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Pharmacognostical and physicochemical analysis of *Asparagus adscendens* Buch. Ham. ex Roxb. (Shweta musali).

Mahesh Kawale, Sandip Ankoliya, R. Saravanan, Tushar Dhanani and P. Manivel

Abstract
Asparagus adscendens is a sub-erect, prickly medicinal plant used as aphrodisiac, antifilarial and against abdominal troubles in Indian System of Medicine. The pharmacognostic parameters were studied for identification of species through macro and microscopical, physicochemical, phytochemical, biochemical analysis and HPTLC fingerprinting. The plant is characterized by sub-erect struggling habit, 30-100 cm long roots and presence of sclerenchymatous fibres outside endodermis, presence of single parenchymatous layered pericycle inside the endodermis, tracheids having thin pointed ends and completely or partially lignified wide pith are important markers for anatomical identification. Tuberous root powder showed 2% of total ash, maximum extractive value 41.25% in ethanol. Powder is also characterized by presence of acicular raphides and raphide bundles. Saponins, flavonoids and glycosides are the main active phytoconstituents present in root and HPTLC fingerprint shows 11 bands in methanolic extract. The study reveals some diagnostic indices for identification of genuine 'safed musli'.

Keywords: Asparagus, Secondary metabolites, phytochemical analysis, Pharmacognosy.

1. Introduction
India is one of the most biologically diverse countries in the world where the uses of herbs for healing diseases is part of a time honored tradition and are even practiced today. Ancient Indian literature incorporates a remarkably broad definition of medicinal plants and considers all plants to be potential sources of medicinal substances [1]. In India, most of these medicinal plants and their products are still in use as home remedies and there is an urgent need to document them using proper scientific research. With this backdrop, it becomes extremely important to make efforts towards standardization of plant materials to be used as medicine. However, standardization of natural product is a complex task due to their heterogeneous compositions, which is true for either the whole plant or any plant part or extracts obtained thereof. According to WHO (World Health Organization) the macroscopic and microscopic descriptions, phytochemical, biochemical and physiochemical characteristics are some steps towards establishing the identity and degree of purity for plant material [2]. Further, quality assurance of the herbal raw drug is also an essential pre-requisite to ensure reproducible quality of final formulations.

Asparagus adscendens Buch. Ham. ex Roxb. (Family Asparagaceae) is also known as shweta musali or satavar bhed which is used to treat female disorders, seminal weakness, importance and as a nutritive tonic [3, 4]. It is distributed in the Western Himalaya, from Himachal Pradesh to Kumaon, between the altitudes ranging from 500 – 1800 m. It is used in treatment of diarrhea and dysentery [5] and is also found to be effective as demulcent [6], galactagogue [7] and antifilarial [8]. Aqueous extract of *A. adscendens* was found to have antidiabetic potentials as it stimulated both the secretion and action of insulin as well as inhibiting starch digestion in clonal pancreatic β cell line [9]. The main active constituents of the roots of *A. adscendens* are steroidal saponins and sapogenins [10]. Although, considerable amount of scientific literature on *A. racemosus*, a closely related species is available [11], systematic studies on *A. adscendens* are negligible. Hence, the present investigation was undertaken to standardize various pharmacognostical and phytochemical parameters of *A. adscendens* which would serve as a measure of authentication and quality control for commercial samples of crude drug.

2. Material and Methods

2.1 Plant Material

Asparagus adscendens was collected in the month of October 2008 from Palampur forest regions (N 32º 08' 54.5" E 76º 23' 32.4" Alt: 1089 m) of Himachal Pradesh, India and domesticated at Directorate of Medicinal and Aromatic Plants Research (DMAPR) experimental field at Anand (N 22^0 35' 56.07" E 072^0 55' 59.64"), Gujarat. Fresh tuberous roots were collected from field and washed, shade dried and packed in a paper bag for further physico-chemical and phytochemical analyses. For anatomical study, material was fixed in FAA (Formalin: Acetic acid: Alcohol, 90:5:5). A voucher specimen of the collected plant material was prepared, authenticated and deposited at NBRI, Lucknow.

2.2 Morphological characters

The macroscopic characters such as size, shape, colour, surfaces features, odour and taste of the fresh stem, cladode and tuberous roots were recorded [12, 13] and photographed using digital camera (DSC W220, Sony Corp, Japan). Size measurements were made using vernier caliper and the microscopic observations were recorded under stereoscopic photo-microscope (Olympus BX50, Olympus corp., Japan) using a micrometer.

2.3 Anatomical Characters

Plant materials such as stem and cladodes were freshly collected from the field and fixed for anatomical studies. Free hand sections of materials were made by using sharp blades, stained with Tolueidene blue (0.5%). Anatomical features were recorded for further analysis as per standard methods [14]. Finely ground root powder was also examined microscopically and photographed under different magnifications.

2.4 Physico-chemical Parameters

Physico-chemical constants such as percentage of total ash, acid-insoluble ash, water soluble ash, yields of petroleum ether, chloroform, methyl alcohol and water as well as powder fineness, bitterness and foreign matter were calculated as per the methods of Indian Pharmacopoeia [15]. Total carbohydrate and protein contents were determined following the methods of Yemm and Willis, 1954 [16] and Lowry *et al*, 1951 [17] respectively.

2.5 Fluorescence Analysis

Fluorescence behavior of root powder was observed and recorded in daylight and ultraviolet light [17]. In a fluorescence study, the dry root powder was taken in a watch glass and treated with various chemicals (Table 2) and observed under 254 and 366 nm.

2.6 Phytochemical Evaluation

Powdered tuberous root (2 g) was extracted separately by cold extraction (keeping overnight on shaker) with 50 ml each of hexane, chloroform, methanol, n-butanol and water. All the extracts were dried and weighed to obtain the extractive value. The presence of different compounds viz. alkaloid (Dragendorff's test), anthraquinone (Borntrager's test), flavonoid (Shinoda test), steroidal glycosides (LB test), proteins (Biuret test), reducing sugar (Fehling solution test) and saponin (foam test) etc. were detected by usual methods prescribed in standard texts [18, 19].

2.7 HPTLC finger printing

The root powder (5 g) was extracted with 85% methanol on a water bath for one hour, concentrated on a rotary evaporator (Heidolph Laborota, 4010 digital) and dried. A stock solution (10 mg ml^{-1}) was prepared in methanol and suitably diluted stock solution was spotted on pre-coated silica gel G60 F_{254} TLC plates (Merck, Germany) using CAMAG Linomat V (CAMAG) applicator and the plates were developed using chloroform: methanol: glacial acetic acid: water (7: 3: 3.5: 1.5) and visualized by treating with anisaldehyde sulphuric acid. Developed plates were scanned using TLC Scanner 3 (CAMAG) at 366 nm and 254 nm. All photographs were made with the help of Reprostar 3 (CAMAG) digital camera. Similarly, chloroform extract of the samples were also processed and scanned at 254 nm.

3. Results

3.1 Morphological characters of the plant

Fig 1: morphological features of aerial parts **A.** Whole plant in natural conditions, **B.** Close up view of stem and branches **C.** Completely developed branch, **D.** Cladodes arrangement on side branch

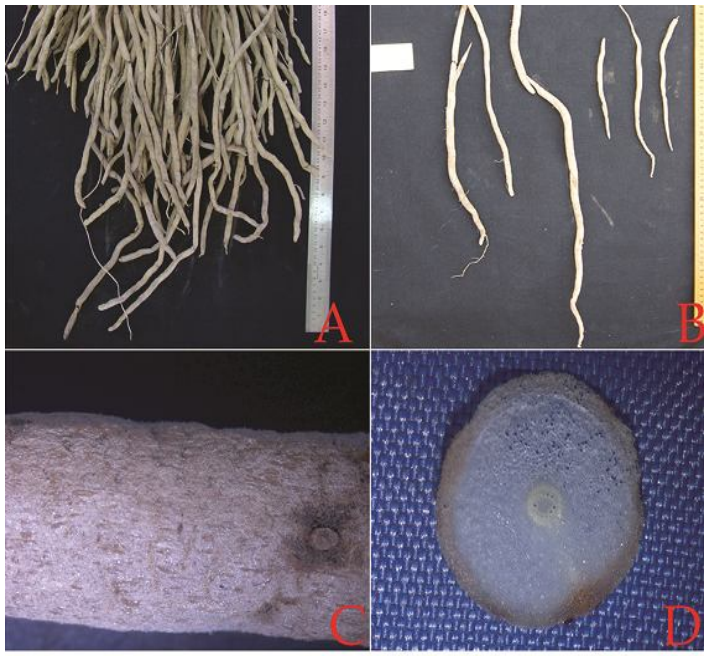

Fig 2: morphological features of tuberous roots **A.** Whole root system of well grown plant, **B.** variations in root length **C.** Close up view surface of roots **D.** Whole mount TS of root

The tuberous roots are 30 – 100 cm long, 0.7 – 1.5 cm diameter (Fig 1d), odourless, cylindrical with slightly tapering ends and sweetish in taste. Older tubers were dark brown whereas young tubers pale yellow in colour. Scars and protuberances of lateral rootlets were seen all over the outer surface with longitudinal wrinkles. Texture was hard and roots breaks with uneven fibrous fracture when dried. Irregular longitudinal furrows developed when root was peeled and dried.

3.2 Anatomical characters of the plant

3.2.1 Microscopic characters of Stem

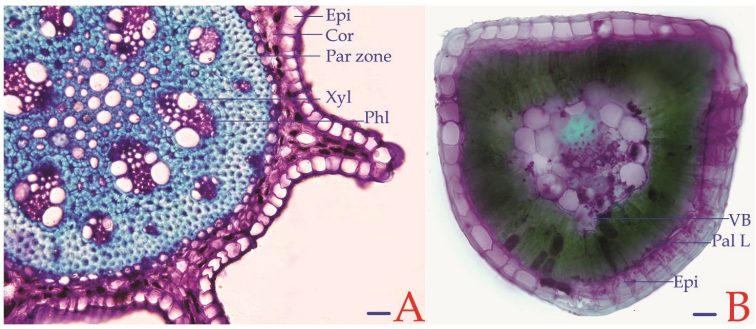

Fig 3: Anatomical features of aerial parts **A.** TS of young stem (400x) (Epi-Epidermis, Cor-Cortex, Par Zone, Xyl - xylem, Phl – phloem) **B.** TS of cladode (100x) VB - Vascular Bundle, Pal L – Palisade layer Epi – Epidermis)

T.S. of young stem showed presence of ridges and furrows along with most of the typical monocot characters such as large pith and irregularly arranged vascular bundles (Fig 2). Epidermis consisted of single layer of dumbbell shaped cells possessing thick layer of cuticle followed by cortex region. Cortex was seen differentiated into outer 3-4 layers of collenchymatous cells below ridges and furrows and an inner continuous layer of parenchymatous cells. Vascular bundles were found scattered irregularly with larger ones placed towards the inner side and smaller ones developing in peripheral region. Vascular bundles were of collateral and closed type with metaxylem present on its outer side. In addition centrally placed pith composed of larger cell size (Fig 2).

3.2.2 Microscopic characters of cladodes

Horizontal section of cladodes viewed triangular in outline with a single cell layer of dumbbell shaped epidermal cells. Below the epidermis 2-3 palisade cell layers with centrally placed vascular tissue zone was present. Xylem and phloem were found intermixed in the central zone.

3.2.3 Microscopic characters of the root

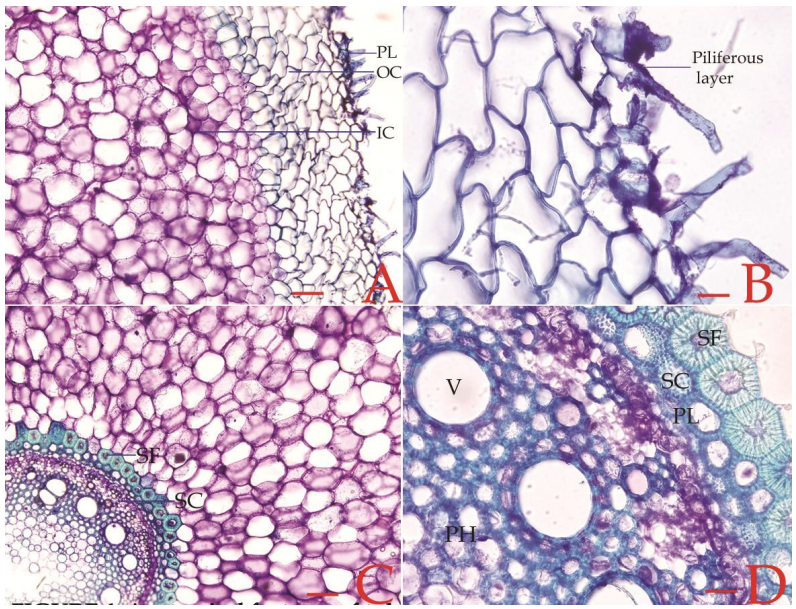

Fig 4: Anatomical features of tuberous roots **A.** Cortex layer divisible in two parts (100x) (PL-piliferous layer, OC- Outer cortex IC – Inner cortex **B.** Close up view of piliferous layer (400x) **C.** piliferous layer **D.** Magnified view of piliferous layer (400x) (v-vessel, SF – Sclerenchymatous fibers, SC – stone cell, PL – Pericycle layer, PH pith)

Transverse section of root clearly showed the outermost layer of epidermal cells, compactly arranged, thick walled cells forming the piliferous layer. Below the epidermis two types of cortex were present i.e. outer lignified cortex and inner parenchymatous cortex. Sclerenchymatous fibres were found scattered in the cortex while some of them on maceration appeared as scattered fibres. A well-developed sheath of stone cells surrounding the endodermis was present at all levels of root. The innermost 1 or 2 layers of cortex immediately outside endodermis comprises of thick walled cells, with numerous circular or oval pits on their walls. The endodermis beneath the sheath shows thickened radial and inner tangential walls. Inner to endodermis, a single layer of thin-walled, parenchymatous cells constituting the pericycle

was present in form of a ring, which surrounds the central stele. Phloem and xylem groups, many in number, were arranged on alternate radii and form a ring. In some root samples, especially from plants growing in shaded places, the cortical sclerenchymatous fibres were confined either to the peripheral region only or were absent. Tracheids with usual thin pointed tapering ends and wide pith comprising of completely or partially lignified rounded cells were present [20].

3.3 Powder study of the root

Tuber powder was pale yellow in colour, odorless and slightly sweetish in taste. Powder showed mostly cortical parenchyma consisting of thin walled polyhedral cells with or without intra cellular spaces. Large number of vessel elements either entire or fragments form showing all kinds of thickenings like scalariform, annular and pitted were found. Fragments of tracheids with linear pits were also found occasionally. Acicular raphides and raphide bundles were seen in large number while, some amount of starch grains was observed throughout the powder.

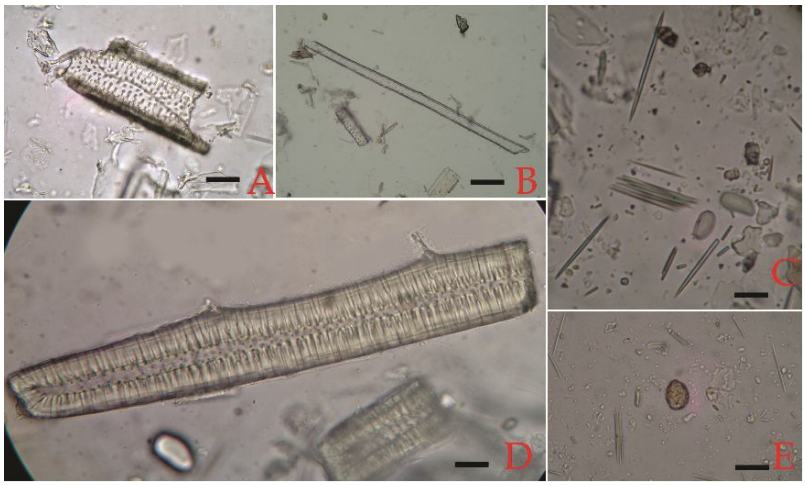

Fig 5: Powder character of the root **A.** Vascular elements (100x) **B.** Tracheids fragments (100x) **C.** Acicular Raphides and Raphide Bundles (100x) **D.** Vessel elements (400x) E. Starch grains (100x)

3.4 Physicochemical studies of root

The percentage of total ash, acid insoluble ash, water soluble ash, bitterness, powder fineness and foreign matter were determined and presented in Table 1. Results of ash analysis indicated the purity of drug, while rest of the parameters gave adulteration or contamination possibility in the drug. Analysis of root powder showed that it contained 2% total ash, 0.25% acid insoluble and 1.3% water soluble ash. The root powder is mildly sweet and did not show any bitterness when tasted. The powder was moderately coarse in nature and without any foreign material (Table 1). Further analysis showed that the roots powder contained carbohydrates (30.65 mg g^{-1}) and protein (0.76 mg g^{-1}) on dry weight basis.

Table 1: Physicochemical properties of root powder of *Asparagus adscendens*

Parameters	Total ash	Acid insoluble ash	Water soluble ash	Bitterness value	Powder fineness/sieve size	Foreign matter
Content	2.0%	0.25%	1.3%	Negligible	Moderately coarse	None

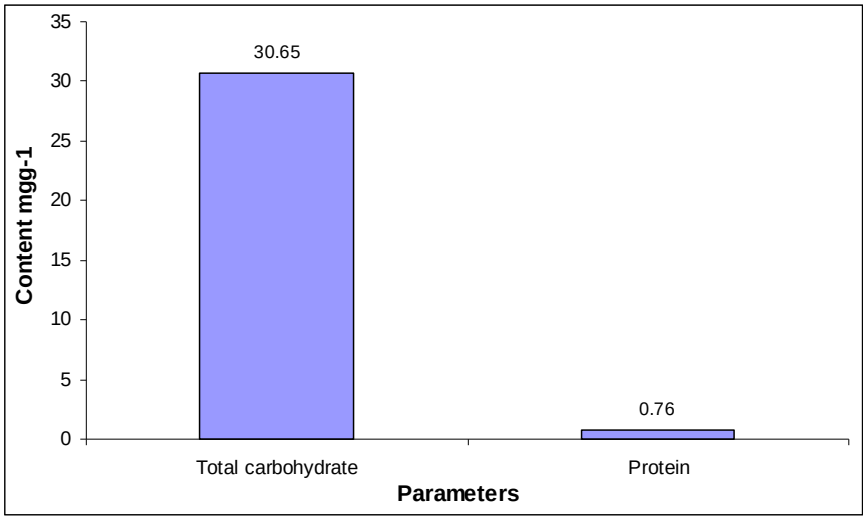

Fig 6: Biochemical constituents of root powder

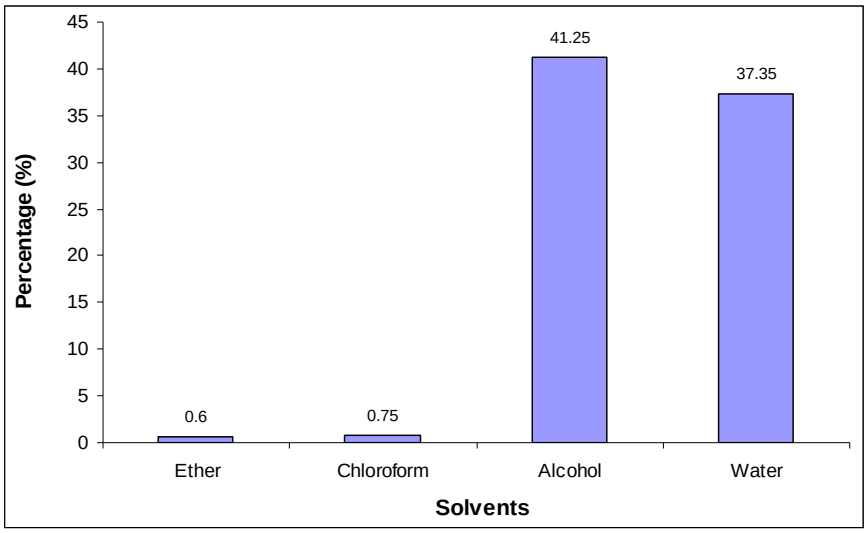

Fig 7: Extractive values of root powder

The powdered tuberous roots contained different extractive values with different solvents and ranges from 0.6 to 41.25%. Maximum yield was obtained using alcohol (41.25%) and minimum (0.6%) in petroleum ether extract.

3.5 Fluorescence analysis of root powder

Table 2: Fluorescence behavior of root powder of *Asparagus adscendens*

Sample	Natural light	Under 254 nm	Under 366 nm
Powder as such	Yellow white	Purple bluish	White
Powder + Saturated picric acid	Yellow	Yellow brown	Dark brown
Powder + Nitric acid	Reddish yellow	Brownish	Brown
Powder + Hydrochloric acid	Light brown	Bluish black	Bluish green
Powder + 50% Sulphuric acid	Dark brown	Black	Blackish green
Powder + glacial acetic acid	Light yellowish	Light brown	Yellow
Powder + 5% Ferric Chloride sol^{n}	Yellowish	Brown	Dark brown
Powder + 2N Sod. hydroxide sol^{n}	Brownish	Brown	Green
Powder + Aqueous iodine sol^{n}	Reddish brown	Brown	Dark brown

3.6 Preliminary phytochemical analysis of root

Table 3: Bio-chemical characteristics of root powder of *Asparagus adscendens.*

Phytoconstituents	Hexane	Chloroform	Methanol	Butanol	Water
Alkaloids	-	-	-	-	-
Anthraquinones	-	-	-	-	-
Flavonoids	-	-	+	+	+
Glycosides	-	-	+	+	+
Proteins	-	-	-	-	+
Reducing Sugars	-	-	-	-	+
Saponins	-	-	+	+	+

3.7 Chromatographic finger print profile

HPTLC profiles of chloroform and methanol extracts were developed (Figure 3 and 4). Both chloroform and methanolic extract showed 9 and 11 bands each on scanning at 254 and 366 nm respectively. Rf values and relative percentage of the separated compounds were recorded and given in Table 4 and 5.

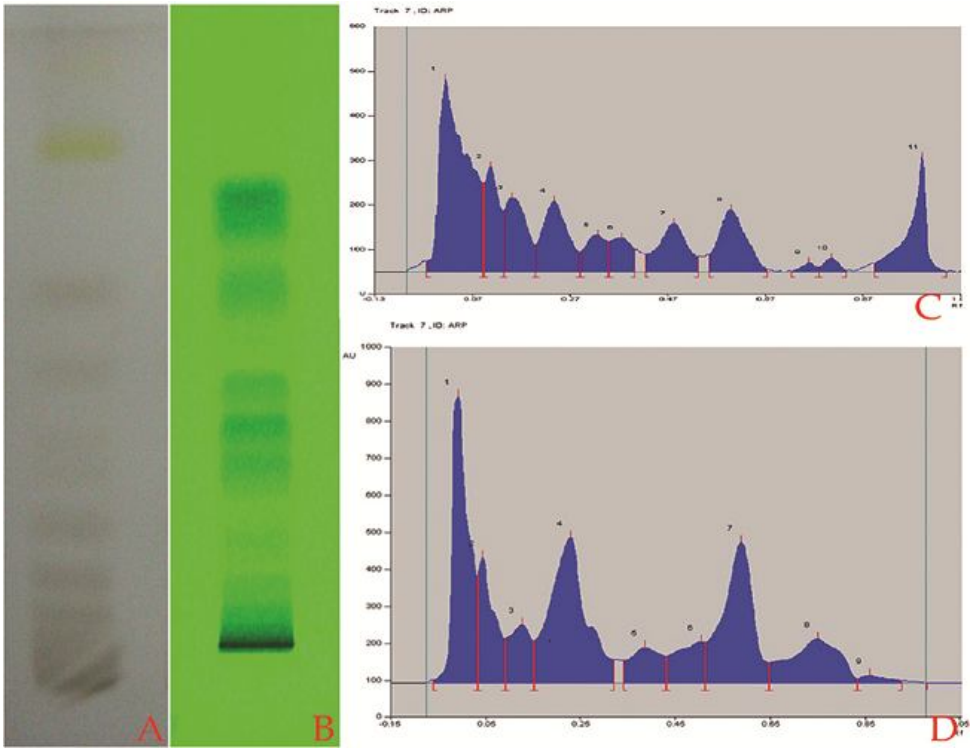

Fig 8: HPLTC Fingerprint profile of Methanol and chloroform extract of roots
A. Banding pattern of methanolic extract
B. Banding pattern of chloroform extract
C. Spectra of methanolic extract bands after densitometric scan at UV 366nm
D. Spectra of Chloroform extract bands after densitometric scan at UV 254nm

Table 4: HPTLC profile of methanol extract of the root powder of *Asparagus adscendens*

Peak no.	Rf	Max Height	Relative amt (%)	Total Area (%)
1	0.01	432.2 AU	25.32	31.91
2	0.10	236.0 AU	13.82	8.83
3	0.15	166.1 AU	9.73	9.34
4	0.23	158.2 AU	9.26	10.34
5	0.32	82.4 AU	4.83	4.46

6	0.37	76.6 AU	4.49	4.17
7	0.48	107.9 AU	6.32	8.13
8	0.60	138.9 AU	8.13	9.96
9	0.76	21.0 AU	1.23	0.71
10	0.80	31.0 AU	1.82	1.18
11	0.99	257.0 AU	15.05	10.96

Table 5: HPTLC profile of chloroform extract of root powder of of *Asparagus adscendens*

Peak no.	Rf	Max Height	Relative amt (%)	Total Area (%)
1	0.01	775.1 AU	32.40	21.14
2	0.04	339.5 AU	14.19	9.04
3	0.13	158.0 AU	6.60	5.74
4	0.23	392.8 AU	16.42	23.81
5	0.39	95.2 AU	3.98	5.03
6	0.51	111.2 AU	4.65	5.39
7	0.59	380.6 AU	15.91	18.93
8	0.75	118.4 AU	4.95	10.03
9	0.86	21.4 AU	0.90	0.89

4. Discussion

It has been reported that genus *Asparagus* has about 300 species and their roots are rich source of sapogenins and saponins [1]. The steroidal saponins were reported to have antitumour, immunostimulant, immunoadjuvant, antiinflammatory and antibacterial properties [21]. Out of the several species available in India, *A. racemosus, A. gonoclados* and *A. adscendens* are most commonly used in indigenous medicine and traded under the name 'Satavari' [22].

Similarly *Chlorophytum borivilianum, C. arundinaceum, C. tuberosum* and *A. adscendens* have been in use as aphrodisiacs under the common name of 'Safed Musli' and because of its white tuberous roots [23] it becomes difficult to distinguish. Therefore some diagnostic features have been described to identify and differentiate *A. adscendens* from crude drugs and adulterants. *A. adscendens* differed by erect to sub-erect shrub type of habit in comparison to climbing habit of other *Asparagus* sp. Besides number of cladodes were more than 6 which is up to 6 in *A. racemosus* and length of tuberous root ranges 30-100 cm in *A. adscendens* while a maximum of 50 cm is observed in *A. racemosus*. Anatomical study confirms that specialized types of sclerenchymatous fibers were found scattered around endodermis of root, which were totally absent in *A. racemosus* instead a single layered pericycle was present under the endodermis of root which was followed by vascular bundles and pith. Macro and microscopic characteristics of root had been earlier studied by some worker[23]. However, only microscopic study did not reveal much about medicinal value of the crude drug therefore physico-chemical, biochemical, phytochemical and HPTLC data can serve as valuable information for studying the purity of the drug. Ash analysis revealed 2% total ash and 0.25% acid insoluble ash in *A. adscendens* while 5% of total ash and 0.6% of acid insoluble ash is obtained in *A. racemosus* [24]. Yield of ethanol soluble extract (41.25%) in root of *A. adscendens* was higher as compared to *A. racemosus* (9%) [25]. Preliminary qualitative analysis showed its root contained flavonoids, glycosides and saponins.

5. Conclusion

In conclusion, it can be stated that the standardization parameters used in the present investigation will provide a way for identification of raw materials and formulation prepared from herbal origin. The structural features like, sub-erect struggling shrubby habit with height upto 3 m, presence of 6-10 cladodes per node and 30–100 cm long tuberous roots are morphological identification markers, whereas presence of sclerenchymatous fibres particularly outside the endodermis, presence of single parenchymatous layered pericycle inside the endodermis, tracheids having thin pointed ends and completely or partially lignified wide pith are important markers for anatomical identification. Dried tuberous root possessed pale yellow colour at immature condition while at mature state it turned dark brown with prominent scars and protuberances. Root powder is characterized by the presence of acicular raphides and raphide bundles. HPTLC profile produced 9 and 11 bands on scanning at 254 and 366 nm respectively which serves as fingerprint of the drug. These parameters could be further useful in preparation of Herbal Pharmacopoeia.

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