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Original Article

Analytical Characterization of *Achyranthes aspera* L. Whole Plant

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ABSTRACT

Background: The therapeutic properties of *Achyranthes aspera* have long been acknowledged and integrated into diverse traditional healthcare like Ayurvedic, Siddha, Unani and Naturopathic medicine for the management of various ailments. In ancient Indian systems of medicine such as Siddha and Ayurveda, the constituents found in this medicinal plant play a pivotal role in treating disorders. Both macro and micro elements are crucial components for plants to carry out their basic functions. **Objectives:** Consequently, the ash of *Achyranthes aspera* was analyzed chemically, with a focus on phytochemical and chromatographic analysis, along with spectral characterization of the entire plant, to determine the presence of inorganic constituents. **Materials and Methods:** Standard methods were followed for all physicochemical parameters. Chromatographic characterization was carried out using High Performance Thin Layer Chromatography (HPTLC) and High-Performance Liquid Chromatography (HPLC). Additionally, elemental characterization was performed using different instruments such as Energy Dispersive X-ray analysis (EDX), X-ray Fluorescence (XRF), Powder X-ray Diffraction (PXRD), X-ray Photoelectron Spectroscopy (XPS) and Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES) to investigate the minerals present in the ash of *Achyranthes aspera*. **Results:** The HPTLC photo documentation revealed the presence of five spots at 254 nm and eight spots at 366 nm. Vanillin sulfuric acid reagent derivatization resulted in the appearance of 9 spots under white light. Furthermore, the HPLC chromatogram showed the separation of 13 peaks at 366 nm. The evaluation techniques also detected various elements such as Carbon (C), Oxygen (O), Sodium (Na), Magnesium (Mg), Aluminium (Al), Silicon (Si), Phosphorus (O), sulphur (S), Chlorine (Cl), potassium (K), Calcium (Ca), Manganese (Mn), Chromium (Cr), Iron (Fe) and Zinc (Zn). **Conclusion:** The analysis primarily focuses on quality control criteria and the mineral composition of the plant.

Keywords: *Achyranthes aspera*, Nayurivi, SEM-EDX, XRF, XPS, PXRD, ICP-OES.

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INTRODUCTION

Achyranthes aspera Linn. is a familiar medicinal plant utilized in various traditional medicine practices such as Ayurvedic, Siddha, Unani and Naturopathic medicine to treat a variety of diseases.

The macro elements are the essential components that plants require to perform their basic functions. The treatment of disorders heavily relies on the crucial contribution of both macro and micro elements found in medicinal plants. *A. aspera* is commonly referred to as Apamarg in Sanskrit, Nayurivi in Tamil and Prickly Chaff flower in English.[1] It is a perennial plant belonging to the *Amaranthaceae* family. It is an easily accessible and significant medicinal plant. It grows widely in the world as a weed, native to Tropical Asia, Africa and South America.[2] *A. aspera* is an erect, much-branched, monoecious perennial subshrub up to 30-90 cm high. The leaves possess an elliptic or obovate shape, with a rounded apex and are characterized by their thickness and soft pubescence. On the contrary, the flowers are small and arranged in slender, elongated spikes positioned at the terminal end and gradually bend downwards. Stems are succulent at first but progressively become woody with increasing age.[3,4] Alkaloids, carbohydrates, flavonoids, Oil and fats, saponins, tannins and phenolic compounds are major secondary metabolites present in *A. aspera*.[5] It contains caffeic acid, chlorogenic acid, ferulic acid, gallic acid, gentisic acid, genistein, isoferulic acid, para-coumaric acid, para-hydroxybenzoic acid, protocatechuic acid, rutin, salicylic acid, syringic acid, trans-cinnamic acid, taxifolin, sinapic acid, vanillic acid as major phenolic compounds.[2] Chemical components 4-methoxyheptatriacont-1-en-10-ol and tetracontanol-2, fatty acid arachidic, behenic, lauric, linoleic, myristic, palmitic, oleic stearic,[6] Acetoxy-6

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benzoyloxyapangamide,[7] diterpenoid ester,[8] bisdesmosidic saponins (I-III), 20-hydroxyecdysone, quercetin-3-O-β-D galactoside[9] oleonolic acid glycosides[10] are various compounds found in different aerial parts, Leaves, seeds, shoots and stem of the plant.

Pharmacologically *A. aspera* this plant shows Antibacterial, antifungal,[11] antimicrobial, anthelmintic,[2] antioxidant and wound healing,[12-14] Antiinflammatory and antiarthritic,[15,16] Anticancer,[17] Anti-allergic,[18] Gastroprotective effect,[19] Hepatoprotective activity,[20] Antiobesity, hypolipidemic, antioxidant property,[21] Diuretic activity,[22] hypoglycaemic effect.[23]

Therapeutically it is used for the treatment of apaci (lymphadenitis-cervical), arśa (haemorrhoids), kandu (itching), medroga (obesity) śula (colic), udararoga (diseases of the abdomen) as texted in API and Cevinōy (ear disease), Cūtakat tatai(secondary amenorrhea), Iyanōy (disease due to seetham), Irumal(cough), Kāmālai(jaundice), Vellai nōy(leucorrhoea), Veluppu nōy(anaemia), Vikkam (oedema)according to the SPI. Apāmārgaksāra, Apāmārgaksāra Taila, Abhayā Lavana, Gudapippali, Jyotismati Taila, Nakāc Centūram, Oon Poochu Thailam are the few formulations where *A. aspera* is used.[1,24,25]

The plant is utilized in traditional systems of practices since ancient days. Various traditional uses and the pharmacological activities of the plant are well documented. There is a deficiency of sufficient data related to the characterization of the plant. A comprehensive analysis of the plant *A. aspera* is accomplished by TLC photo documentation, HPTLC fingerprint profiles, HPLC analysis and elemental analysis by SEM-EDX, XRF, PXRD, XPS and ICP-OES.

MATERIALS AND METHODS

Plant Collection

The whole *A. aspera* plant was obtained from the Arumbakkam locality within Chennai District, Tamil Nadu. Validation and authentication were done by the department of Pharmacognosy at Siddha Central Research Institute in Chennai.

Chemicals and Solvents

Analytical grade solvents including toluene, ethyl acetate, methanol, formic acid, conc. HCl, conc.HNO_3 and HPLC grade methanol were procured from Merck. The HPTLC analysis involved the use of vanillin (1 g) for derivatization in a methanol solution containing 5% sulphuric acid.

Sample Preparation

The powdered sample was collected for physicochemical examination. The whole plant was dried and roughly pulverized at room temperature. The resulting powder was then subjected to a muffle furnace until the ash become devoid of carbon. After cooling in a desiccator, it was carefully transferred to an airtight container and designated AAA for subsequent examination and chemical characterization. 2 g of plant material were sonicated with 20 mL of methanol for 30 min in preparation for HPTLC and HPLC analysis. Following filtration, the collected filtrate was transferred into a sample vial to facilitate its subsequent examination. To prepare for ICP-OES analysis, 0.5 g of the sample was accurately weighed and mixed with 10 mL of conc.HCl and 10 mL of concentrated HNO_3. The resulting solution was then treated in a laboratory microwave. An additional 10 mL of conc. HCl was added to the solution to ensure complete digestion. After filtering with Whatman no.1 filter paper, the solution was diluted to 250 mL in a standard measuring flask. This solution was subsequently stored for later use.

Physico-chemical analysis

Standard procedures were followed to assess the AAWP powder for loss on drying, extractive values, total ash content, water soluble ash content, acid insoluble ash content and pH level.[26]

HPTLC analysis

The CAMAG HPTLC instrument, manufactured in Switzerland, was employed for the HPTLC analysis. A volume of 10 µL of the extract was carefully sprayed onto an aluminum plate coated with silica gel $60F_{254}$ measuring (4×10 cm), forming a band length of 8 mm and positioned 10 mm from the bottom by the ATS4 Autosampler. Subsequently, the plate was developed by a pre-saturated solvent system consisting of toluene, ethyl acetate and formic acid (5:1:0.5, v/v/v) within a twin trough chamber (10×10 cm). Following development, the plate was dried on a hot plate, photographed in the CAMAG Visualizer Chamber and scanned under λ_{254} nm (D_2 lamp, absorbance mode) and λ_{366} nm (Hg lamp, fluorescence mode). The scanning was performed with 6×0.45 mm slit dimension, at 20 mm/s scanning speed (Scanner_210441, software: WINCATS). Subsequently, TLC plate was treated with a derivatizing reagent (vanillin sulfuric acid) and heated on a TLC plate heater at 105°C until coloured bands were visible. A photograph was taken under white light and then scanned at λ_{520} nm (W lamp, absorption mode).[27]

HPLC analysis

The HPLC studies were conducted using the Shimadzu HPLC system (manufactured in Japan), equipped with the Lab Solutions software. The system included a quaternary pump (LC-20 AP), an ultraviolet PDA detector (SPD-M20A 230V) and a vacuum Degasser (DGU-10B). The separation process involved the utilization of an analytical RP-C18 column (250 X 4.6 mm) and 100% methanol in an isocratic method as the stationary and mobile phases, respectively with a flow rate of 1 mL/min. The data were recorded at λ_{366} nm.[28]

SEM-EDX analysis

Utilizing a SEM-EDX (FESEM, Carl Zeiss, SUPRA 55 VP), the sample was subjected to analysis. Before conducting the analysis, a fine layer of gold was applied to the sample. The electron micrographs were recorded at 15 kV. An Oxford Instruments X-MAX (20 mm^2) Energy Dispersive Spectrometer (EDS) was employed for elemental analysis.[29]

XRF analysis

The XGT-2700 X-ray analytical microscope (Horiba, Japan) was used for the examination. The microscope was equipped with an X-ray tube and an Rh-anode. The tube voltage was configured at 50 kV, with a current of 1 mA. A high-purity silicon detector named XEROPHY was utilized to detect a wide range of elements. The analysis had a duration of 60 sec. The XGT diameter was 10 µm.[30]

XPS analysis

Utilizing a Perkin-Elmer PHI 5500 ESCA System the XPS analysis was performed. Analysis was carried out under a vacuum pressure of at least 10-9 Torr. The instrument's calibration was derived from the Au4f7/2 peak binding energy of 84.0 eV. Each spectrum was acquired at a resolution of 0.5 eV. The XPS spectra's binding energies were adjusted using the hydrocarbon component of the C1s spectrum at 285.0 eV as the reference.[31]

Table 1: Physicochemical parameters of *A. aspera*.

Sl. No.	Parameters	Mean value (*n*=2)
1	Loss of Drying	9.7%
2	Total Ash	13.76%
3	Water soluble Ash	6.73%
4	Acid Insoluble Ash	3.96%
5	Water Soluble Extractive	6.76%
6	Alcohol Soluble Extractive	3.32%
7	pH	7.02

PXRD analysis

The Aeries PANalytical diffractometer from the Netherlands was used to collect powder diffraction data. In Bragg-Brentano geometry, diffraction was performed using Ni-filtered copper radiation. A thin layer of the sample was prepared on a silicon zero background holder. Recorded data was obtained at a 2θ angle starting from 10-100°, with an incremental size of 0.01° and a 40-sec recording duration per step employing CuKα (λ=1.5418 A°). The observed pattern was subsequently correlated with the ICDD (International Centre for Diffraction Data) library.[31]

ICP-OES analysis

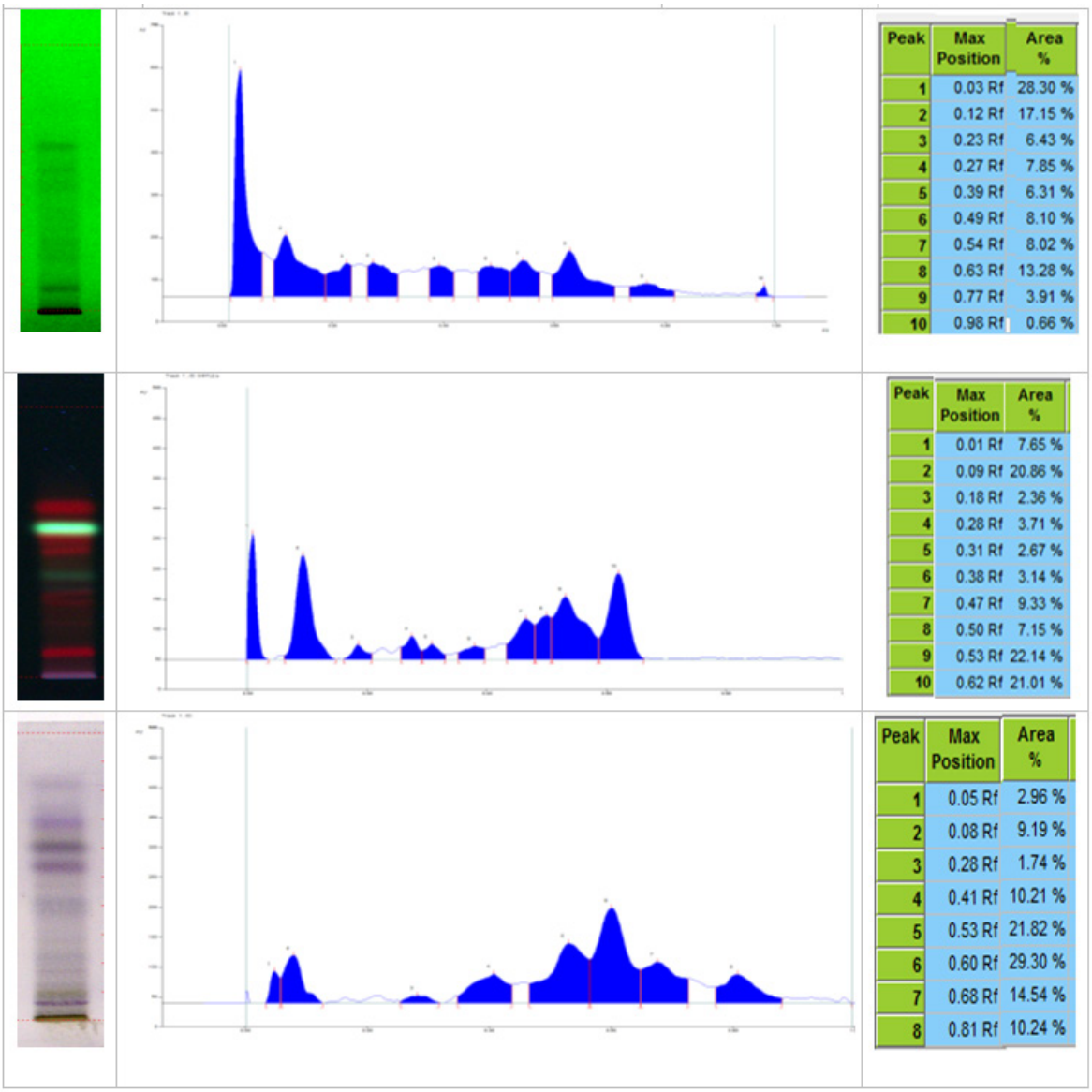
The elements present in the sample were analyzed with the Agilent ICP-OES (Model 720 series, Santa Clara, California, USA). A laboratory microwave digester outfitted with 40 sealed vessels (Mars 6, CEM, USA) was used to wet digest the sample.[31]

RESULTS

The physico-chemical characteristics of *A. aspera* are outlined in Table 1. The methanol extract of the plant sample was analyzed using thin layer chromatography, revealing the existence of five distinct bands with R_f values of 0.09, 0.21, 0.46, 0.54 and 0.62, all appearing green under short UV light. When exposed to long UV light, eight bands were visible with R_f values of 0.09 (red), 0.20 (red), 0.27 (red), 0.33 (red), 0.38 (green), 0.48 (red), 0.55 (green) and 0.64 (red). Post-derivatization, the plate exhibited 6 bands with R_f values of 0.08, 0.17, 0.25, 0.29, 0.59 and 0.65, all appearing violet under white light. Further scanning of *A. aspera* revealed 10 peaks with R_f 0.03 (area 28.30%), 0.12 (17.15%), 0.23 (6.43%), 0.27 (7.85%), 0.39 (6.31%), 0.49 (8.10%), 0.54 (8.02%), 0.63 (13.28%), 0.77 (3.91%) and 0.98 (0.66%) under 254 nm. Scanning under long UV revealed 10 peaks with R_f 0.01 (area 7.65%), 0.09 (20.86%), 0.18 (2.36%), 0.28 (3.71%), 0.31 (2.67%), 0.38 (3.14%), 0.47 (9.33%), 0.50 (7.15%), 0.53 (22.14%) and 0.62 (21.01%) under long UV. White light scanning of the post-derivatized plate displayed eight peaks, each with an R_f 0.05 (area 2.96%), 0.08 (9.19%), 0.28 (1.74%), 0.41 (10.21%), 0.53 (21.82%), 0.60 (29.30%), 0.68 (14.54%) and 0.81 (10.24%) (Figure 1, Table 2).

Table 2: R_f and color of spots.

UV 254 nm		UV 366 nm		After derivatization	
R_f	Colour	R_f	Colour	R_f	Colour
Green	0.09	Red	0.09	Violet	0.06
Green	0.21	Red	0.20	Green	0.08
Green	0.46	Red	0.27	Violet	0.17
Green	0.54	Red	0.33	Violet	0.39
Green	0.62	Green	0.38	Violet	0.42
		Red	0.48	Violet	0.55
		Green	0.55	Violet	0.62
		Red	0.64	Violet	0.70

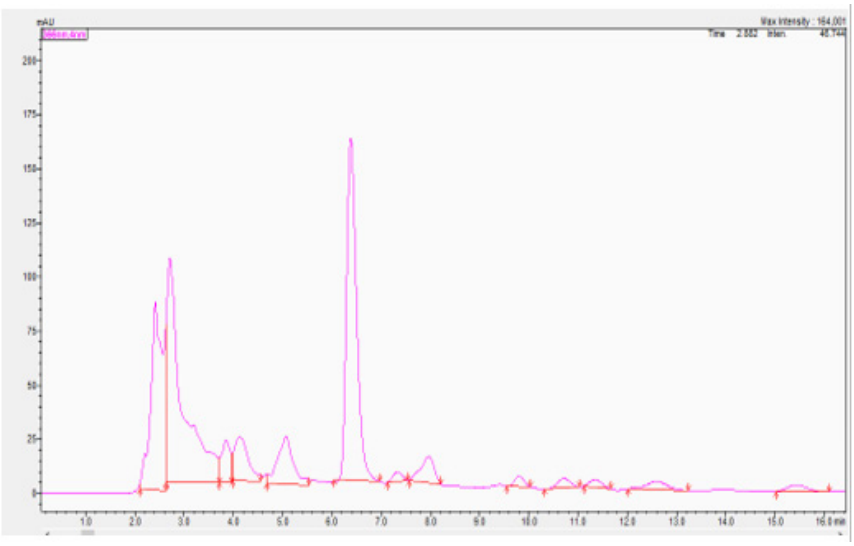


Peak	Max Position	Area %
1	0.03 Rf	28.30 %
2	0.12 Rf	17.15 %
3	0.23 Rf	6.43 %
4	0.27 Rf	7.85 %
5	0.39 Rf	6.31 %
6	0.49 Rf	8.10 %
7	0.54 Rf	8.02 %
8	0.63 Rf	13.28 %
9	0.77 Rf	3.91 %
10	0.98 Rf	0.66 %

Peak	Max Position	Area %
1	0.01 Rf	7.65 %
2	0.09 Rf	20.86 %
3	0.18 Rf	2.36 %
4	0.28 Rf	3.71 %
5	0.31 Rf	2.67 %
6	0.38 Rf	3.14 %
7	0.47 Rf	9.33 %
8	0.50 Rf	7.15 %
9	0.53 Rf	22.14 %
10	0.62 Rf	21.01 %

Peak	Max Position	Area %
1	0.05 Rf	2.96 %
2	0.08 Rf	9.19 %
3	0.28 Rf	1.74 %
4	0.41 Rf	10.21 %
5	0.53 Rf	21.82 %
6	0.60 Rf	29.30 %
7	0.68 Rf	14.54 %
8	0.81 Rf	10.24 %

Figure 1: TLC fingerprint profile at 254 nm, 366 nm and 520 nm and peak area.



Peak#	Ret. Time	Area	Area%
1	2.419	1490204	19.023
2	2.708	2284469	29.163
3	3.847	244425	3.120
4	4.122	390681	4.987
5	5.067	476957	6.089
6	6.382	2260581	28.858
7	7.336	56869	0.726
8	7.966	222979	2.846
9	9.803	62218	0.794
10	10.714	78417	1.001
11	11.342	62580	0.799
12	12.594	118329	1.511
13	15.434	84862	1.083
Total		7833572	100.000

Figure 2: HPLC chromatogram and peak table of methanol extract of *A. aspera.*

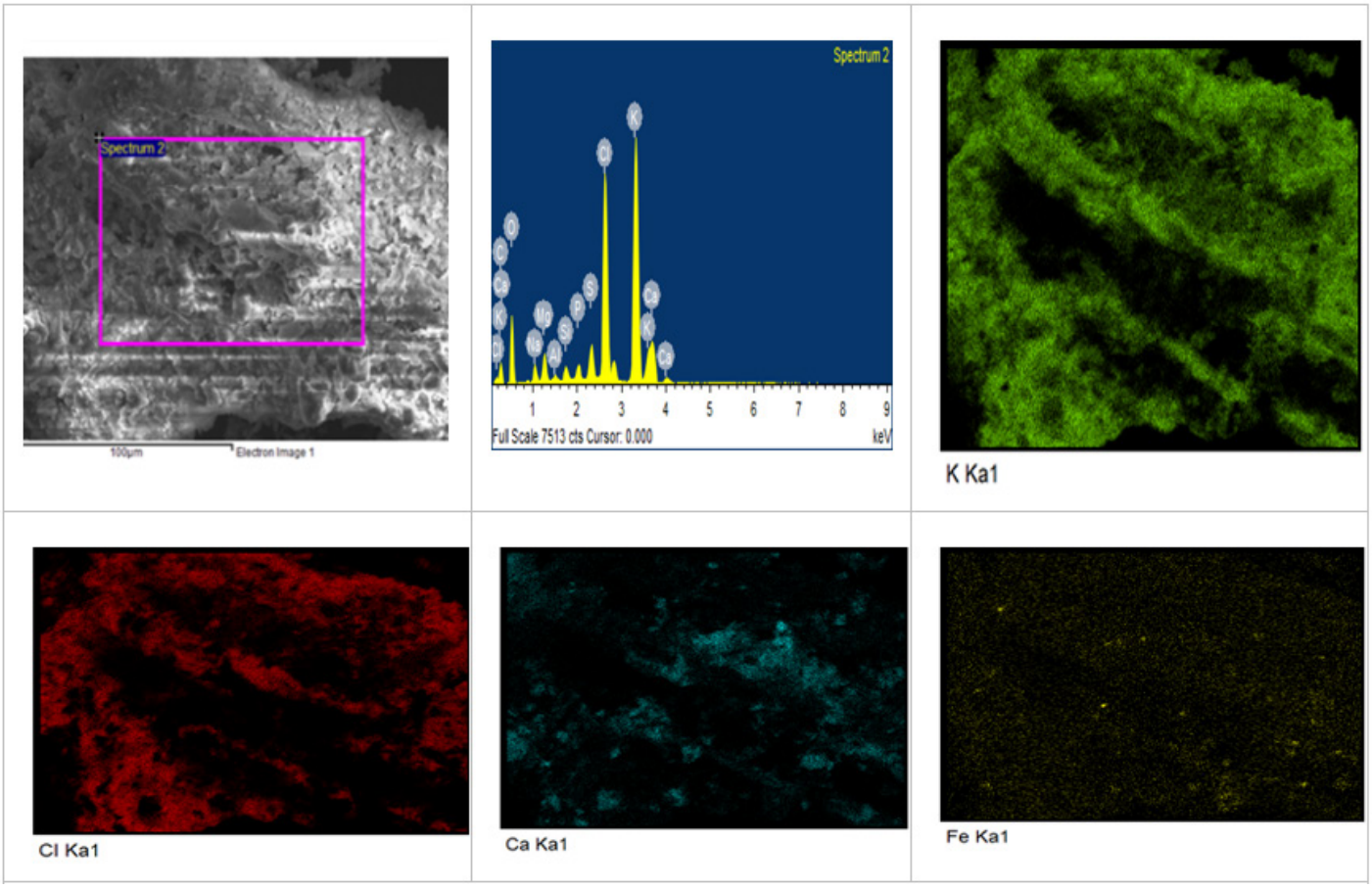


Figure 3: SEM-EDAX graphs of AAA.

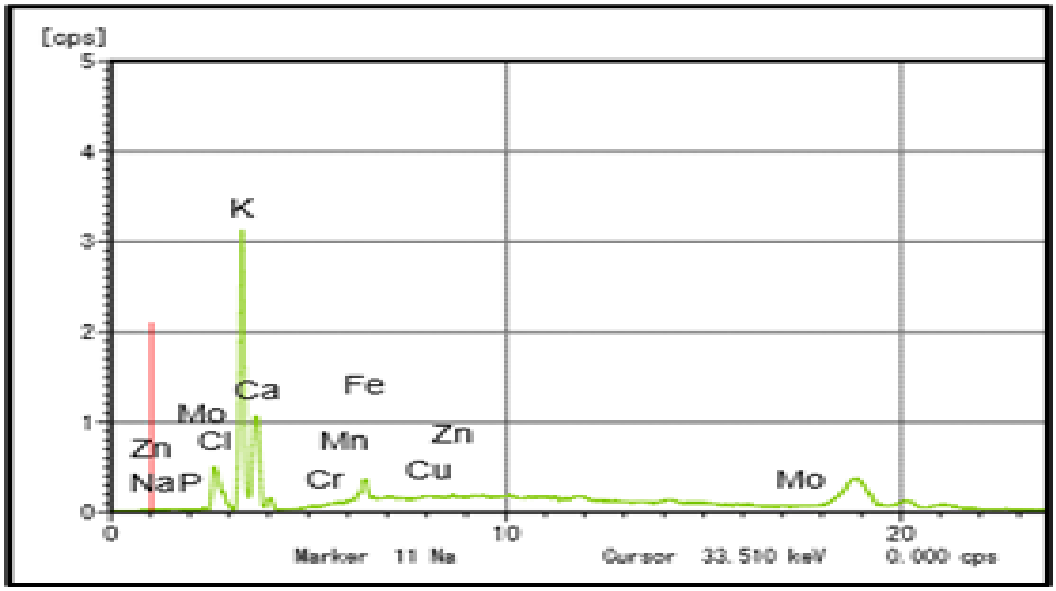


Figure 4: XRF spectra of AAA.

RP-HPLC assessment of methanol extract of *A. aspera* revealed 13 peaks with Rt 2.419 (area 19.023%), 2.708 (29.163%), 3.847 (3.120%), 4.122 (4.987%), 5.067 (6.089%), 6.382 (28.858%), 7.336 (0.726%), 7.966 (2.846%), 9.803 (0.794%), 10.714 (1.001%), 11.342 (0.799%), 12.594 (1.511%) and 15.434 (1.083) at λ_{366} nm (Figure 2).

The EDX results indicated the presence of eleven elements in descending order: oxygen (33.30%), potassium (23.73%), chlorine (16.08%), carbon (14.59%), calcium (4.60%), sulphur (2.21%), magnesium (1.83%), sodium(1.58%), phosphorous (1.08%), silicon (0.77%) and aluminium (0.23%) (Figure 3, Table 3).

XRF data obtained from the sample showed the presence of seven elements in descending order: potassium (53.79%), calcium (38.14%), chlorine (4.53%), iron (2.58%), chromium (0.66%), zinc (0.14%) and phosphorus (0.14%). The constituent elements of the *A. aspera* sample identified by XRF is shown in (Table 4, Figure 4). PXRD results detected elements such as Ca, K, Mg, C, Cl and O existed in the form of potassium chloride, calcium carbonate and dolomite (Figure 5). The peak intensities were observed corresponding to 2θ values and the phases were identified in comparison with ICDD (International Centre for Diffraction Data). XPS analysis of *A. aspera* divulges the presence of Mg, Na, O, Ca, C, Cl, P and Si in the respective different electronic states (Table 5, Figure 6).

ICP-OES measures the elemental analysis of whole sample values were obtained as sodium (0.10%), magnesium (2.41%), aluminium (0.11%), silicon (3.02%), phosphorus (0.006%), sulphur(0.35%), chlorine(0.12%), potassium (16.73%), calcium (3.27%), chromium (<0.001%), manganese (0.02%), iron (0.24%), nickel (<0.002%), copper (<0.001%), zinc (0.03%), cadmium (<0.001%) and lead (<0.001%) (Table 6).

Table 3: Composition by EDX of AAA.

Sl. No.	Elements	Mass%
1	Carbon	14.59
2	Oxygen	33.30
3	Sodium	1.58
4	Magnesium	1.83
5	Aluminium	0.23
6	Silicon	0.77
7	Phosphorous	1.08
8	Sulphur	2.21
9	Chlorine	16.08
10	Potassium	23.73
11	Calcium	4.60

Table 4: XRF Result of AAA.

Sl.No.	Elements	Mass%
1	Phosphorous (P)	0.14
2	Chlorine (Cl)	4.53
3	Potassium (K)	53.79
4	Calcium (Ca)	38.14
5	Chromium (Cr)	0.66
6	Iron (Fe)	2.58
7	Zinc (Zn)	0.14

DISCUSSION

Physico-chemical parameters are essential factors that impact the quality of the drug. Moisture plays an important role in shelf-life determination. The moisture content was found to be 9.7% based on the loss on drying value. The total ash value denotes the presence of total inorganic salt content. It was calculated as 13.76%. Acid insoluble ash which is mainly comprised of silica was estimated as 3.96. The water and alcohol solubility value denotes the more solubility of the drug in water. pH was found to be 7.02 meaning the drug is almost neutral in nature.

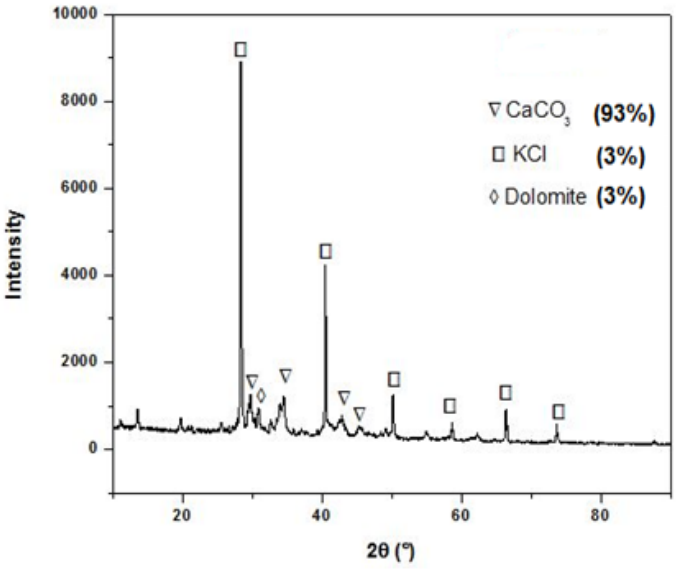


Figure 5: PXRD spectra of AAA.

Table 5: Composition by XPS of AAA.

Sl. No.	Elements	Electronic state	Binding Energy (eV)
1	C	1s	280.0
2	O	1s	535.0
3	P	2p	130.0
4	K	2p	295.0
5	Mg	1s	1300.0
6	Na	1s	1075.0
7	Ca	2p	348.0
8	Si	2p	100.0
9	Cl	2p	200.0

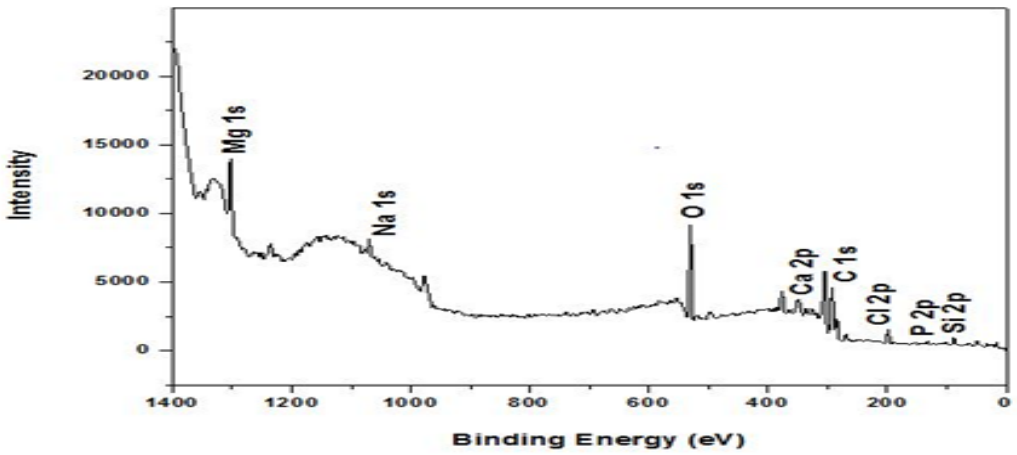


Figure 6: XPS spectra of AAA.

Table 6: ICE-OES Result of AAA.

Sl. No.	Test Parameters	Results (%)	Sl. No.	Test Parameters	Results (%)
1	Aluminium as Al	0.11	10.	Sodium as Na	0.10
2	Calcium as Ca	3.27	11.	Nickel as Ni	<0.002
3	Cadmium as Cd	<0.001	12.	Phosphorous as P	0.006
4	Chromium as Cr	<0.001	13.	Lead as Pb	<0.001
5	Copper as Cu	<0.001	14.	Zinc as Zn	0.03
6	Iron as Fe	0.24	15.	Chloride as Cl	0.12
7	Potassium as K	16.73	16.	Sulphur as S	0.35
8	Magnesium as Mg	2.41	17.	Silica SiO_2	3.02
9	Manganese as Mn	0.02			

Table 7: Comparison of ICP-OES, EDAX, XRF.

Sl. No.	Elements	EDX Mass%	XRF Mass%	ICP-OES Mass%
1	K	23.73	53.79	16.73
2	Ca	4.6	38.14	3.27
3	Mg	1.83	0	2.41
4	Na	1.58	0	0.1
5	Cl	16.08	4.53	0.12
6	S	2.21	0	0.35
7	Si	0.77	0	3.02
8	Al	0.23	0	0.11
9	Fe	0	2.58	0.24
10	Mn	0	0	0.02
11	Ni	0	0	< 0.02
12	P	0.006	0.14	1.08
13	Zn	0.03	0.14	0
14	Cd	<0.001	0	0
15	Cr	<0.001	0.66	0
16	Cu	<0.001	0	0

Comparative elemental analysis by EDX, XRF and ICP OES is summarised in Table 7. EDX analysis revealed O, K, Cl, C and Ca as major elements in the sample. XRF analysis showed K, Ca and Cl were in prominent percentages. In ICP-OES analysis K, Ca, Mg and Si were detected as major. The presence of iron was confirmed through analysis using XRF and ICP-OES. K, Mg and Ca were detected comparatively in more percentage by XPS. These data revealed the presence of K, Cl, Ca, P, Na, Mg, C and O in most predominant in *A aspera*. Toxic metals like Cd, Pd, Cr, Cu and Ni were in BDL (Below the Detection Limit) (by ICP-OES). This result denoted that heavy metal toxicity is absent in this drug material. PXRD confirmed the presence of K, Cl, Ca, Mg, C and O which existed in the form of calcite, sylvine and dolomite as phases of corresponding crystal structures. The *Amaranthaceae* family is known to contain potassium, chlorine, calcium, carbon, oxygen, magnesium and phosphorus in the majority of its plant species, as evidenced by our recent study. These inorganic elements are vital for various biological functions.[32] Chromatograms by HPTLC and HPLC reveal the separation of metabolites of the *A. aspera* whole plant.

CONCLUSION

Current study present a comprehensive guide to the phytochemical parameters, physicochemical parameters, metals and minerals compositions of the sample *A. aspera*. This study is vital for ensuring quality control and mapping the mineral composition according to pharmacopoeial standards. Further biochemical studies are necessary to confirm the mechanism described in ancient texts related to the activity of the plant *A. aspera*.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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ABBREVIATIONS

AAW: *Achyranthes aspera* ash; **HPTLC:** High Performance Thin Layer Chromatography; **HPLC:** High Performance Liquid Chromatography; **EDX:** Energy Dispersive X-ray analysis; **XRF:** X-ray fluorescence; **PXRD:** Powder X-ray Diffraction; **XPS:** X-ray Photoelectron Spectroscopy; **ICP-OES:** Inductively Coupled Plasma Optical Emission Spectroscopy.

SUMMARY

The powdered *A. aspera* whole plant was subjected physicochemical parameters, TLC, HPTLC, HPLC analysis; its ash was subjected to EDAX, XRF, XPS, PXRD and ICP-OES. In HPTLC, revealed the separation of 5, 8 and 9 spots under 254, 366 nm and white light after derivatization with vanillin sulphuric acid reagent respectively. In HPLC a maximum of 13 peaks were separated at 366 nm. Fifteen elements such as carbon, oxygen, sodium, magnesium, aluminium, silicon, phosphorus, sulphur, chlorine, potassium, calcium, manganese, chromium, ironand zinc were identified.

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