ORIGINAL ARTICLE

A comparative pharmacognostical, physicochemical, and heavy metal analysis on *Ashwagandha* root obtained from natural and polluted sources

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Background: *Ashwagandha* root has been ascribed as potent aphrodisiac drug in various Ayurvedic literature and lexicons. The same drug has also been cited in a number of Ayurvedic formularies. Recent studies indicate that heavy metals like As, Cd, Hg, Pb, etc., are toxic to human and environment. Plants are suggested as potential bio sorbents for trace metals removal from the soil. In this work, four samples of *Ashwagandha* have been collected (2 from pollution free areas and 2 from polluted areas) for their pharmacognostic, physicochemical, and heavy metal analysis. **Aims:** Awareness is a valuable tool to wipe out the ignorance and mischief. The present work has been selected with the main objective to create the awareness among people regarding the possible side effect of raw drug obtained from polluted areas. **Settings and Design:** Present work was designed to have an experimental study with three main components which were pharmacognostical study, physicochemical study, and heavy metal analysis. Both of these studies also contained several headings and subheadings, respectively. **Materials and Methods:** Research work was carried out as per standard operating procedures and specified protocols. Pharmacognostical and physicochemical studies were carried out in the respective laboratory of I.P.G.T. and R.A.; Gujarat Ayurved University, whereas Heavy metal analysis was carried out at VASU Pharmaceutical Lab Vadodara, 390010. **Statistical Analysis Used:** All the statistical data used in this research work under the experimental study were given in tabular form with respective table number. **Results and Conclusion:** Heavy metal analysis revealed that Hg content was below the detection limit in sample A and B, while in case of sample C and D, the Hg content were above the permissible limit. With all the data generated in this work, it can be concluded that consumption of drug (*Ashwagandha*) obtained from polluted areas may cause accumulated side effect as well as the toxic effect of the heavy metals, respectively.

Key words: Aphrodisiac, heavy metals, pharmacognosy, root, *Withania*

INTRODUCTION

The use of herbal medicines has been on the rise in recent years due to their low prices and also because of a common perception among people that herbal medicines have little side effects and that "being natural in origin, herbs are safe." These medicines are popular because of long-term effectiveness against many chronic disorders.[1]

The administration of medicinal plant, traditionally has been largely indiscriminative without due regard to possible side effects. Diet has long been considered as the major source of human exposure to trace elements and consequently the levels in basic foodstuff, but medicinal uptakes are of greater interest from toxicological and nutritional points of view.[2]

Heavy metals like As, Cd, Hg, Pb, etc., are very toxic to humans, and the environment and plants are suggested as potential bio sorbents for trace metals removal from the soil. In plants, several groups (i.e., hydroxyl, carboxyl, carbonyl, sulfhydryl, thioether, sulfonate, amine, amide, imidazole, phosphonate, phosphodiester groups, etc.) are suggested to form the complexation of metal ions.[3]

The World Health Organization (1998) recommends that medicinal plants, which form the raw materials for the finished products, may be checked for the presence of heavy metals, pesticides, bacterial or fungal contamination.

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MATERIALS AND METHODS

Root Samples were used for the pharmacognostical evaluation, physicochemical, and heavy metal analysis.

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Macroscopic Evaluation

The samples were cleaned, and macroscopic evaluation of whole plant was carried out. The leaf, stem, and root were then separated and individual macroscopic characters like size, shape, texture were noted in detail.[4,5]

Microscopic Evaluation

Free hand transverse sections of roots were taken and cleared with chloral hydrate solution. Sections were first observed in distilled water then stained with phloroglucinol and conc. HCl. Microphotographs were taken by Carl-Zeiss-trinocular microscope.[6]

Organoleptic Evaluation

Organoleptic characters such as color, odor, taste, snap of fracture, and feel of the drug to touch were performed as per standard procedure.[7]

Powder Microscopy

For microscopic evaluation of the powder, small quantity of each powder was studied under the microscope, first with distilled water to observe crystal system and then stained with iodine solution for starch grains. Another set of slides were stained with phloroglucinol and concentrated HCl for lignified tissues, Microphotographs were taken by Carl-Zeiss-trinocular microscope.[8]

Histochemical Evaluation

To detect the location site of various chemical constituents of the drug, sections of leaf, stem, and root were treated with various reagents like ruthenium red (for mucilage), $FeCl_3$ solution (for tannin), iodine for (starch grains) etc.[9]

Physico-chemical Parameters of the Study Drugs

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 four samples were determined as per standard protocols.[10]

Preliminary Phytochemical Screening

Qualitative tests were performed using appropriate extract. Tests for alkaloids, test for steroid, test for phenolic compound (tannins), tests for carbohydrate, test for reducing sugar, test for saponin glycoside were performed according to standard procedure.[11]

Heavy Metal Analysis

Heavy metal analysis was performed using Atomic Absorption Spectrophotometer (Shimadzu AA-6300). The standards of Lead (Pb), Cadmium (Cd), Arsenic (As), and Mercury (Hg) were prepared and the calibration curve was developed for each of them. Samples were analyzed using these standard curves.[12]

Experimental Studies

Collection and Authentication of Raw Drug

At the beginning of this work, raw samples (2 + 2) of *Withania somenifera* Dunal were collected from the natural and polluted area, respectively. Sample A was collected from Botanical Garden of Jamnagar and sample B was collected from Rakakhatia forest area. In both of the cases, samples were collected from their natural habitats which were free from any drastic pollution. Samples C and D were collected from relatively elevated polluted areas, that is, one is from Bediport road side (sample C) and later one was collected from Sunday market bridge area [Figure 1a-d].

After the collection of all four samples, the drugs were subjected to go through identification and authentication procedure under the supervision of pharmacognosist at the Pharmacognosy laboratory of I.P.G.T. and R.A. Fresh samples were used for various pharmacognostical evaluations. Samples were preserved in A: A: F (alcohol, acetic acid, formaldehyde) solution in a ratio of 90:5:5. Roots were separated, dried in shed and powdered. Powder was collected through 80 mesh sieves and preserved in air-tight bottles along with their respective voucher numbers (originated after proper authentication) for further powder microscopy, physicochemical and heavy metal analysis. Authenticated samples with their respective voucher number are given in Table 1

Comparative Macroscopic Study of the Four Plants

Results of the comparative study regarding different parts of all four plants with their macroscopic characters have been given in Table 2, [Figure 2a].

Comparative Microscopic Study

Results of the comparative study regarding root of the four plants with their transverse microscopic sections have been given in Table 3, [Figures 3-6].

Organoleptic Characters of the Powder

Results of the comparative study regarding powder of the four plants with their organoleptic evaluation have been given in Table 4; [Figures 7a, 8a, 9a, and 10a].

Comparative Powder Microscopy of the Four Plants

Results of the comparative study regarding powder of the four plants with their microscopic characters have been given in Table 5; [Figures 7b-7d, 8b-8d, 9d-9d, and 10a-d].

Histochemical Evaluation

Histochemical tests were conducted using various reagents to identify the specific character from the thick sections of all four samples. Results are given in Table 6.

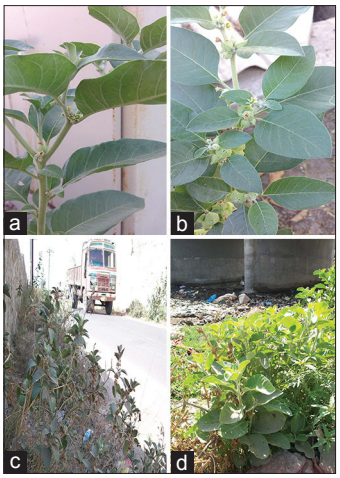

Figure 1: (a) Natural habit of sample A (Unpolluted Area University botanical garden). (b) Natural habit of sample B (Out Skirts of Jamnagar, Rakakhatia forest area). (c) Natural habit of sample C (Polluted area, Bediport road area). (d) Natural habit of sample D (Sunday Market Bridge area)

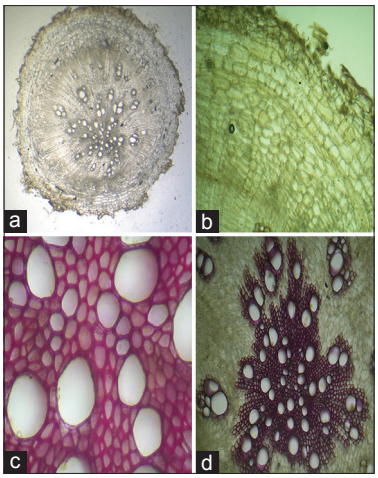

Figure 2: Naturally collected root samples roots samples. (a) Sample A (Unpolluted Area University botanical garden). (b) Sample B (Out Skirts of Jamnagar, Rakakhatia forest area). (c) Sample C (Polluted area, Bediport road area). (d) Sample D (Sunday Market Bridge area)

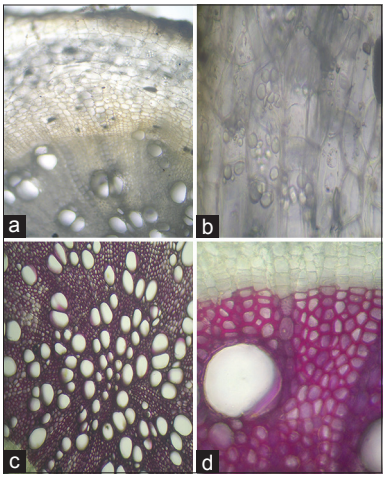

Figure 3: Sample A (Unpolluted Area University botanical garden). (a) Diagrammatic section of the sample A with cork, cortex, steel. (b) Multilayered cork, cortex, phloem, medullary rays. (c) Xylem, xylem fibers, xylem parenchyma. (d) Central stiller portion, xylem with parenchyma fiber

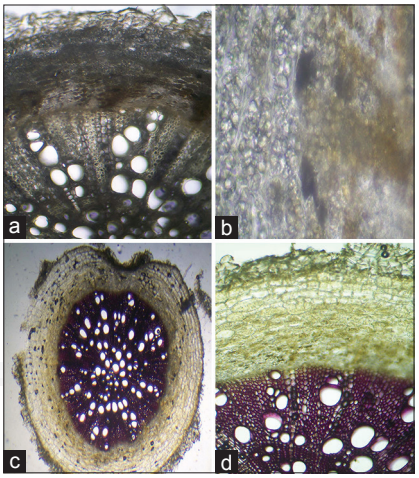

Figure 4: Sample B (Out Skirts of Jamnagar, Rakakhatia forest area). (a) Cork, cortex, steel. (b) Parenchyma cell with starch grain. (c) Central stealer region with xylem parenchyma, xylem fiber with medullary rays. (d) Xylem, xylem parenchyma, fibers, phloem

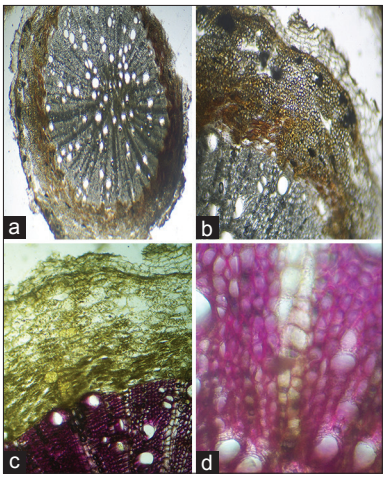

Figure 5: Sample C (Polluted area, Bediport road area). (a) Cork, cortex, steel with black debris. (b) Cork with black debris. (c) Cork, cortex, staler portion shows accumulation of black and brown debris. (d) Multilayered cork, cortex, phloem, xylem with medullary rays

Figure 6: Sample D (Sunday Market Bridge area). (a) Cork, cortex, steel with black debris. (b) Cork with black debris. (c) Cork disturb, cortex with black debris phloem with brown contain. (d) Xylem with xylem parenchyma fiber medullary rays with black and brown contain

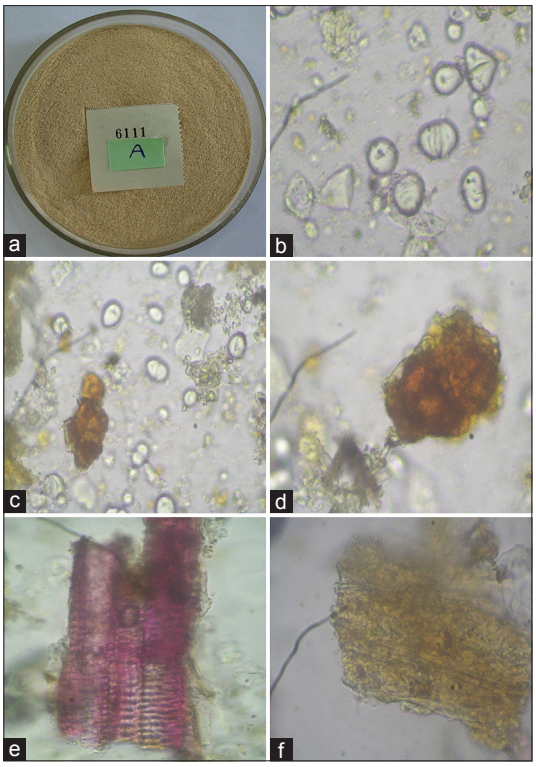

Figure 7: Powder microscopy of Sample A. (a) Powder Sample A. (b) Simple and compound starch grain. (c) Tannin contain. (d) Prismatic crystals along with tannin contain. (e) Lignified bordered pitted vessels. (f) Cork in tangential view

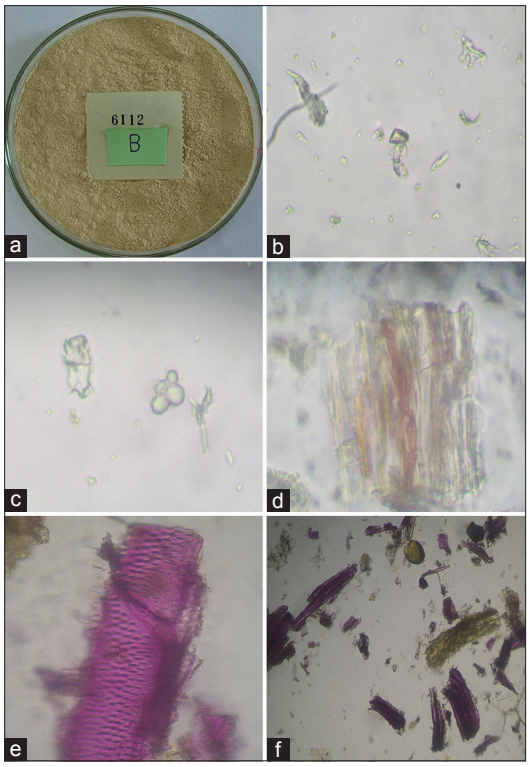

Figure 8: Powder microscopy of Sample B. (a) Powder sample B. (b) Prismatic crystal. (c) Compound starch grain. (d) Tannin contain. (d) Lignified bordered pitted vessels. (e) Cork in tangential view along with fibers

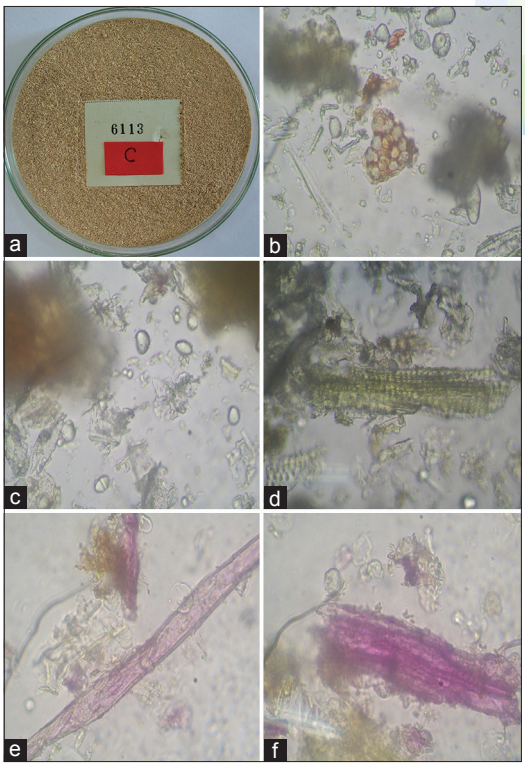

Figure 9: Powder microscopy of Sample C. (a) Powder sample C. (b) Cork in tangential view with black debris. (c) Starch grain with black and brown contain. (e) Lignified bordered pitted vessels with black debris. (f) Lignified fiber with brown contain. (g) Lignified fibers with black debris

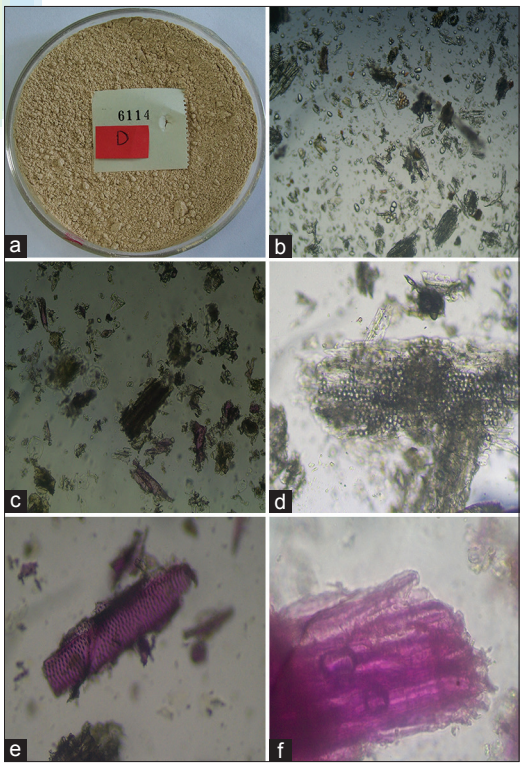

Figure 10: Powder microscopy of Sample D. (a) Powder sample D. (b) Black debris with starch grains. (c) Black and brown debris. (d) Cork along with heavy black debris. (e) Lignified bordered pitted vessels with black debris. (f) Lignified fibered with brown contain

Comparative Physico-Chemical Parameters

Results of the comparative study regarding pH, Loss on drying (L.O.D.), Ash value (A.V.) etc., of the four plants with their physicochemical parameters have been given in Table 7.

Comparative Studies on Secondary Plant Metabolites

Test results of the comparative study regarding the four plants with their secondary plant metabolites have been given in Table 8.

Comparative Heavy Metal Analysis

Results obtained from Heavy Metal analysis regarding the four root sample given in Table 9.

DISCUSSION

Root samples macroscopic parameters like fractures; color taste varies in all four samples. Samples A and B showed that Cork smooth buff grey yellow with longitudinal wrinkles. In the case of Samples C, Cork cells were somewhat hard brown/grey or yellow in color, with longitudinal wrinkles. Lateral roots many be smaller in size like thick hairs, cylindrical in shape, moderately pungent in odor, bitter to mucilaginous in taste. Sample D showed that Cork somewhat hard brown greyish yellow with longitudinal wrinkles. Lateral roots many, smaller in size like thick hairs. Fractures smooth powdery, Center of the root creamy and consists somewhat dark brown and black solid mass with scattered pores.

Transverse sections of samples A and B were circular to wavy in nature, whereas C and D showed more or less wavy circumference. Samples A, C, and D showed that tri-serrate medullary rays and devoid of pith whereas sample B showed tetra serrate medullary rays. Sample A and B showed 3 layers of cork cell whereas sample C and D showed 5–7 layers of cork cells. This factor perhaps indicating the protection against the pollutants. All the samples showed 4-5 layers of cortex, but cortical cell were cleared and field with starch grains in sample A and B, were as heavy deposition of black content along with starch grains in sample C and D. Sample A and B showed healthy vascular bundles and intercepted by medullary rays. Sample C was found with a high black deposition whereas sample

Table 1: Authenticated samples with their respective voucher number

Name of the sample	Voucher number
Sample A - Unpolluted area. University botanical garden	Phm number 6111
Sample B - Out Skirts of Jamnagar. Rakakhatia forest area	Phm number 6112
Sample C - Polluted area. Bediport road area	Phm number 6113
Sample D - Sunday market bridge area	Phm number 6114

Table 2: Comparative macroscopic study of the four plants

Characters	Sample A	Sample B	Sample C	Sample D
Habit	Shrub	Shrub	Shrub	Shrub
Height of the plant	Up to 3-5 ft	Up to 3-4 ft	Up to 2-3 ft	Up to 2-4 ft
Size of the leaf	Up to 10 cm long	Up to 9 cm long	Up to 5 cm long	Up to 8 cm long
Size of the root	Up to 25 cm long	Up to 23 cm long	Up to 19 cm long	Up to 18 cm long
Condition of the leaf	Healthy, green	Healthy, green	Leaves were shrinked, and black patches were found on the surface	Some leaves were affected with chlorosis
Condition of the root	Healthy	Healthy	Shorter and less branched	Shorter and less branched
Branching	Natural, erect, shrubby	Natural erect, shrubby	Moderate	Moderate

Table 3: Comparative microscopic study

Characters	Sample A	Sample B	Sample C	Sample D
SD	Circular to wavy	Circular to wavy	Circular to wavy	Circular to wavy
Cork	3 layers of cork cells, filled with brown content	3 layers of cork cells, filled with brown content	5-7 layers of cork cells, few cells were found with a black deposition	5-7 layer of cork cells, few cells were found with a black deposition
Cortex	4-5 layers of healthy cortex cells which were filled with starch grain	5-6 layers of healthy cortex cells and were filled with starch grains	5 layers of cortex cell with heavy deposition of black content along with starch grains	5 layers of cortex cell with deposition of black and brown content along with starch grains
Phloem	Healthy and intercepted by medullary rays	Healthy and intercepted by medullary rays	Found with high black deposition	Found With deposition of brown content
Cambium	2 layers	1 layer	1 layer	1 layer
Xylem	Well-developed along with fibres and parenchyma	Well-developed along with fibres and parenchyma	Well-developed along with a large number of fibres and more lignified parenchyma	Well-developed along with a large number of fibres and more lignified parenchyma
Medullary rays	Tri serrate	Tetra serrate	Tri serrate	Tri serrate
Pith	Absent	Absent	Absent	Absent

SD – Schematic diagram

Table 4: Organoleptic characters of the powder

Parameters	Sample A	Sample B	Sample C	Sample D
Colour	Light creamish yellow	Light creamish yellow	Dark creamish brown	Brownish yellow
Taste	Bitter	Bitter	Slightly bitter	Slightly bitter
Odour	Horse urine	Horse urine	Characteristic	Characteristic
Touch	Fine	Fine	Fine coarse	Coarse

Table 5: Comparative powder microscopy of the four plants

Characters	Sample A	Sample B	Sample C	Sample D
Starch grain with the hilum	++	++	++	++
Tannin content	++	++	++	++
Calcium oxalate crystal	++	++	−−	−−
Lignified fibre	++	++	++	++
Boarder pitted vessels	++	++	++ (blackish)	++ (blackish)
Disposition of black content	−−	−−	++	++

++ – Present, −− – Absent

Table 6: Comparative histochemical evaluation of the four plants

Reagent	Observation	Character	Results			
			A	B	C	D
Phloroglucinol + concentrated HCl	Red	Lignified cells	++	++	++	++
Iodine	Blue	Starch grains	++	++	++	++
Phloroglucinol + concentrated HCl	Dissolved	Calcium oxalate crystals	++	++	++	++
Fecl3 solution	Dark blue to black	Tannin cells	+	+	++	++

++ – Frequently present, + – Present, HCl – Hydrochloric acid

Table 7: Comparative physico-chemical parameters

Parameters	Sample A	Sample B	Sample C	Sample D
Loss on drying (% w/w)	8.29	8.32	8.11	8.13
Water soluble extractive (% w/w)	14.48	14.53	14.28	14.22
Alcohol soluble extractive (% w/w)	7.99	8.04	7.59	7.52
pH (1% w/v)	5.09	5.07	6.09	6.08

Table 8: Comparative studies on secondary plant metabolites

Parameters	Sample A	Sample B	Sample C	Sample D
Alkaloid	++	++	++	++
Steroid	++	++	++	++
Carbohydrates	++	++	++	++
Tannin	++	++	++	++
Reducing sugars	++	++	++	++
Glycosides	++	++	++	++

++ – Present

Table 9: Comparative heavy metal analysis

Heavy metals	Limits (as per API) ppm	Sample A	Sample B	Sample C	Sample D
Lead (Pb)	10	Not detected	Not detected	10.02	10.01
Cadmium (Cd)	0.3	Not detected	Not detected	Not detected	Not detected
Mercury (Hg)	1	Not detected	Not detected	1.001	1.001
Arsenic (As)	3	Not detected	Not detected	Not detected	3.002

API – Ayurvedic pharmacopoeia of India

D was found with deposition of brown content mainly due to the air and water pollutants.

Organoleptic evaluation revealed that sample A and B were bitter in taste whereas sample C and D were slightly bitter in taste.

Powder microscopy showed that all four samples were found with moderate quantity of starch grains, tannin, and lignified fibers. Sample A and B showed that the presence of calcium oxalate crystals, whereas sample C and D were devoid of calcium oxalate crystals, this may be due to the chemical nature of the soil in polluted area. Border pitted vessels were very common in all 4 samples but in samples C and D the adjacent vascular bundles cells were deposited with black debris due to the air and water pollution. Fragments of parenchyma cells of samples A and B were clear but the parenchyma cells with a black deposition in samples C and D remained even after the treatment with chloral hydrate.

Histochemical evaluation showed that the presence of a large amount of starch grains, presence of lignin in all samples. But sample A and B showed less amount of tannin as compared to the sample C and D.

Results obtained from physicochemical analysis revealed that Sample A and B had a similar pattern of L.O.D value (8.29%, 8.32%) while Sample. C and D showed slightly decrease in L.O.D value (8.11%, 8.13%), respectively. This result emphasized that sample A sample B contained same amount of moisture content while sample C and D contained slightly lesser amount of moisture. P^H value of sample A and B showed simile in value with former at 5.09 and later one at 5.07. Sample C and D showed higher P^H value than the sample A and B, with former at 6.09 and later one at 6.08. This result emphasized that group of chemical constituents obtained from sample A and B were slightly acidic in nature while constituents obtained from sample C and D were closer to a neutral value. This may be because of basic soil nature of the sample C and D, from where they collected. In case of water soluble extractive value, both the sample A and B showed similar pattern in percentage yield (14.48%, 14.53%) while sample C and D showed slightly decrease in percentage yield with the value of 14.28%, 14.22%, respectively. In case of Alcohol soluble extractive value, both the sample A and B showed similar

pattern in percentage yield (7.99%, 8.04%) while sample C and D showed slightly decrease in percentage yield with the value of 7.59%, 7.52%, respectively. These results emphasized that a slight decrease in percentage yield was mostly due to unavailability of adequate nutrition supply. Pattern of extractive value depicted that sample A and sample B had have a similar kind of hydrophilic moiety, at the same time sample C and sample D exhibited the presence of similar kind of hydrophilic moiety.

Results obtained from heavy metal analysis depicted distinctive features between the sample obtained from pollution free natural habitat (sample A and B) and those obtained from polluted areas (sample C and D). Limit test of lead exhibited presence of lead in sample C and D, the numerical values were found to be above the limit (10.02 ppm, 10.01 ppm) as specified in A.P.I.

In the case of sample A and B, lead was not detected. Limit test of cadmium depicted that presence of cadmium in all the four samples was negligible as cadmium was not detected in heavy metal analysis. Limit test for mercury exhibited different results in case of samples obtained from pollution free area and those obtained from polluted areas. In the case of sample A and B, mercury was not detected. In the case of sample C and D, presence of mercury was found to be above the limit (1.001 ppm, 1.001 ppm), as specified in A.P.I.

Limit test for arsenic showed difference in result between the samples obtained from pollution free area and those obtained from polluted area In case of sample A, B, and C arsenic was not detected, but in case of sample D, arsenic was found to be above the prescribed limit as per A.P.I specification. All the above-mentioned results significantly indicate that qualitatively samples A and B were far better than sample C and D. This is because sample A and B were collected from relatively pollution-free natural habitat but sample C and D were collected from roadside and dumped water reservoir, respectively.

CONCLUSION

Pharmacognostical data, generated in this work, suggested that samples (*Ashwagandha*), which were collected from the polluted areas, were unhealthy, growth were retarded, and their tissue function were often found to be affected by the incorporation of various pollutants. Data generated from heavy metal analysis revealed that samples (*Ashwagandha*) obtained from polluted areas were contaminated with Pb, Hg and As. Out of eight cases of Heavy metal analysis for samples obtained from polluted areas, at least 5 times their respective presence was found to be above the specified limit. With these data, it can be concluded that consumption of such drug may cause accumulated side effect as well as the toxic effect of the heavy metal, respectively.

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