

Phytochemical Profiling and Nutritional Characterization of Ashwagandha and Shatavari Root Powders: Implications for Medicinal and Nutraceutical Applications

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Abstract

Ashwagandha (Withania somnifera) and shatavari (Asparagus racemosus) roots are revered gems in the realms of Ayurveda and traditional medicine, where their significance shines brilliantly. These remarkable roots are celebrated for their profound therapeutic value and have been cherished for generations in the pursuit of holistic well-being and health. Based on these phytochemical screenings were conducted on ashwagandha and shatavari root extracts, revealing the presence of various beneficial compounds such as alkaloids, flavonoids, tannins, saponins, carbohydrates, and glycosides. Furthermore, quantitative assessments were performed to measure these phytochemicals in both ashwagandha and shatavari root extract. Furthermore, the study examined the physical properties, color attributes, and proximate composition of ashwagandha and shatavari root powders, highlighting their potential as rich sources of valuable nutraceuticals and phytochemical compounds.

Keywords: Alkaloids, nutraceutical, phytochemical analysis, proximate composition, withanolides, steroidal saponins.

1. Introduction

In Ayurveda, the wealth of knowledge about natural herbal remedies plays a significant role, as these herbs have been an integral part of traditional medicine systems throughout history. Various plant species from diverse categories have been harnessed for medicinal purposes due to their accessibility and cost-effectiveness since ancient times. Ashwagandha, a plant highly esteemed in traditional Ayurvedic medicine in India, is a grayish subshrub that stands erect, featuring inconspicuous yellow or greenish flowers and small, spherical, organic-red berries that contain yellow, kidney-shaped seeds. This plant typically grows to a height of three to five feet, primarily in wastelands, but it is also widely cultivated for its entire plant. While various parts of the ashwagandha plant are used medicinally, the root and leaves are the most commonly utilized (Engels and Brinckmann, 2013). Ashwagandha is renowned as a health food and herbal tonic, and it is employed in ethno medicine for managing cardiovascular diseases. Human doses of ashwagandha generally fall within the range of 4-6 grams per day and are considered safe and non-toxic. Withania, the genus to which ashwagandha belongs, contains bioactive compounds, particularly steroidal alkaloids and lactones known as "Withanolides." Among these, Withaferin A and withanolide D are the two primary Withanolides responsible for many of ashwagandha's biological effects (Matsuda et al., 2001, and Sharma et al., 2011).

Asparagus racehorses, a perennial climbing shrub belonging to the Asparagaceae family and subfamily Liliaceous, is commonly known as Shatavari. This plant is found in tropical and subtropical regions, Africa, Java, Australia, Sri Lanka, southern China, and India and it thrives in the tropical and subtropical regions of India. It is recognized for its elongated, tuberous roots with brown exteriors and sweet-tasting white interiors. Shatavari is highly valued in Ayurvedic tradition as a 'rasayana,' enhancing overall health and vitality. Its medicinal and pharmacological significance is attributed to steroidal saponins and sapogenins content. Shatavari offers a wide range of health benefits, including anti-cancer properties, regulation of hypertension, anti-inflammatory and antibacterial actions, spasm relief, and antioxidant activity Thakur and Sharma, (2015). Additionally, it is used as an aphrodisiac, galactagogue, and nutritive agent, making it a versatile and beneficial herb (Sharma et al., 2012).

A review of the available literature reveals that most research has focused on the medicinal and clinical properties of ashwagandha and shatavari root powder, with limited information available on the physicochemical characteristics of this powder. Plants are deemed medicinal when they exhibit pharmacological properties with potential therapeutic benefits. While some of these properties have been discovered through centuries of trial and experimentation, thorough investigation is essential to develop new drugs that align with modern treatment standards. Therefore, the primary aim of this investigation is to assess the physical, chemical, and other quality attributes of ashwagandha and shatavari root powder.

2. Materials and Methods:

2.1 Collection of Materials:

The required materials for the present investigation were collected from the local markets of the Parbhani.

2.2 Preparation of Powder:

The dried ashwagandha root roots were ground using a disc mill. The resulting powder was then sifted using a rotary sieve shaker equipped with sieves of varying mesh sizes, including 30, 60, and 100.

2.3 Preparation of ethanol and aqueous extracts of ARP (Ashwagandha Root Powder) and SRP (Shatavari root powder):

The powdered Ashwagandha and Shatavari root samples (50 g/250 mL) were subjected to successive extraction using ethanol and water in Soxhlet apparatus. This process was carried out at a temperature range of 55-85°C for duration of 8-10 hours to extract both polar and non-polar compounds, by (Elgorashi and Staden, 2004).

2.4 Proximate Composition:

Ashwagandha and shatavari root powder was analyzed for fat, protein, crude fiber according to AACC (2000), Carbohydrate by difference method and moisture, ash as per methods of AOAC (2005).

2.5 Phytochemical Screening of extracts:

1. Test for Alkaloid:

Wagner's test: Approximately ten mg of the extracts were utilized, and a few drops of Wagner's reagent (prepared by dissolving 2 g of iodine and 6 g of KI in 100 cm³ of water) were introduced. The formation of a reddish-brown precipitate was indicative of the presence of alkaloids.

2. Test for Flavonoid:

Lead acetate test: Ten mg of the extract were used, and a few drops of a 10% lead acetate solution were incorporated. The development of a yellow color precipitate signified the presence of flavonoids.

3. Test for Tannin:

Ferric Chloride testing a 5 ml sample, a few drops of 0.1% ferric chloride solution was introduced. The detection of a brownish-green or blue-black color signified the presence of tannins in the substance.

4. Test for Saponin:

Foam test: 0.5 mg of extract was diluted with 20 ml distilled water and shaken well in a graduated cylinder for 15 min. The formation of foam to a length of 1 cm indicated the presence of saponins.

5. Test for Carbohydrates:

Fehling's test: Five ml of Fehling's solution was added to 0.5 mg of extract and boiled in a water bath. The formation of yellow or red precipitate indicates the presence of reducing power.

6. Test for Glycosides:

Glycoside test: 0.5 mg of extract was dissolved in 1 ml of water and then aqueous NaOH solution was added. Formation of yellow color indicates the presence of glycosides.

7. Test for Phenols:

Ferric chloride test: About 2ml plant extract was taken to water and warmed at 45-50°C. Then 2 ml of 0.3% FeCl₃ was added. Formation of green or blue color indicates the presence of phenols.

2.6 Phyto-chemical analysis of ashwagandha and shatavari root powder extract:

1. Determination of total phenolic content:

According to the Pinelo et al., (2005) total phenolic contents (TPC) from the sample extracts were determined using Folin–Ciocalteu's method. Firstly, 5 ml of Folin Ciocalteu reagent was added in 1 ml extract sample in tube. Then, 4 ml of 7.5 percent sodium carbonate was added to mixture. After 1 hr of incubation at ambient temperature (32±1°C), the absorbance was read at 765

nm against blank. The produced results were taken as as mg gallic acid equivalent per gram of fresh sample (mg GAE/g dw basis). The formula for calculating the total phenolic contents present in samples as follows,

$$C = c V/m$$

Where, C = total phenolic content present (mg GAE/g dry extract

c = gallic acid concentration obtained from calibration curve (mg/ml)

V = Extract volume (ml)

m = Extract mass (g)

2. Determination of total flavonoid content:

The total flavonoid content of sample extracts was quantified by using a procedure reported by Meda et al., (2005). 0.5 ml of completely diluted sample was mixed with 0.5 ml methanol, 50 µL of 10 percent of AlCl₃, 50 µL of 1 mol/l potassium acetate and 1.4 mL water and incubates at ambient temperature for half hour. The absorbance of the sample extract was subsequently measured at 415 nm. The total flavonoid was quantified by use of formula,

$$TFC = \frac{A \times DF}{A^{1\%1cm} \times (w - ld)}$$

Where,

A = Absorbance in spectrophotometer

DF = Factor of dilution

A^{1%1cm} = Absorption by AlCl₃

w = weight of sample

ld = Drying loss

3. Determination of alkaloid content:

Five ml of the sample was weighed into a 250 ml beaker and 200 ml of 10% acetic acid in ethanol was added and allowed to stand for 4 min, this was filtered and extract was concentrated on a water bath to one quarter of the original volume. Concentrated ammonium hydroxide added drop wise to the extract until the precipitation was completed. The whole solution was allowed to settle and the precipitate was collected and washed with dilute ammonium hydroxide and then filtered. The residue was alkaloid which was dried and weighed (Harbone,1973).

$$\text{Alkaloid (\%)} = \frac{W_3 - W_2}{W_1} \times 100$$

Where,

W₁ = Initial weight of sample

W₂ = Weight of the extract

W₃ = Final weight of the residue

4. Determination of Total saponin (Foaming Index):

About 1 g of the sample weighed accurately and transferred to a 500 ml conical flask containing 100 ml of boiling water. Maintain at moderate boiling for 30 min. cooled and filtered into a 100 ml volumetric flask and added sufficient water through the filter to dilute to volume. Poured the decoction into 10 stopper test tubes (height 15 cm, diameter 15 mm) in series of successive portion of 1ml, 2ml, 3ml, up to 10ml and volume in each test tube adjusted with water to 10 ml. Stoppered the tubes and shaken them in a lengthwise motion for 15 seconds, two shakers per

second. After allowed the tubes to stand for 15 minutes and the height of foam was measured by means of a graduated tape with millimetre scale.

2.6 Physical properties of ashwagandha root powder and shatavari root powder:

Physical properties such as bulk density, true density, porosity, carr index, hausner ratio and angle of repose of ashwagandha and shatavari root powder were determined.

1. Bulk density:

The procedure involved placing the sample in a 100 ml measuring cylinder and tapping it 25 to 30 times to ensure even compaction of the grains. Afterward, the volume was recorded, and the weight of the sample within the cylinder was measured to calculate the bulk density.

$$\text{Bulk Density (g/ml)} = \frac{\text{Weight of sample}}{\text{volume of sample}}$$

2. True density:

To determine the true density, about 1 gram of the ground material sample was placed in a measuring cylinder filled with toluene. The increase in the toluene level was measured, and the true density was calculated by averaging the results from two readings.

$$\text{True Density (g/ml)} = \frac{\text{Weight of ground sample}}{\text{Rise in toluene level}}$$

3. Carr Index:

From Carr (1965), Carr Index (Carr I) was calculated as the difference between the tapped and bulk densities divided by the tapped density as shown below,

$$\text{Carr Index (\%)} = \frac{\text{True Density} - \text{Bulk Density}}{\text{True Density}} \times 100$$

4. Angle of repose:

The measurement of the angle of repose for the chosen material was conducted using the heap method, as described in the study by Martin et al., (1991). In this procedure, the powdered material was poured through a glass funnel from a fixed distance onto a flat horizontal surface, allowing it to accumulate into a conical heap of maximum height. The diameter and height of this heap were then measured, and the tangent of the angle was calculated using the provided formula.

$$\text{Angle of Repose } (\theta) = \tan^{-1}[h/r]$$

Where,

θ = Angle of repose,

h- The height of heap.

r- The radius of heap made by powder.

5. Hausner ratio:

Hausner ratio (HR) from Hausner, (1967) was calculated as tapped density divided by bulk density,

$$\text{Hausner ratio (HR)} = \frac{\text{True density}}{\text{Bulk Density}}$$

2.7 Color analysis:

Color represents a visual characteristic, and the analysis of color in the samples was conducted following the procedure outlined by Rajiv et al., (2015). This analysis utilized a Hunter Lab Colorimeter, specifically the Colour Flex EZ model, located in the Department of Horticulture at the College of Horticulture, VNMKV, Parbhani. To ensure accuracy, the instrument underwent calibration using a standard reference tile with a light yellow color (characterized by $L^* = 77.14$, $a^* = 1.52$, $b^* = 21.88$). The colorimeter was set up with 10^0 observers and a $45^0/0^0$ geometry. Readings of L^* (where 0 represents black and 100 indicates white), a^* (positive values denoting red and negative values indicating green), and b^* (positive values representing yellow and negative values suggesting blue) were recorded from a glass cell containing the sample. This cell was positioned above the light source and covered with a white plate. The color index conveyed information about the lightness, redness, and yellowness of the sample. The Hunter Lab colorimeter quantified the color in terms of values for L^* , a^* , and b^* , providing insights into chroma (C) and hue (h) by referencing a standard white tile or board during instrument configuration with the illuminant.

3. Result and discussion:

3.1 Physical properties of ashwagandha and shatavari root powder:

Physical properties	Ashwagandha	Shatavari
Bulk density (g/ml)	1.05 ± 0.11	1.07 ± 0.24
True density (g/ml)	1.13 ± 0.12	1.15 ± 0.11
Carr index (%)	12.53 ± 0.56	17.67 ± 0.62
Angle of repose ($^\circ$)	29.60 ± 1.11	30.39 ± 1.57
Hausner ratio	1.182 ± 0.10	1.147 ± 0.12

*Each value is average of three determinations

True density refers to the density of a solid substance, taking into account only the material itself and not the volume occupied by any open or closed pores within it. The true density of powders can often be different from the density of the bulk material because the process of combination or grinding can alter the crystal structure near the surface of each particle, consequently affecting the density of individual particles within the powder.

The angle of repose serves as an indicator of how easily a substance can flow and predicts the flow behaviour of the formulation during processing. When the angle of repose is less than 30° , it typically signifies a material that flows freely. On the other hand, angles equal to or greater than 40° suggest that the material doesn't flow well and has poor flow properties (Apeji et al., 2013).

The compressibility indices, namely the Carr index and Hausner ratio, are valuable indicators of how powders flow and compress. Carr index (CI) and Hausner ratio (HR) provide insights into the flow properties of powders and are particularly useful in the development of new formulations. They serve as indirect measures of powder flowability (Apeji et al., 2013). When the Carr Index is lower or the Hausner ratio is lower, it indicates better flow properties in the material compared to higher values. A Carr Index of 38 or an HR exceeding 1.60 is considered indicative of very poor flow (Carr, 1965, and Hausner, 1967). Good powder flow is essential as it helps prevent the costly and time-consuming process of unloading powders that have difficulty flowing out of storage containers. It also contributes to achieving optimal formulations and enhancing product quality and consistency.

The data from table represents that the results for bulk density, true density, carr index, angle of repose and hausner ratio were reported for ashwagandha root powder (ARP) as 1.05 ± 0.11 ,

1.13±0.12, 12.53±0.56, 29.60±1.11 and 1.182 ±0.10 respectively. Other material known shatavari root powder (SRP) showed the values of bulk density, true density, carr index, angle of repose and hausner ratio as 1.07 ±0.24, 1.15±0.11, 17.67±0.62, 30.39±1.57 and 1.147±0.12 respectively.

3.2: Hunter color analysis of ashwagandha and shatavari root powder

Color parameter	Ashwagandha Root Powder	Shatavari Root Powder
L*	79.52±0.62	80.86±1.08
a*	2.74±0.08	2.27±0.10
b*	19.84±1.10	21.64±0.87
Hue (h*)	82.41±0.95	83.90±0.94
Chroma (C*)	20.33±0.90	22.28±0.87

*Each value is average of three determinations

Above table has indicated the values of L*, a*, b*, C*, h* for ashwagandha root powder (ARP) and shatavari root powder (SRP) by using Hunter lab colorimeter. The readings for ashwagandha root powder were L* lightness value was 79.52±0.62, a* value was 2.74±0.08 and b* value was 19.84±1.10 while Chroma (C*) and Hue value (h*) were 20.33±0.90 and 82.41±0.95 respectively. This indicated that ashwagandha root powder was light in colour. The L*value for shatavari root powder was 80.86±1.08, a* value was 2.27±0.10, b* value was 21.64±0.87, while Chroma (C*) and Hue (h*) value were 22.28±0.87 and 83.90±0.94 respectively.



Hunter Colour Lab (ColorFlex)

3.3: Proximate composition of ashwagandha and shatavari root powder

Parameter (%)	Ashwagandha	Shatavari
Moisture	7.12±0.10	6.06±1.01
Ash	4.68±0.11	5.06±1.02
Protein	4.56±0.07	7.82±0.83
Fat	1.28±0.09	1.39±0.05
Crude Fiber	33.7±1.2	25.96±1.10
Carbohydrate	48.64±0.95	53.76±0.85

*Each value is average of three determinations

From the table of proximate evaluation, it was revealed that values ashwagandha and shatavari root powder had nutrients viz., moisture content of ashwagandha root powder was 7.12 ± 0.10 per cent whereas moisture content of shatavari root powder was estimated as 6.06 ± 1.01 per cent. The ash content of ashwagandha root powder showed remark levels i.e., 4.68 ± 0.11 per cent and shatavari root powder was estimated as 5.06 ± 1.02 per cent. The protein content of ashwagandha root powder was estimated as 4.56 ± 0.07 per cent and shatavari root powder showed remark levels i.e., 7.82 ± 0.83 per cent. The fat content of ashwagandha and shatavari root powder showed remark levels i.e., 1.28 ± 0.09 per cent and 1.39 ± 0.05 per cent respectively. The crude fibre and carbohydrate content of ashwagandha and shatavari root powder were reported as 33.7 ± 1.2 per cent, 48.64 ± 0.95 per cent and 25.96 ± 1.10 per cent, 53.76 ± 0.85 per cent respectively. From the results it could be noticed that ashwagandha and shatavari root powder is rich source of carbohydrates followed by crude fibre.

3.4 Qualitative analysis of phytochemical from ashwagandha and shatavari root extract

Qualitative analysis of ashwagandha and shatavari root extracts was carried out to determine the presence of phytochemical constituents. This analysis aimed to identify the bioactive components or secondary metabolites present in the root powders of ashwagandha and shatavari.

Phytochemical constituents	Extracts				Name of the Test
	Ashwagandha		Shatavari		
	Aqueous	Ethanolic	Aqueous	Ethanolic	
Alkaloid	+	+	-	-	Dragendroff 's reagent
Flavonoid	—	+	-	+	Lead acetate test
Saponin	+	+	+	+	Foam test
Tannin	-	+	-	+	Ferric chloride test
Total phenol	+	+	+	+	Ferric chloride test
Carbohydrate	+	+	+	+	Fehling’s test
Glycosides	+	+	+	+	Glycoside test

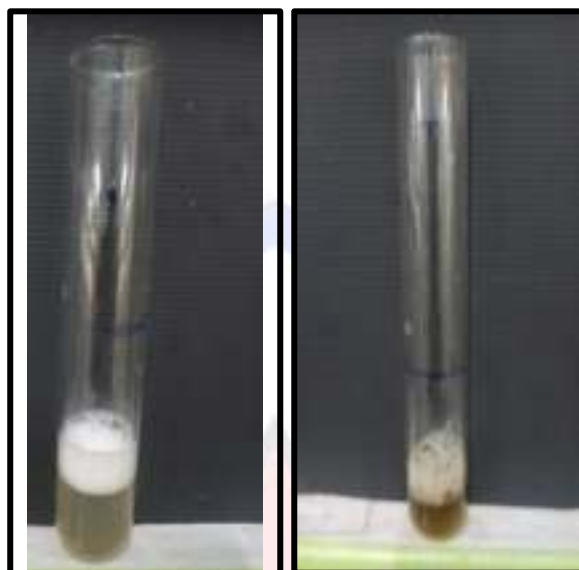
Where, + = Present and - = Absent

Phytochemical testing revealed that various extracts, both ethanollic and aqueous, showed the presence or absence of different phytochemical components. Specifically, the ethanollic extract of ashwagandha root powder was found to contain alkaloids, flavonoids, saponins, and total phenols. In contrast, the aqueous extract tested positive for alkaloids and saponins. These findings align with the research conducted by Veer et al. (2019) and Bargale et al. (2020), who reported that the ethanollic extract of ashwagandha root powder contains alkaloids, flavonoids, carbohydrates, steroids, and saponin glycosides.

In the phytochemical analysis of shatavari root powder, the ethanollic extract was found to contain flavonoids, saponins, and total phenols, while the aqueous extract yielded positive results for saponins and total phenols. These results are consistent with the findings of Selvaraj (2019) and Roy et al., (2014), who identified flavonoids, phytosterols, tannins/phenolic compounds, and glycosides in the ethanollic extract of shatavari root powder.

The presence of these phytochemical constituents in the extracts suggests their potential as nutraceutical components. Each of these phytochemicals is associated with various protective and therapeutic effects. The study demonstrated that the root extracts of ashwagandha and shatavari are

rich sources of different secondary metabolites that contribute to combating various diseases. For instance, alkaloids provide protection against chronic illnesses, while saponins help in managing high cholesterol levels.



Test For Saponins Aqueous Extract (Left: Shatavari, right: Ashwagandha)

3.5 Quantitative estimation of phytochemical component of ashwagandha and shatavari extract:

Phytochemicals can exhibit their health-protective effects in a variety of ways. Research on quantifying ashwagandha and shatavari extracts revealed the existence of alkaloids, flavonoids, saponins, and phenolics. Phytonutrients, which are secondary metabolites of plant materials, are known for their medicinal properties (Viswesari et al., 2013)

Parameters	Ashwagandha extract	Shatavari extract
Total Phenolic content (GAE mg/100g)	9299±2	10522±1
Flavanoid content (QE mg/100g)	8746±1	9412±2
Total alkaloid content mg/100g	786±1	-
Saponins (Foaming Index)	>100	>100

*Each value is average of three determinations

For a long time, medicinal products derived from various parts of plants, including the bark, leaves, fruits, and seeds, have been utilized in phytomedicine due to their specific physiological effects on the human body. Among the crucial natural bioactive components found in plants, alkaloids, tannins, flavonoids, and phenolic compounds are of particular importance. Plant alkaloids, whether derived from plants or produced synthetically, are fundamental medicinal agents known for their pain-relieving, muscle-relaxing, and antibacterial properties (Okwu, 2004).

Saponins, a type of glycoside, possess hypocholesterolemic effects, which can alleviate the metabolic burden on the liver. They also assist the human body in combating fungal, microbial, and viral threats, and they even target specific types of tumor cells, particularly in cases of lung and blood cancer (Olivebever, 1986). Flavonoids are recognized for their antioxidant properties, which help reduce cellular stress. Plant phenolic compounds, including flavonoids, are known for their potent antioxidant capabilities and their potential to prevent genetic mutations and inhibit the development of cancer (Middleton and Kandaswami, 1994).

4. Conclusion

It can be concluded from the study that the ashwagandha and shatavari root powders revealed rich phytochemical compositions in both extracts, including alkaloids, flavonoids, saponins, and phenolics. Root powders of ashwagandha and shatavari are abundant sources of diverse secondary metabolites. These bioactive constituents are associated with various health benefits. Ashwagandha and shatavari are potential sources of nutraceutical compounds with significant therapeutic potential. The quantification of phytochemical components underscores their importance in traditional medicine and their potential applications in developing health-enhancing products. Further investigation highlights that ashwagandha and shatavari root powders exhibit favourable chemical properties that can be advantageous in food processing. This study's outcomes have the potential to be utilized in formulating food products enriched with these bioactive ingredients to combat various diseases.

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