



Chemical and Phytochemical Composition of Ashwagandha (*Withania somnifera* L.) Roots

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The present study was undertaken for estimation of chemical and phytochemical composition of ashwagandha (*Withania somnifera* L.) roots of promising genotypes viz. HWS-08-14, HWS-08-18, HWS-1228, HWS-1229 and selection-2B & varieties JA-20 and RVA-100. Chemical parameters viz. crude fibre, minerals (Fe, Cu, Zn, Mn), starch, total sugars, reducing sugars, non-reducing sugars were analyzed in ashwagandha roots. Crude fibre content ranged from 17.4 to 37.3 %, Fe content ranged from 595.83 to 983.33 ppm, Cu content ranged from 8.40 to 13.72 ppm, Zn content ranged from 16.33 to 41.00 ppm and Mn content ranged from 16.80 to 33.46 ppm. Starch, total sugars, reducing sugars and non-reducing sugars contents ranged from 7.61 to 8.22 mg/g, 5.33 to 6.89 mg/g, 0.40 to 0.64 mg/g and 4.87 to 6.33 mg/g, respectively. Phytochemical parameters viz. total alkaloids and tannins were also analyzed. Total alkaloids and tannins content ranged from 0.26 to 0.31 % and 0.66 to 0.84 mg/g, respectively.

Keywords: Crude fibre, Minerals, Starch, Total sugars, Reducing sugars, Total alkaloids, Tannins.

INTRODUCTION

Since time immemorial, medicinal plants are of great importance to health of individuals and communities. The medicinal plant products, which are derived from plant parts such as stem, bark, leaves, fruits and seeds have been part of phytomedicine that produce a definite physiological action on human body. The most important of these natural bioactive constituents of plants are alkaloids, tannins, flavonoids and phenolic compounds [1].

Among various medicinal plants, *Withania somnifera* is a popular Indian medicinal plant belonging to family Solanaceae and is also known as Ashwagandha, Indian ginseng and Winter cherry. It is one of the most valuable plants in the traditional Indian systems of medicine [2]. The roots are main part of the plant that are widely used as therapeutic agents [3]. The roots are reported to contain alkaloids, amino acids, steroids, volatile oil, starch, reducing sugars, glycosides [4]. Ashwagandha roots contain crude fibre 21.0 to 25.0 %, starch 6.09 to 9.46 mg/g, tannins 0.39 to 0.82 mg/g, minerals K, Mn, Na, Fe, Zn, Cu, Al, Ca, Cd & Ni, total sugars 2.52 to 9.52 mg/g, reducing sugars 0.15 to 2.10 mg/g and non-reducing sugars 2.37 to 7.62 mg/g [5].

Survey of literature reveals that various field studies are being carried out on promising genotypes of ashwagandha but very scarce information is available on chemical and phyto-

chemical composition of ashwagandha roots. Therefore, the objective of present study was to estimate chemical parameters viz. crude fibre, minerals (Fe, Cu, Zn, Mn), starch, total sugars, reducing sugars, non-reducing sugars and phytochemical parameters viz. total alkaloids, tannins in the promising genotypes of ashwagandha roots.

EXPERIMENTAL

Ashwagandha (*Withania somnifera* L.) roots samples of promising genotypes (HWS-08-14, HWS-08-18, HWS-1228, HWS-1229 and Selection-2B) and two varieties (JA-20 and RVA-100) were procured from the experimental area of Medicinal, Aromatic and Potential Crops Section, Department of Genetics and Plant Breeding, Chaudhary Charan Singh Haryana Agricultural University, Hisar. Roots were shade dried. After drying, roots were cut into small pieces of 2-3 inches and were ground. Chemical parameters viz. crude fibre, minerals, starch, total sugars, reducing sugars, non-reducing sugars and phytochemical parameters viz. total alkaloids and tannins were estimated from ashwagandha roots powder.

The commercially available chemicals from Merck, SRL (SISCO Research Laboratories), Qualigens and Sigma-Aldrich, were used for various experimental procedures.

Estimation of moisture content: Moisture content was estimated by the standard procedure of AOAC [6].

Estimation of crude fibre: Crude fibre was estimated by the modified method of Maynard [7]. Two gram of moisture and fat free powdered sample of ashwagandha roots was weighed and transferred to the spoutless 1 L beaker and added 200 mL of 1.25 % (w/v) sulphuric acid. The beaker was then placed on hot plate and allowed to reflux for 30 min timed from onset of boiling and the contents were shaken after every 5 min. After boiling for 30 min beaker was removed from hot plate and filtered through a muslin cloth using suction. The residue was washed with hot water till it became free from acid, then the material was transferred to the same beaker and added 200 mL of 1.25 % NaOH solution and the contents were again refluxed for 30 min. It was filtered again through muslin cloth with the help of vacuum or suction pump and the residue was washed with hot water till it became free from alkali. The residue was then transferred to a crucible and placed in hot air oven, allowed to dry to constant weight at 80-110 °C and recorded its weight. The residue was ignited in muffle furnace at 550-660 °C for 2-3 h, then cooled and weighed again. The loss of weight due to ignition is weight of crude fibre.

$$\text{Crude fibre content (\%)} = \frac{\text{Weight of crude fibre}}{\text{Original weight of sample}} \times 100$$

Estimation of starch: Starch content was estimated by the method of McCready *et al.* [8]. 0.2 g of finally ground sample of ashwagandha roots was taken in a 50 mL centrifuge tube for extraction of sugar and starch and added 20 mL of hot 80 % alcohol. The tubes were then shaken for 5-10 min, centrifuged at 3000 rpm for 10 min. and supernatant was decanted. The supernatant was free of sugars as judged by negative test with anthrone reagent. The residue was cooled in ice water and added 6.5 mL of 52 % perchloric acid while stirring the contents with a glass rod. It was allowed to stand for 15 min. with occasional stirring, centrifuged at 4 °C and supernatant fractions were collected final volume was made up to 100 mL with water. The above extract was diluted so that it contains 5-20 µg of glucose per mL. Then 5 mL aliquot of this diluted extract was taken in tubes and the tubes were placed in cool water bath and added 10 mL freshly prepared anthrone reagent, mixed properly and the tubes were transferred to boiling water bath for 7.5 min. After cooling the tubes under running tap water the absorbance of these solutions was noted at 630 nm. A standard curve was prepared using 0 to 100 µg glucose as per the procedure described above and amount of glucose was calculated in the sample aliquot. Results were expressed as mg/g on dry weight basis.

Estimation of minerals: Minerals content were estimated by the method of Jackson [9] and Ruig [10]. 1 g powdered sample of ashwagandha roots was digested with 15 mL of diacid mixture (4HNO₃:HClO₄) in a conical flask by heating on hot plate in open space till clear white precipitates settled down at bottom of conical flask. The precipitates were dissolved in 1 % HCl prepared in double glass distilled water, filtered and final volume of filtrate was made up to 50 mL with double glass distilled water. Results were expressed as ppm on dry weight basis.

Estimation of total sugars: Total sugars were estimated by the modified method of Dubois *et al.* [11]. 1 mL of aqueous

extract was diluted with distilled water to adjust the absorbance within calibration limits. Then, 2 mL of phenol solution (2 %, w/v) was added followed by 5 mL concentrated sulphuric acid. Acid was added in such a way that it directly pours on the solution. The test tubes were allowed to cool for 30 min and absorbance of the solution was measured at 490 nm using UV-visible double beam spectrophotometer Model 2203 (Systronics Co.) against a blank prepared similarly but containing respective solvent instead of extracts. The amount of total sugars present in the extracts were calculated from the standard curve and the results are expressed as milligrams per gram on dry weight basis.

Estimation of reducing sugars: Reducing sugars were estimated by the method of Nelson [12] as modified by Somogyi [13]. 1 mL of aqueous extract was diluted with distilled water to adjust the absorbance within calibration limits. Then, 1 mL distilled water was added, followed by addition of 1 mL alkaline copper reagent, solution was mixed, covered with aluminum foil and heated in boiling water bath for 20 min. The tubes were cooled to room temperature and 1 mL of arsenomolybdate reagent was added. The contents were mixed thoroughly and volume was made up to 10 mL with distilled water. The absorbance of the solution was measured at 520 nm using UV-visible double beam spectrophotometer Model 2203 (Systronics Co.) against a blank prepared similarly but containing respective solvents instead of extracts. The amount of reducing sugars present in the extracts were calculated from the standard curve and the results are expressed as milligrams per gram on dry weight basis.

Estimation of non-reducing sugars: The content of non-reducing sugars was calculated from the difference between the content of total sugars and that of reducing sugars.

$$\text{Non-reducing sugars} = \text{Total sugars} - \text{Reducing sugars}$$

Estimation of total alkaloids: The total alkaloids content was estimated by the method of Mishra [14]. 1 g of ashwagandha roots powder was weighed and put in stoppered test tube, then added 10 mL chloroform and three drops of ammonia, mixed well and kept overnight. Next day after shaking, the contents were filtered through cotton wool in a small beaker and the residue was washed with chloroform thrice (10 mL each), which ensured complete removal of alkaloids. The extract was dried on water bath and added 10 mL ethyl alcohol and mixed the contents with clean glass rod. Then the liquid portion was evaporated which confirms removal of ammonia and added 10 mL of standard acid solution (0.01 N H₂SO₄) in the beaker and warmed slightly to dissolve the alkaloids in acid solution. It was cooled and the unused acid was titrated with standard NaOH (0.01 N) and note down the volume of alkali used in titration. A blank was run to find out the exact volume of acid neutralized by the alkaloids.

Total alkaloid (%) = 0.415 × volume of acid consumed by alkaloids.

Estimation of tannins: Tannin contents was estimated as catechin equivalent by vanillin-hydrochloric acid method [15]. 200 mg dried plant material was taken in a 50 mL test tube and 25 mL of methanol was added to it. The tubes were closed with pith corks. The contents of the tubes were shaken occasio-

nally and allowed to stand overnight at 25 to 32 °C. 1 mL of clear supernatant was then pipetted in a test tube and 5 mL of vanillin-HCl reagent was added to it. The absorbance of brownish red colour so produced was measured at 525 nm after 25 min on a spectronic 20 colorimeter. A blank containing methanol was also run simultaneously. A standard curve of catechin was prepared simultaneously in order to calculate amount of tannin. Results were expressed as mg/g on dry weight basis.

RESULTS AND DISCUSSION

Moisture content: Moisture content in ashwagandha roots of various genotypes/varieties on fresh weight basis (f.w.b.) ranged from 6.25 to 7.42 % (Table-1) and it was maximum in genotype HWS-1228 (7.42 %) followed by HWS-08-18 (7.18 %), Selection-2B (6.83 %), HWS-08-14 (6.34 %) and HWS-1229 (6.25 %) in comparison to control varieties RVA-100 (7.27 %) and JA-20 (7.10 %). Our finding is in agreement with previous investigation which reported 5.54 % moisture content in ashwagandha root powder [16].

TABLE-1
MOISTURE CONTENTS (%), CRUDE FIBRE (%) AND STARCH (mg/g) IN ASHWAGANDHA ROOTS OF VARIOUS GENOTYPES/VARIETIES

Genotypes/varieties	Moisture content (%) on f.w.b.	Crude fibre (%) on d.w.b.	Starch (mg/g) on d.w.b.
HWS-08-14	6.34 ± 0.19	37.3 ± 0.53	8.00 ± 0.06
HWS-08-18	7.18 ± 0.23	21.5 ± 0.17	8.17 ± 0.03
HWS-1228	7.42 ± 0.26	18.7 ± 0.15	7.61 ± 0.07
HWS-1229	6.25 ± 0.06	23.6 ± 0.09	8.07 ± 0.04
Selection-2B	6.83 ± 0.13	23.0 ± 0.21	7.67 ± 0.12
JA-20 (C)	7.10 ± 0.03	17.4 ± 0.29	7.78 ± 0.09
RVA-100 (C)	7.27 ± 0.09	21.7 ± 0.38	8.22 ± 0.07

Crude fibre: Crude fibre contents in ashwagandha roots of various genotypes/varieties on dry weight basis (d.w.b.) ranged from 17.4 to 37.3 % (Table-1) and it was maximum in genotype HWS-08-14 (37.3 %) followed by HWS-1229 (23.6 %), Selection-2B (23.0 %), HWS-08-18 (21.5 %) and HWS-1228 (18.7 %) in comparison to control varieties RVA-100 (21.7 %) and JA-20 (17.4 %). Other research workers have also reported similar findings. Crude fibre contents ranged from 22 to 34 % at 150 day after planting and from 32.0 to 38.7 % at 210 day after planting in dry roots of five divergent accessions of *Withania somnifera* collected from various locations in India [5].

Starch: Starch content in ashwagandha roots of various genotypes/varieties on dry weight basis (d.w.b.) ranged from 7.61 to 8.22 mg/g (Table-1) and it was maximum in genotype HWS-08-18 (8.17 mg/g) followed by HWS-08-14 (8.00 mg/g), HWS-1229 (8.07 mg/g), Selection-2B (7.67 mg/g) and HWS-1228 (7.61 mg/g) in comparison to control varieties RVA-100 (8.22 mg/g) and JA-20 (7.78 mg/g). Our finding is in agreement with previous investigation which reported that starch content varied between 6.09 to 9.46 mg/g in roots of *Withania somnifera* (L.) Dunal [5].

Minerals (Fe, Cu, Zn, Mn) content: Fe, Cu, Zn and Mn content in ashwagandha roots of various genotypes/varieties

on dry weight basis (d.w.b.) is shown in Table-2. Iron contents ranged from 529.16 to 983.33 ppm and it was maximum in genotype HWS-08-18 (983.33 ppm) followed by HWS-1228 (958.33 ppm), HWS-1229 (627.08 ppm), Selection-2B (604.16 ppm) and HWS-08-14 (529.16 ppm) in comparison to control varieties RVA-100 (795.83 ppm) and JA-20 (595.83 ppm). Cu content ranged from 8.40 to 13.72 ppm and it was maximum in genotype HWS-08-18 (13.72 ppm) followed by HWS-08-14 (12.47 ppm), HWS-1229 (10.88 ppm), HWS-1228 (9.60 ppm) and Selection-2B (9.35 ppm) in comparison to control varieties RVA-100 (8.77 ppm) and JA-20 (8.40 ppm). Zn content ranged from 16.33 to 41.00 ppm and it was maximum in genotype HWS-08-18 (41.00 ppm) followed by HWS-08-14 (27.33 ppm), HWS-1228 (27.17 ppm), Selection-2B (23.50 ppm) and HWS-1229 (22.67 ppm) in comparison to control varieties RVA-100 (25.33 ppm) and JA-20 (16.33 ppm). Mn content ranged from 12.20 to 33.46 ppm and it was maximum in genotype HWS-08-18 (33.46 ppm) followed by HWS-1228 (25.45 ppm), HWS-1229 (18.98 ppm), Selection-2B (17.52 ppm) and HWS-08-14 (12.20 ppm) in comparison to control varieties RVA-100 (22.80 ppm) and JA-20 (16.80 ppm). Results of other research workers showed 35.91 ppm Zn and 12.96 ppm Cu content in dried roots of ashwagandha [17]. Fe, Mn and Cu content ranged from 280 to 945 ppm, from 15 to 49 ppm and from 14 to 21 ppm, respectively in roots of *Withania somnifera* of Sondikola regions and from 349.5 to 602.0 ppm, 19 to 59 ppm and 18 to 42 ppm, respectively in roots of *Withania somnifera* of Karthikere regions [18].

TABLE-2
MINERALS (Fe, Cu, Zn, Mn) CONTENT (ppm) IN ASHWAGANDHA ROOTS OF VARIOUS GENOTYPES/VARIETIES ON DRY WEIGHT BASIS

Genotypes/varieties	Minerals content (ppm) on dry weight basis			
	Fe	Cu	Zn	Mn
HWS-08-14	529.16 ± 15.02	12.47 ± 0.69	27.33 ± 0.93	12.20 ± 0.61
HWS-08-18	983.33 ± 45.12	13.72 ± 0.68	41.00 ± 0.50	33.46 ± 0.95
HWS-1228	958.33 ± 37.90	9.60 ± 0.19	27.17 ± 0.83	25.45 ± 0.28
HWS-1229	627.08 ± 27.56	10.88 ± 1.20	22.67 ± 1.01	18.98 ± 0.55
Selection-2B	604.16 ± 11.02	9.35 ± 0.38	23.50 ± 0.50	17.52 ± 0.52
JA-20 (C)	595.83 ± 19.87	8.40 ± 0.38	16.33 ± 0.60	16.80 ± 1.01
RVA-100 (C)	795.83 ± 13.66	8.77 ± 0.16	25.33 ± 1.01	22.80 ± 0.28

Total sugars: Total sugars content in ashwagandha roots of various genotypes/varieties on dry weight basis (d.w.b.) ranged from 5.33 to 6.89 mg/g (Table-3) and it was maximum in genotype HWS-08-18 (6.89 mg/g) followed by HWS-08-14 (6.82 mg/g), HWS-1228 (6.15 mg/g), HWS-1229 (5.51 mg/g) and Selection-2B (5.33 mg/g) in comparison to control varieties RVA-100 (5.91 mg/g) and JA-20 (5.83 mg/g). Our finding is in agreement with previous investigation which reported that total sugars content varied between 2.52 to 9.72 mg/g in roots of *Withania somnifera* (L.) [5].

TABLE-3

TOTAL SUGARS (mg/g), REDUCING SUGARS (mg/g) AND NON-REDUCING SUGARS (mg/g) IN ASHWAGANDHA ROOTS OF VARIOUS GENOTYPES/VARIETIES ON DRY WEIGHT BASIS

Genotypes/ varieties	Total sugars (mg/g)	Reducing sugars (mg/g)	Non-reducing sugars (mg/g)
HWS-08-14	6.82 ± 0.05	0.52 ± 0.04	6.30 ± 0.01
HWS-08-18	6.89 ± 0.04	0.56 ± 0.04	6.33 ± 0.01
HWS-1228	6.15 ± 0.04	0.64 ± 0.03	5.51 ± 0.02
HWS-1229	5.51 ± 0.06	0.54 ± 0.03	4.97 ± 0.05
Selection-2B	5.33 ± 0.03	0.46 ± 0.02	4.87 ± 0.01
JA-20 (C)	5.83 ± 0.06	0.46 ± 0.01	5.37 ± 0.05
RVA-100 (C)	5.91 ± 0.04	0.40 ± 0.01	5.51 ± 0.03

Reducing sugars: Reducing sugars content in ashwagandha roots of various genotypes/varieties on dry weight basis (d.w.b.) is shown in Table-3. Reducing sugars content ranged from 0.40 to 0.64 mg/g and it was maximum in genotype HWS-1228 (0.64 mg/g) followed by HWS-08-18 (0.56 mg/g), HWS-1229 (0.54 mg/g), HWS-08-14 (0.52 mg/g) and Selection-2B (0.46 mg/g) in comparison to control varieties JA-20 (0.46 mg/g) and RVA-100 (0.40 mg/g). Other research workers have also reported similar findings. Reducing sugars content have been reported to varied between 0.21 to 2.10 mg/g in roots of *Withania somnifera* (L.) Dunal [5].

Non-reducing sugars: Non-reducing sugars content in ashwagandha roots of various genotypes/varieties on dry weight basis (d.w.b.) is shown in Table-3. It was observed that non-reducing sugars content ranged from 4.87 to 6.33 mg/g and it was maximum in genotype HWS-08-18 (6.33 mg/g) followed by HWS-08-14 (6.30 mg/g), HWS-1228 (5.51 mg/g), HWS-1229 (4.97 mg/g) and Selection-2B (4.87 mg/g) in comparison to control varieties RVA-100 (5.51 mg/g) and JA-20 (5.37 mg/g). Results of other research workers showed that non-reducing sugars content varied between 2.33 to 7.62 mg/g in roots of *Withania somnifera* (L.) Dunal [5].

Total alkaloids: Total alkaloids content in ashwagandha roots of various genotypes/varieties on dry weight basis (d.w.b.) is shown in Table-4. Total alkaloids content ranged from 0.26 to 0.31 % and it was maximum in genotype HWS-08-18 (0.31 %) followed by HWS-08-14 (0.30 %), HWS-1229 (0.29 %), HWS-1228 (0.28 %) and Selection-2B (0.26 %) in comparison to control varieties RVA-100 (0.26 %) and JA-20 (0.26 %). Other research workers have also reported similar findings. Total alkaloids content in *Withania somnifera* (L.) roots have been reported to varied between 0.13 to 0.31 % [19].

Tannins: Tannins content in ashwagandha roots of various genotypes/varieties on dry weight basis (d.w.b.) is also shown

TABLE-4

TOTAL ALKALOIDS (%) AND TANNINS (mg/g) IN ASHWAGANDHA ROOTS OF VARIOUS GENOTYPES/VARIETIES ON DRY WEIGHT BASIS

Genotypes/varieties	Total alkaloids (%)	Tannins (mg/g)
HWS-08-14	0.30 ± 0.01	0.73 ± 0.04
HWS-08-18	0.31 ± 0.00	0.77 ± 0.06
HWS-1228	0.28 ± 0.01	0.84 ± 0.04
HWS-1229	0.29 ± 0.00	0.66 ± 0.06
Selection-2B	0.26 ± 0.01	0.73 ± 0.04
JA-20 (C)	0.26 ± 0.01	0.81 ± 0.04
RVA-100 (C)	0.26 ± 0.01	0.66 ± 0.06

in Table-4. It was observed that tannins content ranged from 0.66 to 0.84 mg/g and it was maximum in genotype HWS-1228 (0.84 mg/g) followed by HWS-08-18 (0.77 mg/g), HWS-08-14 (0.73 mg/g), Selection-2B (0.73 mg/g) and HWS-1229 (0.66 mg/g) in comparison to control varieties JA-20 (0.81 mg/g) and RVA-100 (0.66 mg/g). Our finding is in agreement with previous investigation which reported 0.82 mg/g tannins content in roots of Poshita variety of *Withania somnifera* (L.) under *in vivo* conditions and 0.39 mg/g under *in vitro* conditions, respectively [20].

Conclusion

The present study showed that among various genotypes of Ashwagandha roots, genotypes HWS-08-18 and HWS-1228 were found better in terms of crude fibre, minerals (Fe, Cu, Zn, Mn), starch, total sugars, reducing sugars, non-reducing sugars, total alkaloids and tannins.

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