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PHARMACOGNOSTICAL, PHYSICOCHEMICAL AND ELEMENTAL INVESTIGATION OF AN AYURVEDIC POWDERED FORMULATION: SHATAVARYADI CHURNA

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ABSTRACT: Most of the traditional systems of medicines are effective but the need is just to validate them to assess the quality, quantity and purity of the drugs. To support the same a polyherbal Ayurvedic powdered formulation Shatavaryadi churna used as immunomodulator, galactagogue, aphrodisiac and rejuvenator was taken for pharmacognostical, physicochemical and elemental investigation. Shatavaryadi churna is the composition of five ingredients, i.e. powder of *Asparagus racemosus* Willd. tubers-1part, *Tribulus terrestris* Linn. fruits- 1 part, *Mucuna pruriens* (L.) DC seeds- 1 part, *Withania somnifera* Dunal. roots- 1 part and *Chlorophytum tuberosum* Baker bulbs- 1 part. Ingredients of the formulation, In-house formulation and Marketed formulation have been pharmacognostically, physicochemically and elementally characterized by microscopy, physical constant values and Energy Dispersive Spectroscopy. Selected parameters for the investigation were anatomical study; powder microscopy; Extractive values i.e. alcohol soluble, water soluble, chloroform soluble, ethyl acetate soluble and petroleum ether soluble; Ash values i.e. total ash, water insoluble ash, acid insoluble ash; moisture content value, foreign matter and elemental content. The set parameters were found to be simple to evaluate the churna and can be used as reference standards for the quality control/quality assurance of Shatavaryadi churna and other related Ayurvedic formulations.

INTRODUCTION: Traditional medicine incorporates health practices of plant, mineral and animal based medicines, applied singularly or in combination to treat and prevent illnesses/maintain well-being [1].

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Herbal medicines have been enjoying renaissance among the customers throughout the world. However, one of the impediments in the acceptance of the Ayurvedic medicines is the lack of standard quality control profiles.

The quality of herbal medicine, i.e. the profile of the constituents in the final product, has implication in efficacy and safety [2, 3]. According to an estimate of WHO nearly 80% populations of developing countries rely on traditional medicines, mostly on plant drugs for their primary health care need [4].

Most of the traditional systems of medicines are effective but the need is just to validate them. Shatavaryadi churna is an Ayurvedic proprietary medicine mentioned in the Ayurveda Sarsangraha and used as immunomodulator, galactagogue, aphrodisiac and rejuvenator.

Shatavaryadi churna is the composition of *Asparagus racemosus* Willd. Tubers (AR)-1part, *Tribulus terrestris* Linn. Fruits (TT)-1part, *Mucuna pruriens* (L.) DC seeds (MP)-1part, *Withania somnifera* Dunal. roots (WS)-1part and *Chlorophytum tuberosum* Baker bulbs (CT)-1part [5].

For present study, all the ingredients of Shatavaryadi Churna, In house formation and Marketed formulation were taken for the investigation. Extensive investigation of this formulation is very important, especially if one knows that analysis of Herbal formulations has drawn great attention from scientists in recent years both at national and international level.

MATERIALS AND METHODS:

Materials:

Plant Materials: The plant materials of Shatavaryadi churna were collected from the different sources and authenticated by Dr. E. Roshini Nayar, Principal Scientist, National Herbarium of Cultivated Plants, National Bureau of Plant Genetic Resources, Indian Council of Agricultural Research, Pusa Campus, New Delhi (India) i.e. Shatavari *(Asparagus racemosus* Willd.) Collected from the Narayanpur district of Chhatisgarh (India) (Identification Voucher No.: NHCP/NBPGHR/2011-7/); Goksura *(Tribulus terrestris* Linn.) Purchased from local traders of New Delhi (India) (Identification Voucher No.: NHCP/NBPGHR/2011-9/); Atmagupta *(Mucuna pruriens* (L.) DC) Collected from the Narayanpur district of Chhatisgarh (India) (Identification Voucher No.: NHCP/NBPGHR/2011-6/); Ashwagandha *(Withania somnifera* Dunal.) Purchased from local traders of New Delhi (India) (Identification Voucher No.: NHCP/NBPGHR/ 2011-10/) and Safed *Musli (Chlorophytum tuberosum* Baker.) Collected from the Narayanpur district of Chhatisgarh (India) (Identification Voucher No.: NHCP/NBPGHR/ 2011-8/).

Parts of the ingredients were crushed and powdered using grinder and passed through sieve number#85. In-house Shatavaryadi Churna was prepared from these powders by mixing them in one part for each ingredient and named as IH. Marketed Shatavaryadi Churna was also procured and named as M.

Methods:

Anatomical study of each ingredient of Shatavaryadi Churna: Microscopic studies were carried out by preparing the transverse section of the selected plant parts. The sections were cleared with alcohol and stained as per protocol [6, 7, 8, 9, 10]. The anatomy of the transverse section were observed and identified under Leica DME microscope (10*x*X10*x*). Anatomical studies for each ingredients of Shatavaryadi Churna were performed on transverse sections.

Powder microscopy of Shatavaryadi Churna: A judicious quantity of powder was taken on a glass slide and added a few drop of chloral hydrate and was heated for 1-2 minute after placing a cover slip, care should be taken to avoid air bubbles and to see that there was sufficient chloral hydrate under the cover slip. Excess of chloral hydrate outside the cover slip is to be withdrawn using a blotting paper. (Chloral hydrate is used to clear the tissues and to bring in clarity of the view).

Lignified tissues were confirmed by staining the powder by few drop of mixture of 1:1 phloroglucinol + concentrated hydrochloric acid; starch grains were confirmed by staining the powder with 0.1N iodine solution and finally mounted in chloral hydrate/glycerin and observed under Leica DME microscope (10*x*iX40*x*) Powder microscopy for each ingredients of Shatavaryadi Churna were done and unique identifying characters were studied [11].

Determination of Foreign matter: 500 g of the drug sample was examined by spread it out in a thin layer. The foreign matter was detected by inspection with the unaided eye or by the use of a lens (6*x*). Separated and weighed the foreign matters and calculate the percentage of foreign matter present [11].

Determination of Extractive Values: 5g of air dried Powder of ingredients of Shatavaryadi Churna and Shatavaryadi Churna formulations were taken and macerated with 100ml of solvent (i.e. methanol, water, chloroform, ethyl acetate and petroleum ether) in a closed flask for 24 hours, shaking frequently for the first 6 hrs and allowed to stand for 18 hrs; then filtered with taking precautions against loss of solvent. 25ml of the filtrate was evaporated to dryness in a tarred flat bottomed shallow dish and finally dried at 105°C and weighed.[12] The percentage of the alcohol soluble, water soluble, chloroform soluble, ethyl acetate soluble and petroleum ether soluble extractive values were calculated with reference to air dried powder of ingredients of Shatavaryadi Churna and Shatavaryadi Churna formulations.

Determination of Moisture Content: 10 g of sample (without preliminary drying) were placed after accurately weighing it in a tarred evaporating dish. After placing the above said amount of the sample in the tarred evaporating dish dry at 105^{o}C and continue the drying and weighing at 10 minutes interval until difference between two successive weightings corresponds to not more than 0.25 per cent. Constant weight is reached when two consecutive weightings after drying for 30 minutes and cooling for 30 minutes in a desiccator, show not more than 0.01 g difference [12]. Finally moisture content was measured directly in percentage.

Determination of Ash values:

- **Total Ash values:** 3g of air dried Powder of ingredients of Shatavaryadi Churna and Shatavaryadi Churna formulations were taken in a tarred silica dish and incinerated at the temperature not exceeding 450°C until free from carbon; cooled and weighed [12]. The percentage of total ash value was calculated with reference to air dried powder of ingredients of Shatavaryadi Churna and Shatavaryadi Churna formulations.

- **Insoluble ash values:** The total ash was boiled with 25 ml water for five minutes and filtered through an ash less filter paper (Whatman filter paper#1). The filter paper was ignited in the silica crucible. Then cooled and the water insoluble ash was weighed. The water soluble ash was calculated by subtracting the water insoluble ash from the total ash [12]. Then the percentage of water soluble ash was calculated with reference to the air dried drug.

- **Acid insoluble ash values:** The total ash was boiled for five minutes with 25 ml of dilute hydrochloric acid and filtered through ash less filter paper (Whatman filter paper #1).The filter paper was ignited in the silica crucible, cooled and acid soluble ash was calculated by weighing [12].

Elemental Analysis: The human body needs a variety of elements (often called minerals) for almost all aspects of body function. These elements are required in amounts that range from 50 µg to 18 mg per day. There are more than 20 chemical elements necessary for humans. Deficiency in such essential nutrients Leads to a wide range of symptoms depending on the deficient mineral [13].

The Energy Dispersive Spectrometer (EDS) was used to irradiate the samples and to collect its characteristic spectra. The system is fully controlled by an IBM PC using the software package XpertEase running under windows 3:1:1 and with the conditions like EDX Detector: Silicon, Window: SATW, Tilt (deg): 0.0, Elevation (deg): 33.0, Azimuth (deg): 0.0, Magnification: 750X, Accelerating voltage (kV): 20.00, working distance: 10mm. Before each run, the spectrometer is programmed by the user to operate under the appropriate fixed conditions for the sample using XpertEase.

In the present work, the samples were irradiated under five different fixed conditions, namely Very light elements (VLE), Solids (S-V), Steels (ST), Medium elements (ME) and Very heavy elements (VHE). It is suitable for measuring the concentrations of the elements S through V using their K-Lines and the elements Ba through Sn using their L-lines.

RESULTS AND DISCUSSION:

Anatomical Study: *Asparagus racemosus* Wild.: Single layered epidermis, tangentially elongated thin walled cells were present. Major portion of the transverse section was covered by cortex and identified as thick walled cells (collenchymas) and thin walled cells (parenchyma).

Endodermis was present with 3-4 layered cells followed by pericycle. Vascular tissue was composed of alternate strands of xylem and phloem (radial vascular bundle). Pith contains thin walled parenchymatous cells (**Figure 1**).

Tribulus terrestris Linn.: Transverse section contains small rectangular epidermal cells. Unicellular trichomes were found on the surface, 6-10 layers of large parenchymatous cells were present. Endosperm was present at the centre (**Figure 2**).

Mucuna pruriens (L.) DC: Transverse section contains anticlinical walls in epidermis. Hypodermis comprises of bearer cells, which is followed by parenchyma with tangentially elongated cells. Cotyledons comprise of epidermis, and parenchymatous cells containing oil globules and oval starch grains (**Figure 3**).

Withania somnifera Dunal: Transverse section contains cork cells in outer part followed by cortex. Few layers of tangentially elongated cells were present and identified as cambium followed by phloem and xylem. Multiseriate medullary ray was present (**Figure 4**).

Chlorophytum tuberosum Baker.: Transverse section contains a outermost layer of epiblema followed by 2-4 rows of oval, tangentially elongated, radially arranged compact cells of the exodermis. Endodermis and cortex layer was distinct. Groups of phloem tissue were alternating with xylem bundles in a ring encircled by lignified xylem parenchyma. Pith was very narrow (**Figure 5**).

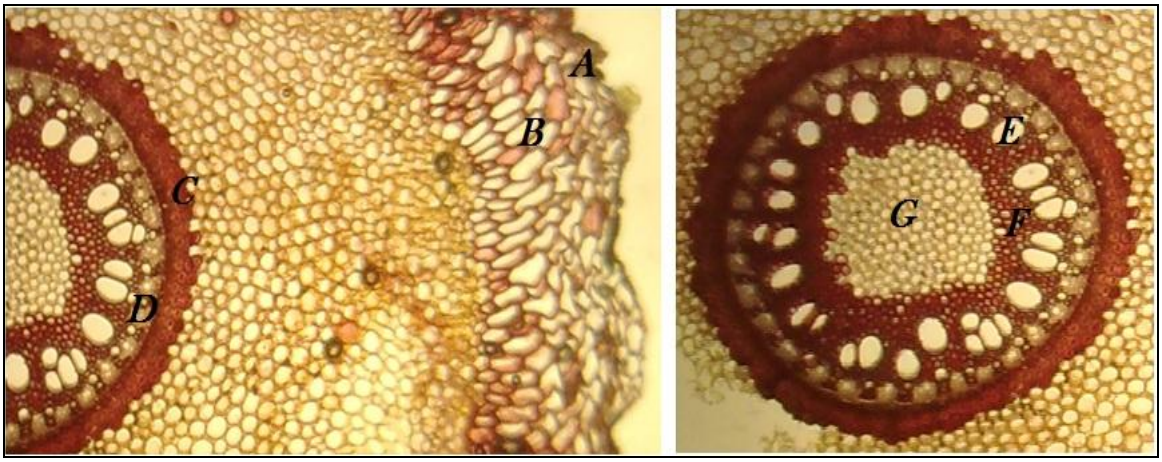

FIGURE 1: TRANSVERSE SECTION OF *ASPARAGUS RACEMOSUS* WILLD. (A) EPIDERMIS, (B) CORTEX, (C) ENDODERMIS, (D) PERICYCLE, (E) XYLEM VESSELS, (F) PHLOEM, (G) PITH

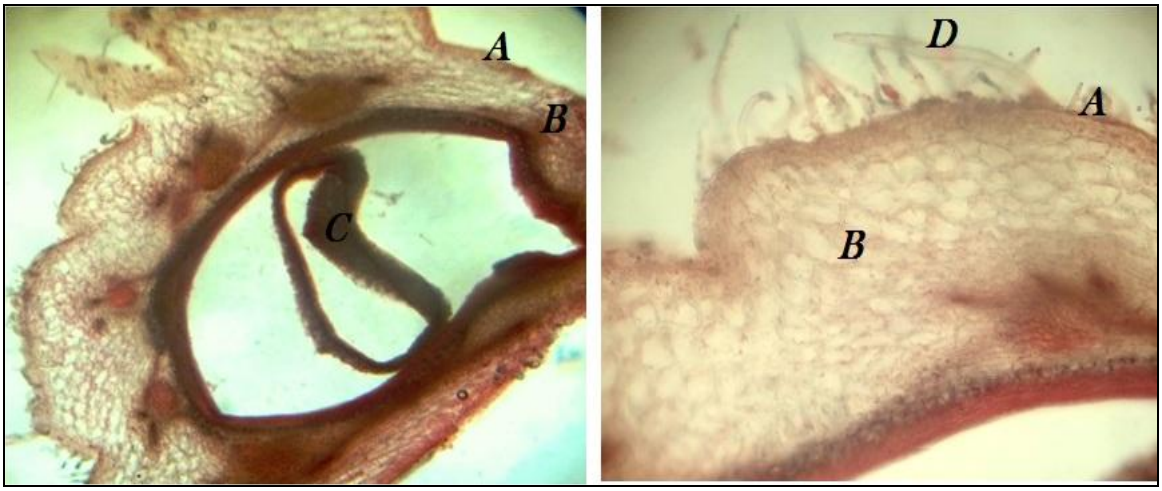

FIGURE 2: TRANSVERSE SECTION OF *TRIBULUS TERRESTRIS* LINN. (A) EPIDERMIS, (B) PARENCHYMATOUS LAYER, (C) ENDOSPERM, (D) UNICELLULAR TRICHOMES

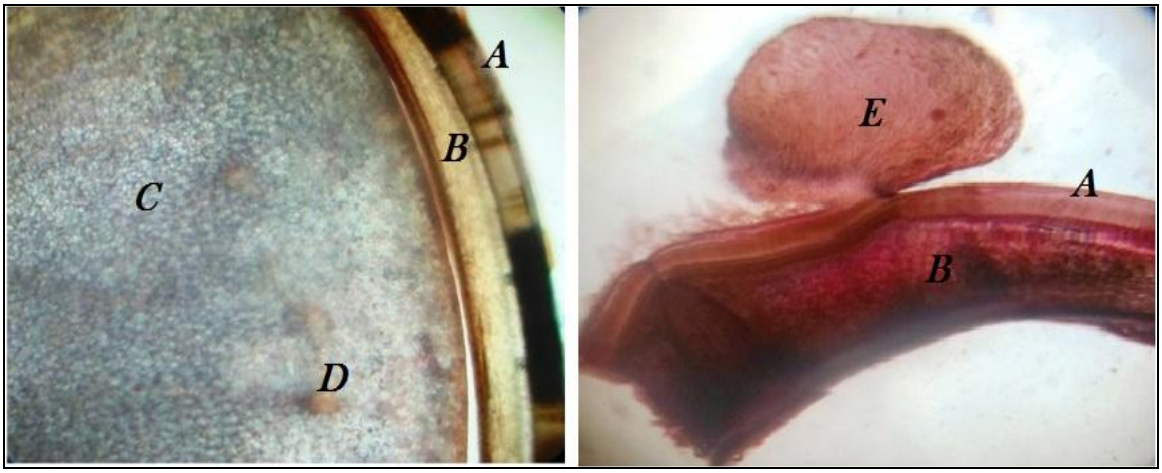

FIGURE 3: TRANSVERSE SECTION OF *MUCUNA PRURIENS* (L.) DC. (A) EPIDERMIS, (B) BEARER CELL, (C) PARENCHYMA, (D) OIL GLOBULE, (E) HILUM.

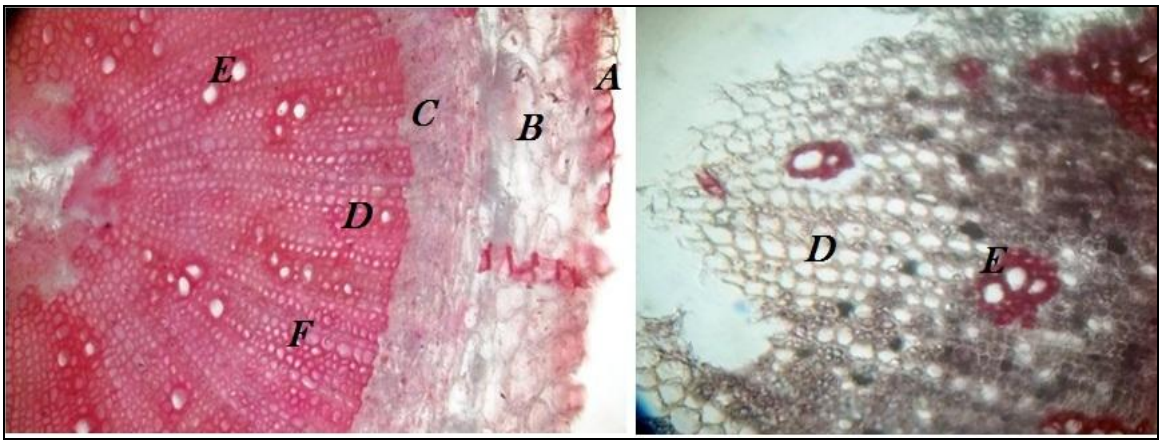

FIGURE 4: TRANSVERSE SECTION OF *WITHANIA SOMNIFERA* DUNAL. (A) CORK, (B) CORTEX, (C) CAMBIUM, (D) PHLOEM, (E) XYLEM VESSELS, (F) MEDULLARY RAYS.

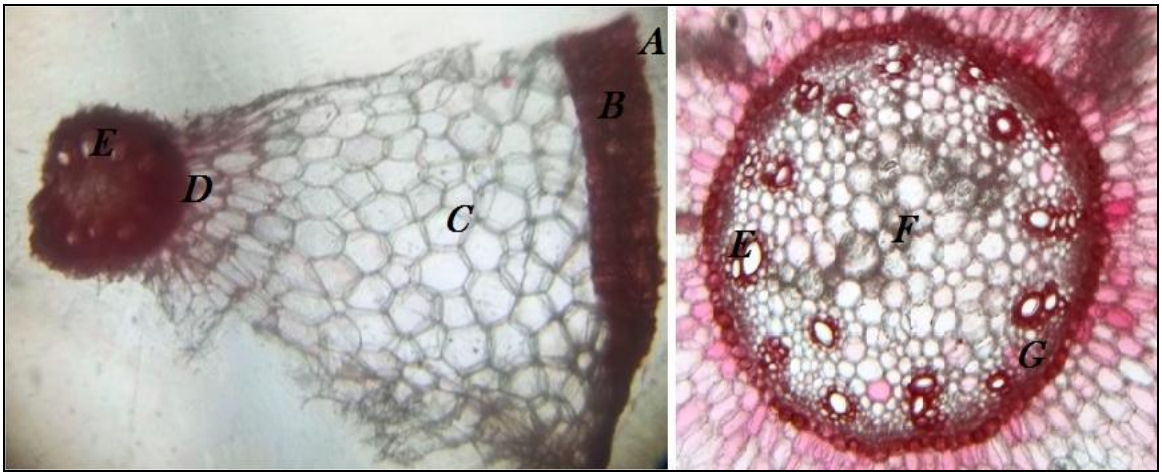

FIGURE 5: TRANSVERSE SECTION OF *CHLOROPHYTUM TUBEROSUM* BAKER. (A) EPIBLEMA, (B) EXODERMIS, (C) CORTEX, (D) ENDODERMIS, (E) XYLEM VESSELS, (F) PITH, (G) PHLOEM

Powder Microscopy: Powder microscopy of the ingredients of Shatavaryadi Churna contains unique identifying characters i.e. Pitted vessel and needle shaped calcium oxalate crystals of *Asparagus racemosus* Willd (**Figure 6**), Unicellular trichome and rosette shaped calcium oxalate of *Tribulus trrestris* Linn (Figure 6), Epidermis with bearer cells and oil gland of *Mucuna pruriens* (L.) DC (Figure 6), Cork cells and starch grains of *Withania somnifera* Dunal (Figure 6), Epiblema and Acicular calcium oxalate crystals of *Chlorophytum tuberosum* Baker. (Figure 6). Powder microscopy was done for the formulations and same characters were identified.

FIGURE 6: POWDER MICROSCOPY OF INGREDIENTS OF SHATAVARYADI CHURNA

(A) Pitted vessel and (B) Needle shaped calcium oxalate crystals of *Asparagus racemosus* Willd, (C) Unicellular trichome and (D) Rosette shaped calcium oxalate of *Tribulus trrestris* Linn., *(E)* Epidermis with bearer cells and (F) Oil gland of *Mucuna pruriens* (L.) DC., (G) Cork cells and (H) Starch grains of *Withania somnifera* Dunal., (I) Epiblema and (J) Acicular calcium oxalate crystals of *Chlorophytum tuberosum* Baker.

Foreign matter values: Foreign matter value for all the ingredients were found between the ranges of 0.583±0.033-1.775±0.104 (**Table 1**).

Extractive values: Alcohol soluble, water soluble, chloroform soluble, ethyl acetate soluble and petroleum ether soluble extractive values were determined. Water soluble extractive values were found to be maximum for all the ingredients except *Withania somnifera* Dunal.; which has given maximum extractive value in alcohol (**Table 2**).

Moisture content: Moisture content values were found between the ranges of 2.867±0.666 to 6.967±0.441% (**Table 3**).

Ash values: Total ash value, water soluble as value and acid insoluble ash value were investigated for all the ingredients and formulations of Shatavaryadi churna and shown in **table 4**.

Elemental Analysis: Elements modify the action of drug on the body. So the elemental analysis was carried out. Presence of different elements in the ingredients and formulations were shown in **figure 7 and table 5**.

TABLE 1: FOREIGN MATTERS OF VARIOUS INGREDIENTS OF SHATAVARYADI CHURNA

S. No.	Sample	Foreign Matter (%w/w) ±SEM	Reference Value[12]
1	AR	0.733±0.088	Not more than 1%
2	TT	0.833±0.033	Not more than 1%
3	MP	0.583±0.033	Not more than 1%*
4	WS	1.775±0.104	Not more than 2%
5	CT	0.700±0.058	-

TABLE 2: EXTRACTIVE VALUES OF VARIOUS INGREDIENTS OF SHATAVARYADI CHURNA AND FORMULATIONS

S. No.	Sample	Extractive value (%w/w)±SEM				
		Alcohol soluble	Water soluble	Chloroform soluble	Ethyl acetate soluble	Petroleum ether
1	AR	10.133±0.267	45.333±0.267	20.533±0.267	3.333±0.027	0.853±0.027
2	TT	10.533±0.133	13.333±0.267	1.627±0.027	7.920±0.046	4.827±0.071
3	MP	4.373±0.208	30.400±0.924	6.347±0.053	6.187±0.053	5.867±0.133
4	WS	15.067±0.353	9.467±0.353	2.480±0.080	12.533±0.533	4.160±0.080
5	CT	3.280±0.046	30.667±0.267	2.453±0.027	3.013±0.071	0.853±0.053
6	IH	7.893±0.027	27.653±0.096	3.547±0.053	6.747±0.053	3.733±0.096
7	M	7.627±0.071	26.320±0.167	3.227±0.071	6.640±0.080	4.507±0.107

TABLE 3: MOISTURE CONTENT OF VARIOUS INGREDIENTS OF SHATAVARYADI CHURNA AND FORMULATIONS

S. No.	Sample	Moisture content (%w/w) ±SEM	Reference value (%w/w) [14]
1	AR	6.967±0.441	Not more than 15%
2	TT	4.467±0.333	Not more than 5%
3	MP	3.933±0.333	-
4	WS	5.933±0.333	Not more than 12%
5	CT	5.733±0.666	-
6	IH	3.067±0.333	-
7	M	2.867±0.666	-

TABLE 4: ASH VALUES OF VARIOUS INGREDIENTS OF SHATAVARYADI CHURNA AND FORMULATIONS

S. No.	Sample	Ash value (%w/w)±SEM		
		Total ash	Water soluble ash	Acid insoluble ash
1	AR	4.911±0.120	3.022±0.088	0.489±0.033
2	TT	12.611±0.088	7.655±0.120	0.933±0.058
3	MP	4.267±0.346	2.511±0.033	0.289±0.033
4	WS	6.400±0.173	4.200±0.058	0.956±0.033
5	CT	5.178±0.145	2.689±0.033	0.356±0.033
6	IH	5.511±0.145	2.9997±0.033	0.622±0.033
7	M	5.344±0.088	2.966±0.033	0.578±0.033

TABLE 5: WEIGHT PERCENTAGE OF DIFFERENT ELEMENTS IN VARIOUS INGREDIENTS OF SHATAVARYADI CHURNA AND FORMULATIONS

S. No.	Name of element	Name of lines	Weight percentage of elements.						
			AR	TT	MP	WS	CT	IH	M
1	C	*K*	42.30	51.37	66.70	45.06	46.38	47.85	51.42
2	O	*K*	50.02	36.81	27.67	47.18	47.84	38.97	41.47
3	Al	*K*	0.27	-	-	-	0.18	0.80	-
4	S	*K*	0.23	0.42	0.31	0.15	0.21	1.08	0.27
5	Fe	*K*	-	-	-	-	0.28	-	-
6	Na	*K*	-	-	-	-	-	-	0.23
7	Mg	*K*	-	0.37	0.15	0.15	-	-	0.45
8	Si	*K*	0.42	-	-	-	0.31	-	0.52
9	Cl	*K*	0.32	0.42	-	0.14	0.31	0.42	0.15
10	K	*K*	0.98	0.68	1.02	1.49	2.32	1.61	2.56
11	Ca	*K*	0.24	6.50	-	1.72	-	0.60	0.83
12	Ir	*M*	-	-	0.43	-	-	1.63	-
13	Cu	*K*	1.01	0.54	0.79	0.98	-	1.55	-
14	Zn	*K*	0.85	0.56	0.46	0.64	-	1.01	-
15	Zr	*L*	-	1.28	1.58	-	1.40	3.83	1.32
16	Pt	*M*	3.37	1.05	-	2.50	0.77	0.65	0.77

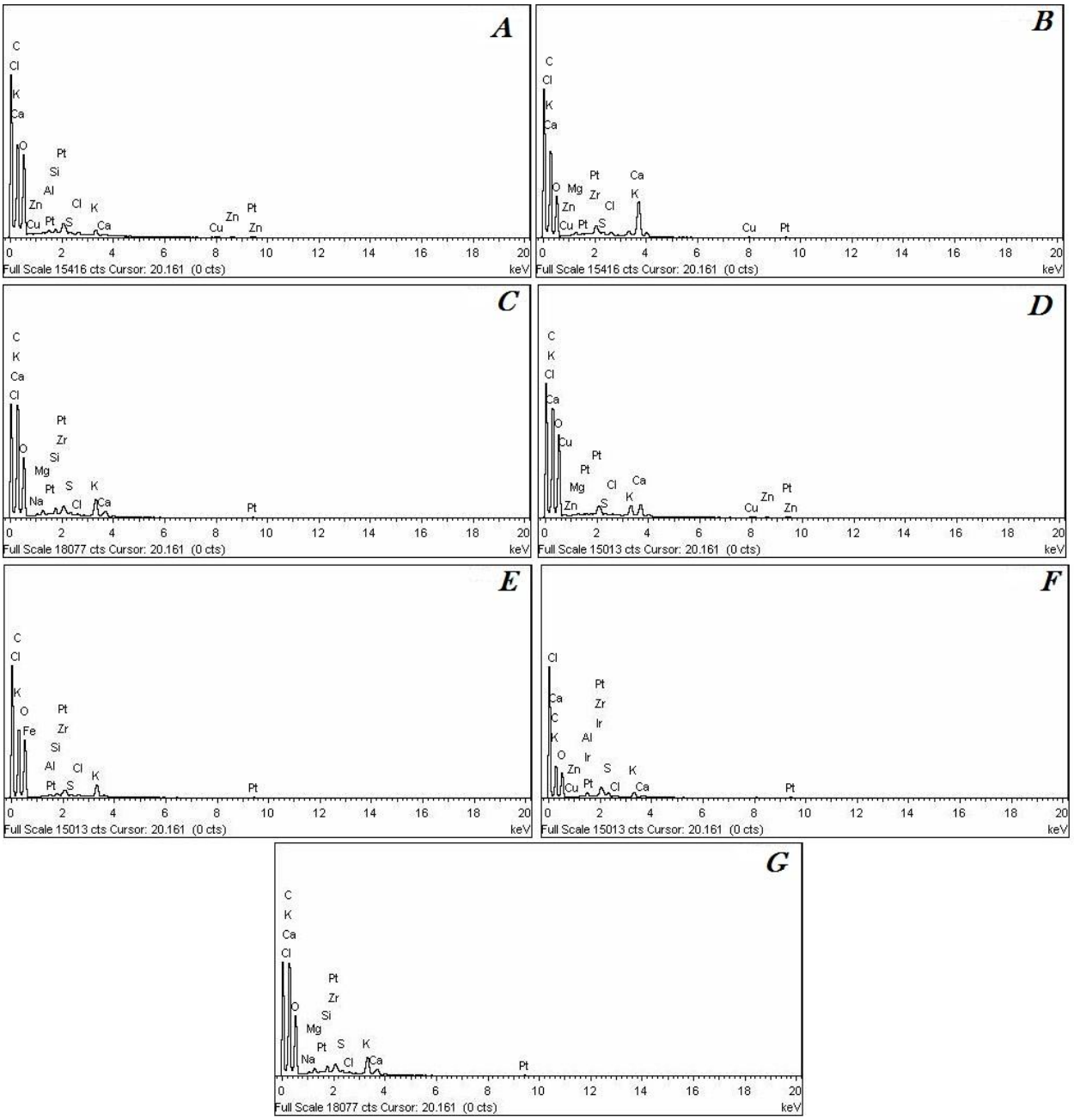

FIGURE 7: EDS SPECTRA OF VARIOUS INGREDIENTS OF SHATAVARYADI CHURNA AND FORMULATIONS. (A) *Asparagus racemosus* Willd. (B) *Tribulus terrestris* Linn. (C) *Mucuna pruriens* Linn. (D) *Withania somnifera* Dunal. (E) *Chlorophytum tuberosum* Baker. (F) Formulation IH (G) Formulation M

CONCLUSION: The present study revealed that the set parameters for investigation can be used for correct identification of the ingredients of Shatavaryadi churna. All the ingredients were found to be genuine and passed the pharmacognostical, physicochemical and elemental studies. Ash values, Extractive values were passed the Ayurvedic Pharmacopoeial standard and moisture content value were passed Indian Pharmacopoeial standard for the ingredients, while all these standards were established for the formulations of Shatavaryadi churna and can be used for routine standardization purpose.

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