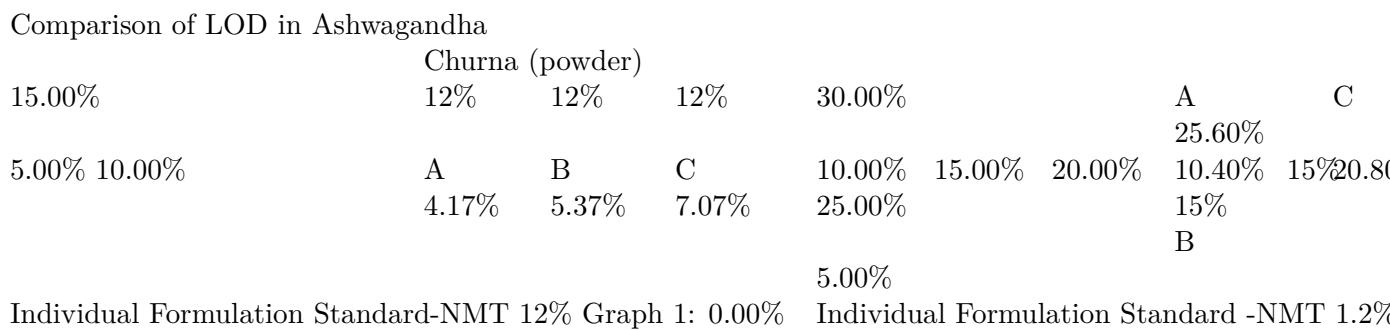


Figure 10: Comparison of Acid Insoluble Ash in Ashwagandha Churna (powder)

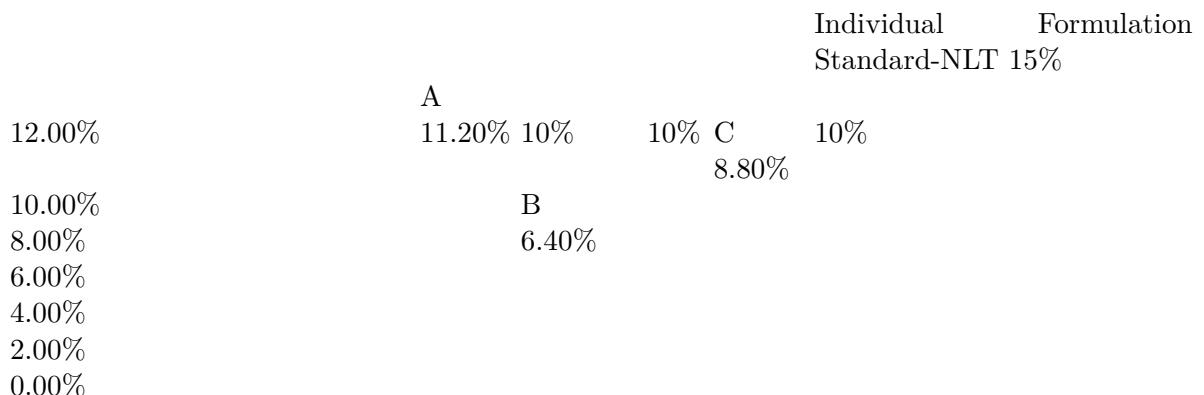


Figure 11: Comparison of Water Soluble Extractives in Ashwagandha Churna (powder)

Individual Formulation Standard-NLT 10%

Figure 12: Comparison of Alcohol Soluble Extractives in Ashwagandha Churna (powder)

235 [Anonymous. U.S. Pharmacopoeia-National] , *Anonymous. U.S. Pharmacopoeia-National*
236 [Formulary] , USP 39 NF 34. *Formulary*
237 [Kokate et al. ()] , C K Kokate , A P Purohit , S B Gokhale , Pharmacognosy . 2009. (44th ed. Pune (IN):Nirali
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