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

By Princy Agarwal, Rajat Vaishnav & Anju Goyal

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Methods: Three different and popular marketed formulations of Ashwagandha Churna (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 Ashwagandha Churna (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.

Keywords: quality evaluation, ashwagandha churna (powder), pharmaceutical, physico-chemical, phytochemical, heavy metal analysis.

GJMR-B Classification: NLMC Code: QV 17

Strictly as per the compliance and regulations of:
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Princy Agarwal α, Rajat Vaishnavα & Anju Goyalα

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Conclusion: Therefore the present investigation reveals that there is a need to standardize the complete manufacturing procedure and to make more stringent quality control parameters to reduce variation among different Ayurvedic preparations.

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1. Introduction

Withania somnifera, 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 well-being. 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 (With anolides, with aferin), saponins containing an additional acyl group (Sitoindoside VII and VIII), and with anolides 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 with anolides, with aferin A and With anolide D. Other constituents include: Anaf erine, Anahydrine, Beta-Sisterol, Chlorogenic acid (in leaf only), Cysteine (in fruit), Cuscohygrine, Iron, Pseudo tropine, Scopoletin, Somniferinine, Somniferiene, with anine and with anolides 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, anti-venom, anti-inflammatory, anti-tumor, immunomodulation, hypolip idemic, antibacterial, cardiovascular protection properties. [3]

II. Need for Evaluation

In the traditional medicine system, plants in raw form, both fresh and dried, are used for their healing effects against a variety of human disorders. The quality control of medicinal herbs and their biological components is critical to justify their acceptability in the modern medical system. The fundamental problem faced by the user industry is the lack of availability of rigid quality control profiles for herbal raw materials and their formulations. With the emergence of revolutionary analytical tools and instrumental technologies, it is possible to suggest a practical quality assurance profile for a raw drug or its bioactive component. [4, 5]

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This paper reports the comparative determination of pharmaceutical, physicochemical and phytochemical parameters like bulk density, tapped density, the angle of repose, ash values, extractive values, loss on drying, etc. of three marketed preparations of Ashwagandha Churna (powder).

III. MATERIALS AND METHODS: [5-13]

a) Procurement of Samples

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

b) Organoleptic Evaluation

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

c) Pharmaceutical Evaluation

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

i. Determination of Bulk Density and Tapped Density

Bulk density is defined as the mass of many particles of the material divided by the total volume they occupy. The total volume includes particle volume, inter-particle void volume, and internal pore volume. Tapped density is the term used to describe the bulk density of powder (or granular solid) after consolidation/compression prescribed regarding “tapping” the container of powder measured number of times, usually from a predetermined height.

The term bulk density refers to a measure used to describe a packing of particles or granules and the term Tapped density refers to the true density of the particles or granules.

The Formula for calculation:

\[
\text{Bulk Density} = \frac{\text{Weight of powder taken}}{\text{Bulk Volume of powder}} = \frac{10}{\pi r^2 h_b}
\]

\[
\text{Tapped Density} = \frac{\text{Weight of powder taken}}{\text{Tapped Volume of powder}} = \frac{10}{\pi r^2 h_t}
\]

Where,

\[
\pi r^2 h = \text{Volume of Graduated Cylinder}
\]

\[
h_b = \text{Bulk height of the powder}
\]

\[
h_t = \text{Tapped height of the powder}
\]

ii. Determination of Carr’s Compressibility Index

Carr index is an indication of the compressibility of a powder. It is another indirect method of measuring the powder flow from bulk and tapped density.

The Formula for calculation:

\[
\text{Carr’s Index} (\%) = \frac{\text{Tapped Density} - \text{Bulk Density}}{\text{Tapped Density}} \times 100
\]

iii. Determination of Hausner’s Ratio

Hausner’s ratio is related to inter-particle friction and as such can be used to predict the powder flow properties.

The Formula for calculation:

\[
\text{Hausner’s Ratio} = \frac{\text{Tapped Density}}{\text{Bulk Density}}
\]

iv. Determination of Angle of Repose

The angle of repose is a parameter used to estimate the flow ability of a powder. It is defined as the maximum angle possible between the surface of the pile of powder and the horizontal plane. Powders with low angles of repose will flow freely, and powders with high angles of repose will flow poorly.

The Formula for calculation:

\[
\tan \theta = \frac{h}{r}
\]

Where,

\[
\theta = \text{Angle of repose}
\]

\[
h = \text{Height of pile}
\]

\[
r = \text{radius of the base of the pile}
\]

Table 1: Relationship of Angle of Repose, Carr’s Index & Hausner’s Ratio with Flow Properties of Powder

<table>
<thead>
<tr>
<th>Angle of Repose</th>
<th>Carr’s Index</th>
<th>Hausner’s Ratio</th>
<th>Flow Properties</th>
</tr>
</thead>
<tbody>
<tr>
<td>25-30</td>
<td>&lt;10</td>
<td>1.00-1.11</td>
<td>Excellent</td>
</tr>
<tr>
<td>31-35</td>
<td>11-15</td>
<td>1.12-1.18</td>
<td>Good</td>
</tr>
<tr>
<td>36-40</td>
<td>16-20</td>
<td>1.19-1.25</td>
<td>Fair</td>
</tr>
<tr>
<td>41-45</td>
<td>21-25</td>
<td>1.26-1.34</td>
<td>Passable</td>
</tr>
<tr>
<td>46-55</td>
<td>26-31</td>
<td>1.35-1.45</td>
<td>Poor</td>
</tr>
<tr>
<td>56-65</td>
<td>32-37</td>
<td>1.46-1.59</td>
<td>Very Poor</td>
</tr>
<tr>
<td>&gt;66</td>
<td>&gt;38</td>
<td>&gt;1.60</td>
<td>Very Very Poor</td>
</tr>
</tbody>
</table>
IV. PHYSICO-CHEMICAL EVALUATION

Physicochemical parameters like Foreign matter, Moisture content (Loss on Drying), pH, Total ash, Acid-Insoluble ash, Water-soluble extractive, Alcohol-soluble extractive values of all three samples were determined as per standard protocols. All the procedures are described as follows:

a) Determination of Foreign Matter

100 g of sample was taken and spread in a thin layer on a suitable platform and was examined in daylight with the unaided eye (or using 6x or 10x magnifying glass), and the foreign matter was separated and weighed. The percentage of foreign matter was calculated with reference to the drug sample.

Standard: The sample should not contain more than 2% of foreign matter unless otherwise specified in the individual monograph.

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

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

The Formula for calculation:

\[ \% \text{LOD} = \frac{W_2 - W_3}{W_3 - W_1} \times 100 \%
\]

Where,

- \( W_1 \) = weight of container (g)
- \( W_2 \) = weight of container + wet sample (g)
- \( W_3 \) = weight of container + dried sample (g)
- \( W_2 - W_3 \) = weight of moisture
- \( W_3 - W_1 \) = weight of dried sample

c) Determination of Loss on Ignition (LOI)

An accurately weighed 5g of polyherbal formulation powder was taken in a previously ignited and tared silica dish/crucible. The material was evenly spread and ignited in a Muffle-Furnace by gradually increasing the temperature to not more than 450°C - 600°C till the carbon-free ash was not obtained. The total ash value was calculated with reference to the air-dried powdered drug material.

The Formula for calculation:

\[ \% \text{LOI} = \frac{W_5 - W_4}{W_5 - W_c} \times 100 \%
\]

Where,

- \( W_c \) = weight of crucible (g)
- \( W_5 \) = weight of the sample (g)
- \( W_4 \) = weight of ash (g)

\( W_5 - W_4 \) is the weight of the ash obtained.

\( W_c \) is the weight of crucible.

\( W_5 - W_c \) is the total weight of ash.

d) Determination of Total ash

An accurately weighed 3 g of the sample was taken in a previously ignited and tared silica dish/crucible. The material was evenly spread and ignited in a Muffle-Furnace by gradually increasing the temperature to not more than 450°C - 600°C till the carbon-free ash was not obtained. The total ash value was calculated with reference to the air-dried powdered drug material.

The Formula for calculation:

\[ \% \text{Total Ash} = \frac{\text{Weight of Ash}}{\text{Weight of the sample taken}} \times 100 \%
\]

e) Determination of Acid Insoluble ash

Ash above obtained, was boiled for 5 min with 25ml of 1M Hydrochloric acid and filtered using an ash less filter paper. Insoluble matter retained on filter paper was washed with hot water, and filter paper was burnt to a constant weight in a Muffle-Furnace. The percentage of acid insoluble ash was calculated with reference to the air-dried powdered drug material.

The Formula for calculation:

\[ \% \text{Acid - Insoluble Ash} = \frac{\text{Weight of acid insoluble residue}}{\text{Weight of the sample taken}} \times 100 \%
\]

f) Determination of Water-Soluble ash

1g of ash obtained in Total ash experiment was boiled for 5 min with 25ml water and insoluble matter collected on an ashless filter paper which was then washed with hot water and ignited for 15 min at a temperature not exceeding 450°C in a Muffle-Furnace. The difference in weight of ash and weight of insoluble matter was determined as difference represents the value. The percentage of water-insoluble ash was calculated with reference to the air-dried powdered drug material.

The Formula for calculation:

\[ \% \text{Water Soluble Ash} = \frac{\text{Weight of watersoluble residue}}{\text{Weight of the sample taken}} \times 100 \%
\]

g) Determination of Extractive Values

i. Determination of Alcohol Soluble Extractives

5 gm of churna (powder) was accurately weighed and placed inside a glass-stoppered conical flask. It was then macerated with100ml of ethanol. The flask was shaken frequently during the first 6 hours and was kept aside without disturbing for 18 hours. It was then filtered, and about 25ml of the filtrate was transferred into a tared flat-bottomed shallow dish and
was evaporated to dryness on a water bath. It was then dried to 105° C for 6 hours, cooled and finally weighed. The percentage of Alcohol Soluble extractives was calculated with reference to the air-dried powdered drug material.

The Formula for calculation:

\[
\% \text{ Alcohol Soluble Extractive} = \frac{\text{Weight of residue} \times 100 \times 100}{25 \times \text{Weight of the sample taken}} \%
\]

ii. Determination of Water-Soluble Extractives

Proceed as directed for determination of Alcohol–Soluble Extractive, using chloroform-water (2.5 ml chloroform in purified water to produce 1000 ml) instead of ethanol.

h) Determination of pH Value

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

V. Phytochemical Evaluation

The aqueous and alcoholic extracts of the respective formulations were prepared and were subjected to preliminary phytochemical screening. These tests reveal the presence of various bioactive secondary metabolites which might be responsible for their medicinal attributes. Methods for preliminary qualitative phytochemical tests of the plant extracts are given below in Table 2.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Phyto-Constituents</th>
<th>Name of Tests</th>
<th>Procedure</th>
<th>Observation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Alkaloids</td>
<td>Mayer's test</td>
<td>2 ml extract + few drops of HCl + Mayer's reagent</td>
<td>Cream Precipitation</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Hager's test</td>
<td>2 ml extract + few drops of HCl + Hager's reagent</td>
<td>Yellow Precipitation</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Wagner's test</td>
<td>2 ml extract + few drops of HCl + Wagner's reagent</td>
<td>Reddish brown color</td>
</tr>
<tr>
<td>2.</td>
<td>Carbohydrates</td>
<td>Molisch test</td>
<td>2 ml extract + 2 Drops of Molisch reagent + few drops of Conc. H₂SO₄</td>
<td>Violet or Reddish color</td>
</tr>
<tr>
<td>3.</td>
<td>Reducing sugars</td>
<td>Fehling’s test</td>
<td>1 ml extract + 1 ml Fehling Solution (A and B)</td>
<td>First a Yellow and then Brick Red Precipitation</td>
</tr>
<tr>
<td>4.</td>
<td>Flavonoids</td>
<td>Alkaline reagent test</td>
<td>2 ml extract + few drops of 40% NaOH solution</td>
<td>Intense yellow color forms which become colorless on the addition of dilute acid</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Lead acetate test</td>
<td>2 ml extract + few drops of the Lead Acetate solution</td>
<td>Yellow precipitation</td>
</tr>
<tr>
<td>5.</td>
<td>Saponins</td>
<td>Foam test</td>
<td>2 ml extract + 4 ml distilled H₂O Mix well and shake vigorously</td>
<td>Foam formation</td>
</tr>
<tr>
<td>6.</td>
<td>Tannins</td>
<td>Braymer’s test</td>
<td>2 ml extract + 2 ml H₂O₂ + 2-3 drops of 5% FeCl₃</td>
<td>Black green or bluish color</td>
</tr>
<tr>
<td>7.</td>
<td>Steroids</td>
<td>Salkowski’s test</td>
<td>2 ml extract + 2 ml Chloroform + 2 ml Conc. H₂SO₄</td>
<td>Chloroform layer appears red, and acid layer shows greenish-yellow fluorescence</td>
</tr>
<tr>
<td>8.</td>
<td>Proteins</td>
<td>Millon’s test</td>
<td>3 ml extract + 5 ml Millon’s reagent</td>
<td>White precipitate which turns brick red on warming</td>
</tr>
<tr>
<td>9.</td>
<td>Glycosides</td>
<td>Keller Killian’s test</td>
<td>2 ml extract + Glacial Acetic Acid + 1 drop of 5% FeCl₃ + Conc. H₂SO₄</td>
<td>Reddish brown color appears at the junction of 2 layers, and upper layer appears bluish green</td>
</tr>
<tr>
<td>10.</td>
<td>Phenols</td>
<td>-</td>
<td>2-3 ml of extract + few drops of 5% FeCl₃ solution</td>
<td>Deep blue-black color</td>
</tr>
</tbody>
</table>

Table 2: Preliminary Phytochemical Tests for Plant Extracts
11. Amino acids Ninhydrin test
3 ml of extract + 3 drops of 5% Ninhydrin solution
Keep in boiling water bath for 10 min.
Purple or bluish color appears

12. Terpenoids Copper Acetate test
2 ml extract dissolved in water + 3-4 drops of Copper Acetate solution
Emerald green color

VI. Determination of Heavy Metals (Lead and Cadmium)

a) Method (Direct Calibration Method)
Three reference solutions of the element being examined having different concentrations were prepared to cover the range recommended by the instrument manufacturer. Separately the corresponding reagents were added to the test solution, and the blank solution was prepared with the corresponding reagents. The absorbance of the blank solution and each reference solution were measured separately, and the readings were recorded. A calibration curve was prepared with the average value of 3 readings of each concentration on the ordinate and the corresponding concentration on the abscissa. A test solution of the substance being examined was prepared as specified in the monograph. The concentration was adjusted such that it falls within the concentration range of the reference solution. The absorbance was measured three times, and the readings were recorded, and the average value was calculated. The mean value was interpolated on the calibration curve to determine the concentration of the element.

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

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

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

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

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

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Pb</th>
<th>Cd</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wavelength (nm)</td>
<td>217</td>
<td>228.8</td>
</tr>
<tr>
<td>Slit width (nm)</td>
<td>1.0</td>
<td>0.5</td>
</tr>
<tr>
<td>Light Source</td>
<td>Hollow Cathode Lamp</td>
<td>Hollow Cathode Lamp</td>
</tr>
<tr>
<td>Flame type</td>
<td>Air/C(_2)H(_2)</td>
<td>Air/C(_2)H(_2)</td>
</tr>
<tr>
<td>Current</td>
<td>10</td>
<td>3.5</td>
</tr>
<tr>
<td>AAS Technique</td>
<td>Flame</td>
<td>Flame</td>
</tr>
</tbody>
</table>

VII. RESULTS

a) Organoleptic Evaluation

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

Table 4: Results for Organoleptic Evaluation of different brands of Ashwagandha Churna (powder)

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

b) Pharmaceutical Evaluation

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

Table 5: Results for Pharmaceutical Evaluation of different brands of Ashwagandha Churna (powder)

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Properties</th>
<th>Brand A</th>
<th>Brand B</th>
<th>Brand C</th>
<th>Standard (IP)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Bulk Density</td>
<td>0.478</td>
<td>0.361</td>
<td>0.584</td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td>Tapped Density</td>
<td>0.641</td>
<td>0.612</td>
<td>0.751</td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td>Hausner’s Ratio</td>
<td>1.34</td>
<td>1.37</td>
<td>1.29</td>
<td></td>
</tr>
<tr>
<td>4.</td>
<td>Carr’s Index</td>
<td>25.43%</td>
<td>27.17%</td>
<td>22.24%</td>
<td></td>
</tr>
<tr>
<td>5.</td>
<td>Angle of Repose</td>
<td>37.715°</td>
<td>32.619°</td>
<td>29.052°</td>
<td></td>
</tr>
</tbody>
</table>

c) Physico-Chemical Evaluation

The observations for the physicochemical evaluation of three brands of Ashwagandha Churna (powder) are reported in Table-6.

Table 6: Results for Physico-chemical Evaluation of different brands of Ashwagandha Churna (powder)

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Properties</th>
<th>Brand A</th>
<th>Brand B</th>
<th>Brand C</th>
<th>Standard (IP)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Foreign Matter</td>
<td>Nil</td>
<td>Nil</td>
<td>0.4%</td>
<td>NMT 2.0%</td>
</tr>
<tr>
<td>2.</td>
<td>pH</td>
<td>5.0</td>
<td>5.6</td>
<td>5.0</td>
<td>-</td>
</tr>
<tr>
<td>3.</td>
<td>Loss on Drying/ Moisture Content</td>
<td>4.17%</td>
<td>5.37%</td>
<td>7.07%</td>
<td>NMT 12.0%</td>
</tr>
<tr>
<td>4.</td>
<td>Water Soluble Extractive</td>
<td>25.6%</td>
<td>10.4%</td>
<td>20.8%</td>
<td>NLT 15.0%</td>
</tr>
<tr>
<td>5.</td>
<td>Alcohol Soluble Extractive</td>
<td>11.2%</td>
<td>6.4%</td>
<td>8.8%</td>
<td>NLT 10.0%</td>
</tr>
<tr>
<td>6.</td>
<td>Loss on Ignition</td>
<td>94.54%</td>
<td>94.79%</td>
<td>95.09%</td>
<td>-</td>
</tr>
<tr>
<td>7.</td>
<td>Total Ash Value</td>
<td>6.06%</td>
<td>5.52%</td>
<td>5.1%</td>
<td>NMT 7.0%</td>
</tr>
<tr>
<td>8.</td>
<td>Acid Insoluble Ash</td>
<td>0.97%</td>
<td>0.84%</td>
<td>0.60%</td>
<td>NMT 1.2%</td>
</tr>
<tr>
<td>9.</td>
<td>Water Soluble Ash</td>
<td>0.85%</td>
<td>0.79%</td>
<td>1.60%</td>
<td>-</td>
</tr>
</tbody>
</table>

d) Phytochemical Evaluation

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

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Phyto-Constituent</th>
<th>Name of Tests</th>
<th>Brand A</th>
<th>Brand B</th>
<th>Brand C</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Alkaloids</td>
<td>Hager’s test</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Wagner’s test</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mayer’s test</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>2.</td>
<td>Glycosides</td>
<td>Keller Killani’s test</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>3.</td>
<td>Carbohydrates</td>
<td>Molisch’s test</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>4.</td>
<td>Proteins</td>
<td>Biuret’s test</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Milon’s test</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5.</td>
<td>Amino Acids</td>
<td>Ninhydrin’s test</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>6.</td>
<td>Steroids</td>
<td>Salkowski’s test</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>7.</td>
<td>Flavonoids</td>
<td>Alkaline Reagent test</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Lead acetate test</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>8.</td>
<td>Terpenoids</td>
<td>Copper Acetate test</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>9.</td>
<td>Tannins</td>
<td>Ferric Chloride test</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>10.</td>
<td>Saponins</td>
<td>Foam test</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>11.</td>
<td>Phenols</td>
<td>Ferric Chloride test</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Table 8: Heavy metal analysis of Ashwagandha Churna (powder)

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Properties</th>
<th>Brand A</th>
<th>Brand B</th>
<th>Brand C</th>
<th>Standard (API)</th>
</tr>
</thead>
<tbody>
<tr>
<td>a.</td>
<td>Lead</td>
<td>4.825</td>
<td>5.786</td>
<td>4.253</td>
<td>10 ppm</td>
</tr>
<tr>
<td>b.</td>
<td>Cadmium</td>
<td>0.363</td>
<td>0.325</td>
<td>0.334</td>
<td>0.3 ppm</td>
</tr>
</tbody>
</table>

e) Determination of Heavy Metals (Lead And Cadmium)

The observations for the Heavy metal determination of three brands of Ashwagandha Churna (powder) are reported in Table-8.
Comparative Quality Evaluation of Three Different Marketed Brands of Ashwagandha Churna (Powder)

Graph 1: Graphs for various Pharmaceutical and Physico-chemical parameters of different brands of Ashwagandha Churna (powder)
VIII. Discussion

Ashwagandha churna (powder) of Brand A was of the powder form of Creamish color with a characteristic odor and bitter taste. This preparation had pH value of 5.0, and Loss on drying value of 4.17% w/w. Preparation has Alcohol-soluble extractives and Water-soluble extractives values of 11.2% w/w and 25.6% w/w respectively. The bulk density and tapped density of the powder were 0.478 and 0.641 respectively. The powder flow was fair-passable as it had the Carr’s Index of 25.43% (Passable), Hausner’s ratio of 1.34 (Passable) and Angle of repose of 37.71° (Fair). It had Total Ash value of 6.06% w/w, and Acid-insoluble ash and Water-soluble ash value of 0.97% w/w and 0.85% w/w respectively. Loss on ignition was found 94.54% w/w. The concentration for heavy metals Lead and Cadmium were found to be 4.825 and 0.227 respectively of which Lead concentrations were within the prescribed limits and of Cadmium was a little more than the standard value. Phytochemical screening revealed the presence of Glycosides, Carbohydrates, Steroids, and Terpenoids in both the extracts; Alkaloids in alcoholic extract only and Saponins in aqueous extract only.

Ashwagandha churna (powder) of Brand B was of the powder form of Yellowish brown color with a characteristic odor and very bitter taste. This preparation had pH value of 5.6, and Loss on drying value of 5.37% w/w. Preparation had Alcohol-soluble extractives and Water-soluble extractives values of 6.4% w/w and 10.4% w/w respectively. The bulk density and tapped density of the powder were 0.381 and 0.612 respectively. The powder flow was poor-good as it had the Carr’s Index of 25.43% (Poor), Hausner’s ratio of 1.37 (Poor) and Angle of repose of 32.61° (Good). It had Total Ash value of 5.52% w/w, and Acid-insoluble ash and Water-soluble ash value of 0.84% w/w and 0.79% w/w respectively. Loss on ignition was found 94.79% w/w. The concentration for heavy metals Lead and Cadmium were found to be 5.786 and 0.363 respectively of which Lead concentrations were within the prescribed limits and of Cadmium was a little more than the standard value. Phytochemical screening revealed the presence of Glycosides, Carbohydrates, Steroids, and Terpenoids in both the extracts; Alkaloids in alcoholic extract only and Saponins in aqueous extract only.

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

IX. Conclusion

Thus, all the parameters of three brands of Ashwagandha Churna (powder) had approximately similar values and were compatible with the standard values mentioned in the Pharmacopoeias except the value of Water-soluble and Alcohol-soluble extractives of Brand B i.e. 10.4% and 6.4% which was lesser than the standard values of 15% and 10% respectively. The pH of the formulation was also higher than the other two with the value of 5.6. 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.

Hence, it can be concluded that the present study on the pharmaceutical, physicochemical and phytochemical characters can serve as a vital source of information and provide suitable reference standards for the quality control of these formulations for future investigations. It is also emphasized to perform quality checks on every batch to optimize the final product according to the Pharmacopoeial standards.

Abbreviations
API – Ayurvedic Pharmacopoeia of India
IP – Indian Pharmacopoeia
Pb - Lead
Cd - Cadmium

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Conflict of Interest: We declare that we have no conflict of interest.
References Références Referencias