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PHARMACOGNOSTICAL IDENTIFICATION OF ASPARAGUS RACEMOSUS WILLD. (ROOT) WITH THE HELP OF HPTLC METHOD

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ABSTRACT

Asparagus racemosus commonly known as Shatavari is used in traditional systems of medicine as a uterine tonic, as a galactogogue, in hyperacidity, and as a general health tonic. The present study provides a detailed pharmacognostic study based on its physicochemical, macroscopic, microscopic and chromatographic features. The physicochemical parameters such as loss on drying, solubility in different solvents, ash content, acid insoluble ash, water soluble ash, volatile oil, fibre content etc. were determined by standard methods. Anatomical features of the roots of *Asparagus racemosus* were determined. For this the sample was fixed in FAA, cast into paraffin blocks and sectioned with the help of Rotary Microtome. The stomata morphology, venation pattern and trichome distribution were studied. Microscopic descriptions of tissues were supplemented with micrographs wherever necessary. Photographs of different magnifications were taken with Nikon Labphot 2 Microscopic Unit. Powder microscopy was carried out using standard methods. HPTLC profile of the ethyl acetate plant extract was carried out in short UV, long UV and using vanillin-sulphuric acid as detection reagent. The R_f values of the spots developed were noted

which is an important parameter for identification of plant materials. The pharmacognostical parameters along with the HPTLC profile may be utilized to identify the drug material and for laying down the pharmacopoeial standards.

KEY WORDS: *Asparagus racemosus* , pharmacognostic study, microscopic features, physicochemical parameters, HPTLC profile

INTRODUCTION

Asparagus racemosus is an important plant in traditional medicine in tropical and subtropical India. Its medicinal usage has been reported in the Indian and British Pharmacopoeias and in traditional systems of medicine such as Ayurveda, Unani and Siddha. *Asparagus racemosus* is the accepted botanical source of Shatavari [1]. *Asparagus racemosus* (Satavar, Shatavari, or Shatamull) (Figure 1) is common throughout Sri Lanka, India and the Himalayas. It grows one to two metres tall and prefers to take root in gravelly, rocky soils high up in piedmont plains, at 1,300–1,400 metres elevation[2]. Shatavari has small pin-needle-like phylloclades (photosynthetic branches) that are uniform and shiny green. In July, it produces minute, white flowers on short, spiky stems, and in September it fruits, producing blackish-purple, globular berries. It has an adventitious root system with tuberous roots that measure about one metre in length, tapering at both ends, with roughly a hundred on each plant.

Figure 1: Asparagus racemosus

Due to its multiple uses, the demand for *Asparagus racemosus* is constantly on the rise. Due to destructive harvesting, combined with habitat destruction, and deforestation, the plant is now considered 'endangered' in its natural habitat.

The roots are used in medicine, following a regimen of processing and drying. It is generally used as a uterine tonic, as a galactogogue (to improve breast milk), in hyperacidity, and as a general health tonic. *Asparagus racemosus* is recommended in Ayurvedic texts for the prevention and treatment of gastric ulcers, dyspepsia and as a galactogogue. *A. racemosus* has also been used for nervous disorders [3]. The name Shatawari means "curer of a hundred diseases" (shat: "hundred"; vari: "curer").Fasciculated tuberous roots of Shatavari is considered as one of the Rasayana (adaptogenic) drugs, having cooling, diuretic, emollient, galactogogue, nervine tonic, rejuvenating and stomachic properties [4].

Phytochemical reports show that Asparagamine A, a polycyclic alkaloid , two new steroidal saponins, shatavaroside A and shatavaroside B together with a known saponin, filiasparoside C, were isolated from the roots of *Asparagus racemosus* [5]. Five steroidal saponins, shatavarins VI-X, together with five known saponins, shatavarin I (or asparoside B), shatavarin IV (or asparinin B), shatavarin V, immunoside and schidigerasaponin D5 (or asparanin A), have been isolated from the roots of *Asparagus racemosus* [6]. Isoflavone, 8-methoxy-5,6,4'-trihydroxyisoflavone 7-O-beta-D-glucopyranoside was also isolated [7].

MATERIALS AND METHODS

Plant material

Fresh root tubers of *A. racemosus* (Figure 2) were collected from the out skirts of Chennai. Botanical identification was carried out using local floras [8].

Figure 2: *Asparagus racemosus*: Exomorphic features of Tuberous storage root

Anatomical studies of *A. racemosus*

The samples were fixed in FAA (Formalin - 5 mL + Acetic acid - 5 mL + 70% Ethyl alcohol - 90 mL). After 24 hour of fixing, the specimens were dehydrated with graded series of

tertiary-Butyl alcohol (TBA) as per standard procedure [9]. Infiltration of the specimens was carried by gradual addition of paraffin wax (melting point 58-60°C) until TBA solution attained super saturation. The specimens were cast into paraffin blocks.

Sectioning

The paraffin embedded specimens were sectioned with the help of Rotary Microtome. The thickness of the section was 10 - 12 µm. Dewaxing of the sections were done by customary procedure [9]. The sections were stained with toluidine blue as per the method published by O'Brien *et al*., (1964) [10]. Since toluidine blue is a polychromatic stain, the staining results were remarkably good; and some cytochemical reactions were also obtained. The dye rendered pink colour to the cellulose walls, blue to the lignified cells, dark green to suberin, violet to the mucilage, blue to the protein bodies etc. Wherever necessary the sections were also stained with safranin and Fast green and IKI (for starch). To study the stomata morphology, venation pattern and trichome distribution, para dermal sections (sections taken parallel to the surface of leaf) as well as clearing of leaf with 5% sodium hydroxide or epidermal peeling by partial maceration employing Jeffrey's maceration fluid [9] were prepared. Glycerine mounted temporary preparations were made for macerated/ cleared materials.

Photomicrographs

Microscopic descriptions of tissues were supplemented with micrographs wherever necessary. Photographs of different magnifications were taken with Nikon Labphot 2 Microscopic Unit. For normal observations bright field was used. For the study of crystals, starch grains and lignified cells, polarized light was employed. Since these structures have birefringent property, under polarized light they appeared bright against dark background. Magnifications of the figures were indicated by the Scale-bars. Descriptive terms of the anatomical features are as given in the standard Anatomy books [11].

Powder microscopy

Powder microscopy was carried out following the methods of [12]. To study the epidermal tissues, fragments of leaves measuring 1-2 mm^2 were treated with Jeffrey's maceration fluid (5% chromic acid + 5% nitric acid in equal volumes) and partial maceration resulted in the separation of upper and lower epidermis. The peelings were stained with safranin and mounted on drop of glycerine. To visualize the venation system under the microscope, small

bits of the lamina were boiled in alcohol to remove chlorophyll. Then, the material was soaked in warm 10% sodium hydroxide for several hours till the materials became transparent. After total clearing was achieved, the material was washed to remove the alkali from the cells. The cleared materials were stained and mounted in glycerine. Maceration of xylem elements was carried out with the maceration fluid mentioned earlier.

Physico-chemical parameters

The physico-chemical examinations include determination of total ash, acid insoluble ash, and water soluble ash, extractable matter in water and alcohol, loss on drying at 105˚C, volatile oil, swelling index, foaming index, and fibre content. All the physico-chemical parameters and the limit test for arsenic and heavy metals were determined by the methods of WHO (1998) guidelines [13].

Qualitative and quantitative analysis of organic constituents

The chemical tests for different organic constituents were carried out using alcohol extract of the plant material by standard methods [14]. The quantitative analysis of sugar was carried out by Fehling`s solution method. All the reagents used were of GPR grade.

Development of high performance thin layer chromatographic (HPTLC) profile

HPTLC profile of the alcohol extract of the plant material was performed on silica gel 60 F_{254} pre-coated aluminium sheets using CAMAG instrument using HPTLC system (CAMAG, Switzerland) made up of a Linomat sample applicator, a CAMAG twin – trough plate development chamber, CAMAG TLC Scanner 3, CAMAG Reprostar 3 photo document system and WinCATS Software 4.03.The extract for the study was prepared by soaking 4 g of the powdered plant material in 40 ml of alcohol and kept overnight. The solution was boiled for 10 minutes and filtered. The filtrate was concentrated and made up to 10 ml in standard flask The plate was developed in Toluene: Ethyl acetate (5:1.5). The plate was dried and visualised under UV 254 nm and 366 nm, and derivatised using vanillin-sulphuric acid reagent & heated at 105° C till the colour of the bands appeared and photodocumented [15].

RESULTS AND DISCUSSION

Macroscopic characters of the root tuber

The roots of *A. racemosus* are borne in a compact bunch and are fleshy, and spindle-shaped. They are silvery white or light ash-coloured externally and white internally, more or less

smooth when fresh, developing longitudinal, wrinkles when dry. They have no well-marked odour, but sweet and bitter in taste.

Microscopic characters

The root tuber has three anatomical zones Periderm, Cortex / Stele and Pith. Periderm is the narrow outermost zone of tissue measuring less than 50 µm thick. The cells are thin walled, submersed and are radially oblique and oblong (Fig. 3). Cortex / Stele is the central hollow cylinder comprising of wide pith, several radial rows of xylem elements alternating with small nests of phloem elements, a thin layer of pericycle and sclerotic endodermis (Fig. 3a, 3b, 4a). Pith contains the circular and thin walled as well as thick walled cells, which are in random distribution (Fig. 4b). Xylem elements occur in radial files of 2-4 cells, the protoxylem being exarches and the metaxylem elements are thick walled measuring 100 µm in diameter. The metaxylem elements and part of the protoxylem elements are surrounded by sclerenchymatous elements. Phloem elements are in small clusters situated inner to the pericycle and in between the protoxylem cells. Pericycle is a thin, single layer of tabular cells, the walls being cellulostic. Endodermis is two or three-layered sclerotic zone with thick, lignified cells.

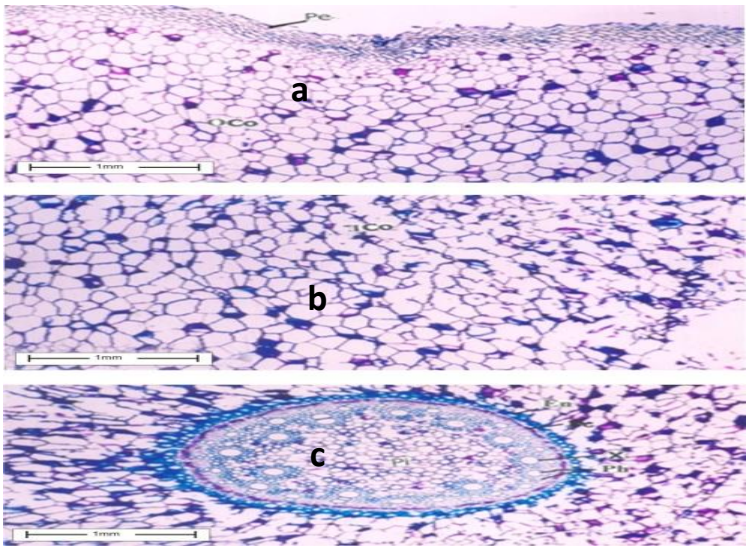


Figure 3: *Asparagus racemosus*: Anatomy of the tuberous root

a. Periderm zone enlarged

b. Inner cortex enlarged

c. Stele enlarged

En – Endodermis; ICO – Innercortex; OCO- Outer cortex; Prx – Protoxylem ; Ph- Phloem; X- xylem; Pe- periderm; C- Cortex

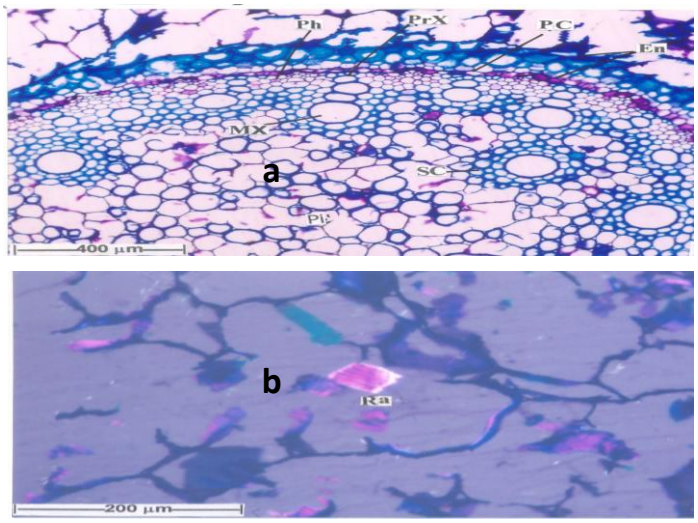


Figure 4: *Asparagus racemosus:* Enlarged stele and pith

a. Stele a sector enlarged

b. Raphides in the pith region

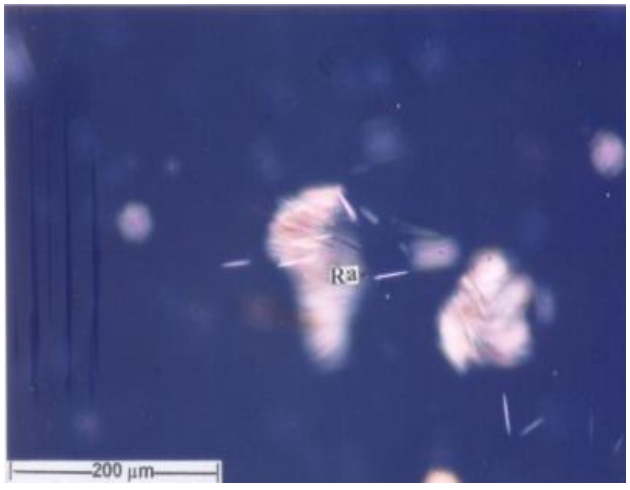
En – Endodermis; MX –Metaxylem; PC-Pericycle; Ph – Phloem; Pi –Pith;

Prx – Protoxylem; Ra – Raphide; Sc – Sclerenchyma

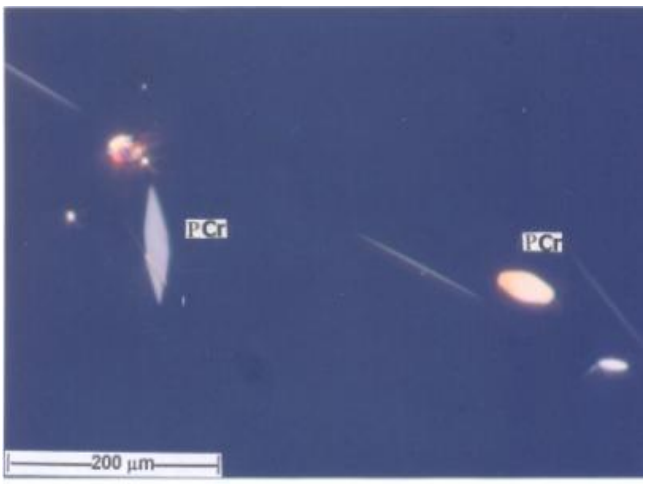
Powder microscopy

Crystals in the root stock

Calcium oxalate raphides are frequently seen in the cortical cells (Fig. 5a). The raphide is short and thick, measuring 40 × 50 µm. The individual crystals are thin needles with pointed ends. Other than the raphides, prismatic crystals of other type are also occasionally seen in the ground tissue. Elongated rhomboidal type and short thick double pyramidal type were seen in the powdered material of the root stock (Fig. 5b).



a



b

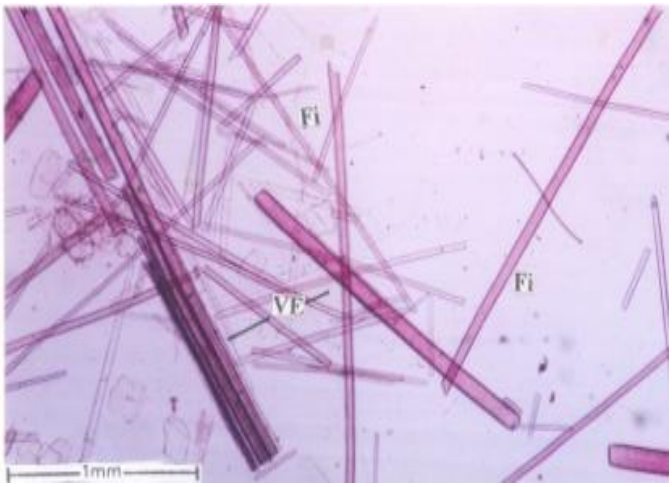
Figure 5: *Asparagus racemosus*: Raphide and Prismatic crystals distribution

a. Broken raphides

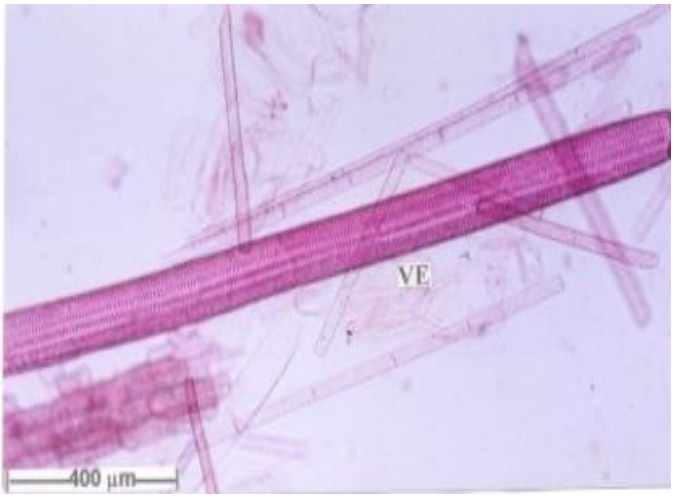
b. Two types of prismatic crystals

Macerated powder of the root stock shows fibre, vessel elements and parenchyma cells (Fig. 6 a) .The vessel elements are long, narrowly cylindrical and many sided. The end wall of the vessel element has circular, horizontal perforation. The lateral wall has several vertical rows of scalariform thickenings (Fig. 6b). The fibres are longer and narrower than the vessel elements. Some of the fibres have dense simple pits and others have no pits. The parenchyma cells are rectangular, thick walled and densely pitted (Fig. 6c).

a



b



c

Figure 6: *A. racemosus*: Powder microscopy of the root

a. Xylem parenchyma and fibres

b. Fibres and vessel elements

c. Vessel element enlarged

Fi – Fibres; VE- Vessel element; XP –Xylem parenchyma

Physico-chemical parameters

The physico-chemical parameters of three samples of the plant material were determined and the mean values obtained were taken. The values obtained for Loss on drying at 105˚C, Total ash, Acid insoluble ash, Water and alcohol soluble extractives, pH of water extract, Volatile oil, Fibre content, Swelling index, Foaming index and Particle size are given in Table 1.

Table 1: Physico-chemical parameters of *A. racemosus* (root)

Sl. No.	Test	Result
1	Loss on drying at 105˚C (%)	11.00
2	Total ash (%)	3.90
3	Acid insoluble ash (%)	0.15
4	Water soluble ash (%)	2.60
5	Volatile oil	Nil
6	Water soluble extractives (%)	27.70
7	Alcohol soluble extractives (%)	21.78
8	pH of water extract	5.00
9	Fibre content (%)	16.50
10	Swelling index (ml/gm)	4.50
11	Foaming index	<100

Qualitative and quantitative analysis of organic constituents

The qualitative organic analysis of the plant material showed the presence of carbohydrates, flavonoids, glycosides, alkaloids, phenolics. Saponins, phenolics, alkaloids and sugar. The estimation of total sugar and reducing sugar was carried out and was found to be 6.42% and 5.13% respectively.

High Performance Thin Layer Chromatography (HPTLC)

The HPTLC of the ethyl alcohol extract of the plant material was carried out. The plates were viewed under UV short, UV long and developed in anisaldehyde sulphuric acid reagent. HPTLC profile is a valuable parameter for identification of plant materials. HPTLC profile of *A. racemosus* is given in Figure 7. The scanned Peak table at 254nm is given in Table 2, 366nm is given in Table 3, at 580nm after derivatisation using anisaldehyde sulphuric acid and heating at105ºC for 5 minutes is given in Table 4.

(a) **(b)** **(c)**

Figure 7: HPTLC profile of ethyl alcohol extract *A. racemosus* at (a) 254 nm, (b)366 nm and (c) Day light after derivatisation and heating at 105°C for 5 minutes (spray reagent - anisaldehyde sulphuric acid) and scanned it at 580 nm

Table 2: Scanned Peak table-After development the plate was scanned at 254nm

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	-0.00 Rf	0.0 AU	0.02 Rf	428.8 AU	35.98 %	0.06 Rf	0.2 AU	13555.2 AU	41.57 %
2	0.07 Rf	0.1 AU	0.09 Rf	241.5 AU	20.27 %	0.11 Rf	33.6 AU	4058.4 AU	12.45 %
3	0.11 Rf	96.0 AU	0.11 Rf	108.8 AU	9.13 %	0.14 Rf	0.5 AU	1777.9 AU	5.45 %
4	0.15 Rf	0.1 AU	0.18 Rf	33.2 AU	2.78 %	0.20 Rf	0.0 AU	723.7 AU	2.22 %
5	0.21 Rf	3.8 AU	0.26 Rf	69.9 AU	5.87 %	0.28 Rf	10.2 AU	1868.9 AU	5.73 %
6	0.28 Rf	10.3 AU	0.32 Rf	73.5 AU	6.17 %	0.36 Rf	0.1 AU	2216.3 AU	6.80 %
7	0.36 Rf	0.0 AU	0.39 Rf	14.1 AU	1.18 %	0.41 Rf	1.1 AU	249.8 AU	0.77 %
8	0.44 Rf	0.4 AU	0.46 Rf	6.6 AU	0.55 %	0.47 Rf	4.0 AU	131.3 AU	0.40 %
9	0.48 Rf	3.8 AU	0.51 Rf	9.3 AU	0.78 %	0.53 Rf	1.5 AU	203.7 AU	0.62 %
10	0.62 Rf	1.6 AU	0.67 Rf	82.5 AU	6.93 %	0.70 Rf	76.9 AU	2857.1 AU	8.76 %
11	0.70 Rf	77.3 AU	0.71 Rf	78.5 AU	6.58 %	0.75 Rf	44.3 AU	2508.7 AU	7.69 %
12	0.75 Rf	43.7 AU	0.76 Rf	45.0 AU	3.78 %	0.87 Rf	0.5 AU	2459.6 AU	7.54 %

Table 3: Scanned Peak table-After development the plate was scanned at 366nm

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	-0.00 Rf	5.6 AU	0.01 Rf	70.0 AU	5.91 %	0.03 Rf	0.0 AU	890.9 AU	2.40 %
2	0.04 Rf	0.9 AU	0.06 Rf	36.0 AU	3.04 %	0.08 Rf	24.5 AU	900.2 AU	2.43 %
3	0.09 Rf	24.7 AU	0.11 Rf	40.6 AU	3.43 %	0.13 Rf	19.8 AU	1045.9 AU	2.82 %
4	0.13 Rf	19.9 AU	0.15 Rf	87.1 AU	7.36 %	0.18 Rf	33.8 AU	2128.8 AU	5.75 %
5	0.18 Rf	34.0 AU	0.22 Rf	108.4 AU	9.15 %	0.28 Rf	16.1 AU	4267.0 AU	11.52 %
6	0.28 Rf	16.1 AU	0.30 Rf	17.9 AU	1.51 %	0.32 Rf	12.4 AU	440.8 AU	1.19 %
7	0.32 Rf	12.5 AU	0.36 Rf	44.4 AU	3.75 %	0.42 Rf	18.5 AU	2315.5 AU	6.25 %
8	0.43 Rf	18.5 AU	0.49 Rf	779.8 AU	65.84 %	0.56 Rf	4.8 AU	25062.4 AU	67.64 %

Table 4: Scanned Peak table-After development the plate was derivatised using anisaldehyde sulphuric acid as spray reagent and heated at105ºC for 5m) and scanned it at 580nm

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.00 Rf	32.0 AU	0.02 Rf	155.7 AU	12.20 %	0.04 Rf	0.3 AU	2451.3 AU	6.34 %
2	0.06 Rf	1.0 AU	0.08 Rf	74.5 AU	5.84 %	0.09 Rf	2.1 AU	864.3 AU	2.24 %
3	0.11 Rf	0.5 AU	0.14 Rf	149.4 AU	11.70 %	0.18 Rf	0.1 AU	4521.8 AU	11.70 %
4	0.21 Rf	0.3 AU	0.26 Rf	157.9 AU	12.37 %	0.29 Rf	31.0 AU	4937.6 AU	12.77 %
5	0.30 Rf	31.3 AU	0.35 Rf	274.6 AU	21.51 %	0.42 Rf	0.0 AU	11894.1 AU	30.76 %
6	0.42 Rf	0.0 AU	0.45 Rf	14.2 AU	1.11 %	0.46 Rf	11.8 AU	231.8 AU	0.60 %
7	0.46 Rf	11.8 AU	0.50 Rf	101.6 AU	7.96 %	0.54 Rf	4.2 AU	2961.1 AU	7.66 %
8	0.57 Rf	1.9 AU	0.59 Rf	5.4 AU	0.42 %	0.60 Rf	0.1 AU	85.9 AU	0.22 %
9	0.61 Rf	0.7 AU	0.67 Rf	64.6 AU	5.06 %	0.70 Rf	27.6 AU	2496.8 AU	6.46 %
10	0.71 Rf	27.8 AU	0.73 Rf	39.7 AU	3.11 %	0.80 Rf	0.2 AU	1758.3 AU	4.55 %
11	0.82 Rf	0.4 AU	0.86 Rf	239.0 AU	18.72 %	0.88 Rf	35.6 AU	6460.0 AU	16.71 %

CONCLUSION

Asparagus racemosus, is more popular in Siddha and Ayurveda for their stimulant, tonic and strengthening properties. Besides these properties, the plant is used to cure many other diseases. Hence the plant was taken up for detailed study pharmacognostical standardization. The physico-chemical parameters obtained for *Asparagus racemosus* serves the purpose of reasonable and dependable standards for the plant material. The macro- and microscopical characters and the powder microscopy of the root tubers along with detection by HPTLC is also presented. The overall result can be utilized to identify the drug material, to differentiate from substitute or adulterant and also in laying down pharmacopeial standards.

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