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ORIGINAL RESEARCH ARTICLE

Asparagus racemosus Willd. – "A Comparative Phytochemical Analysis of Fresh Dried Roots of Shatavari"

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ABSTRACT

Asparagus racemosus Willd. (*Shatavari*) is a well known plant in Ayurvedic systems of medicine. '*Shatavari*' is a reputed classical drug and said to possess therapeutic properties as Rasayana drugs of Ayurveda. One of the Ayurvedic Samhita namely Sharangdhar samhita, it was suggested to use *Shatavari*, only in fresh conditions. At the same time, certain formulations included it in Churna form also. In classics, fresh root of this plant is said to be used for the treatment. But in practice dry roots used for the preparation. The roots have been taken into for investigated thoroughly its macroscopic and microscopic characters and phytochemical study in both dry condition and fresh condition. Keeping this in view, an attempt has been made to identify the significant phytochemical differences among the fresh and dry samples of *Shatavari*. The present paper deals with the detailed macroscopic and microscopical characters and phytochemical study of the fresh and dry root of the plant.

Key words: *Asparagus racemosus* Willd., *Shatavari*, Phytochemistry, Dry root, Fresh root.

INTRODUCTION

Medicinal plants are the nature's gift to human being to make disease free healthy life. India is one of the most medico-culturally diverse countries in the world where the medicinal plant sector is part of a time-honoured tradition that is respected even today. Owing to the global trend towards improved 'quality of life', there is considerable evidence of an increase in demand for medicinal plant [1]. India is richly endowed with a wide variety of plants having medicinal value. These plants are widely used by all sections of the society either directly as folk remedies or indirectly as pharmaceutical preparation of modern medicine [2].

Allopathy has already accepted the potential of the herbs as a source of new bioactive constituents. There are number plant drugs possessing good therapeutic properties but have not been found to be explored therapeutically hence scientific investigations are needed for them to comprehend their therapeutic properties. India can contribute to the global market for herbals, raw materials and Phytochemical because of its extensive flora, expert trained technocrats, great plant heritage and other resources. The *Asparagus* genus is considered to be of medicinal importance because of the presence of steroidal saponins and sapogenins in various parts of the plant [3]. Root is widely used in many formulations. Therapeutic properties mainly depend upon the constituents present in the root. Root mainly contains alkaloids, glycosides, carbohydrate, mucilage, starch, steroids and calcium carbonate.

In the *Ayurvedic* classics named *Sharangdhar Samhita* [4], it was suggested to use certain drugs like Guduchi, Kutaj, Vasa, *Shatavari*, etc. only in fresh conditions i.e. *Sadaiv ardravastha*. At the same time, certain formulations included them in *Churna* form. Keeping this in view, an attempt has been made to identify the significant

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phytochemical differences among the fresh and dry samples of *Shatavari (Asparagus racemosus* Willd.).

Now a days whatever researches are done and published in journals, magazines and scientific seminar proceedings mostly deals only with therapeutic evaluation of drug. But nobody pays attention towards the basic state of the drug whether it is fresh or dry. This really forms the most important part and parcel of the study.

MATERIALS AND METHODS

Collection and authentication

In Month of July, 2011, *Asparagus racemosus* Willd. W.S.R. was collected from its natural habitat i.e. 1km away from Ranjitsagar Damm, Jamnagar, Gujarat, India, in fully matured condition, after being identified as Shatavari. The correct botanical identity and authenticity of the plant as *Asparagus racemosus* Willd. W.S.R. (Liliaceae) was done by studying its morphological characters and comparing them with the characters mentioned in various floras. Collected samples were matched with herbarium of Pharmacognosy Department, I.P.G.T. & R.A., Gujarat Ayurved University, Jamnagar. Roots were washed properly under running water to make them free from foreign matter like sand, soil etc. Leaves were dried under shade, powdered to 60# and few whole leaves were preserved in solution of FAA (70% Ethyl alcohol: Glacial acetic acid: Formalin) in the ratio of (90:5:5) [5]. Later, subject experts of Dravyaguna department and Pharmacognosy department, I.P.G.T. & R.A., Gujarat Ayurved University further confirmed the identification.

Phytochemical studies:

Physicochemical parameters [6]:

Physicochemical study of two samples was carried out by using various physiochemical parameters as mentioned in Ayurvedic Pharmacopoeia of India, Indian Pharmacopoeia.

1. Determination of Loss on drying.
2. Determination of Total ash.
3. Determination of Acid insoluble ash.
4. Determination of Extractive value.
    a) Methanol soluble extractive value.
    b) Water soluble extractive value.
    c) Chloroform soluble extractive value.
    d) Hexane soluble extractive value.

Preliminary qualitative chemical test [9]:

The methods employed to isolate these substances are termed as extraction methods. Crude extracts obtained from such extraction can be qualitatively tested to ascertain the presence of different types of components.

Preparation of Plant Extracts:

5 gm of dry root powder and fresh root paste was extracted with methanol (100ml), keeping it for overnight with initial occasional shaking up to 6 hours and then set aside. After 24 hours it was filtered and alcoholic extract was collected. Similarly chloroform extract and water extract were prepared and collected.

- Series of tests were performed in this section to identify the presence of different chemical constituents.

Quantitative estimation [7]:-

1. Quantitative estimation of Tannin.
2. Quantitative estimation of Sugar.
3. Quantitative estimation of Saponin

Chromatographic study:- HPTLC STUDY [8]

For the HPTLC study following samples were prepared which were titled as Track 1 and Track-2.

Track-1:-*Asparagus racemosus* Willd fresh root paste methanol extract

Track 2:-*Asparagus racemosus* Willd. dry root powder methanol extract

Mobile phase [8]: -Toluene: Ethyl acetate: Water (5: 2.5: 2)

Spray reagent [8]:- Vanillin Sulphuric acid (5%)

RESULTS & DISCUSSION

Qualitative tests:

S. No	Qualitative tests	Sample (A)	Sample(B)
1	Test for Alkaloids		
(i)	Dragendorff's reagent	(-ve)	(-ve)
(ii)	Mayer's reagent	(-ve)	(-ve)
(iii)	Wagner's reagent	(-ve)	(-ve)
2	Test for Tannins	(+ve)	(+ve)
3	Test for Triterpenes (steroids)		
(i)	Salkowski reaction:	(+ve)	(+ve)
(ii)	Libermenn Buchard:	(-ve)	(-ve)
4	Test for Saponins	(+ve)	(+ve)
5	Test for Flavonoids / Shinoda's test	(-ve)	(-ve)
6	Test for Carbohdyrates / Fehling's test	(+ve)	(+ve)
7	Test for Aminoacids	(-ve)	(-ve)
8	Test for Protein	(+ve)	(+ve)

Sample (A) - Fresh paste of *Shatavari* roots (*Asparagus racemosus* Willd.)

Sample (B) - Dry powder of *Shatavari* roots (*Asparagus racemosus* Willd.)

This table shows the presence of tannin, saponin, protein and carbohydrate in both samples. While amino acid, flavonoids, steroids, alkaloids were absent in both samples.

Quantitative estimation of tannin: 0.83 % tannin present in both the samples A and B.

Quantitative estimation of sugar:

Sample	% of Reducing sugar	% of Non-reducing sugar	% of Total sugar
A	0.94	4.69	5.53
B	3.91	24.54	28.55

This table indicates that there is more amount of reducing, non-reducing and total sugar in dry sample as compared to the fresh sample.

Quantitative Estimation of Saponin:

Sample	% of Saponin
Sample – A	0.9%
Sample – B	4.04%

HPTLC RESULTS

Solvent system: Toluene: Ethyl acetate: Water (5**:** 2.5**:** 2)

Sample: Methanolic extract of sample.

Track	Mobile phase	Observation under U.V				Post chromatographic Visualization	
		254nm		366nm		After spray	
		No. of spots	R_f	No. of spots	R_f	No. of spots	R_f
1	Toluene: Ethyl acetate: Water 5:2.5:2	7	0.01, 0.11, 0.15, 0.27, 0.45, 0.59, 0.67	17	0.01, 0.04, 0.11, 0.13, 0.22, 0.27, 0.30, 0.38, 0.42, 0.44, 0.47, 0.57, 0.59, 0.62, 0.67, 0.70, 0.72	4	0.09, 0.17, 0.22, 0.38
2		11	0.08, 0.13, 0.24, 0.27, 0.31, 0.35, 0.45, 0.55, 0.59, 0.64, 0.70	15	0.08, 0.11, 0.14, 0.17, 0.27, 0.31, 0.35, 0.38, 0.40, 0.44, 0.49, 0.55, 0.59, 0.64, 0.68	3	0.03, 0.26,0.39

- In track-1, 7 spots were seen under 254nm at R_f 0.01, 0.11, 0.15, 0.27, 0.45, 0.59 and 0.67. While in track-2 11 spots were seen under 254 nm at R_f 0.08, 0.13, 0.24, 0.27, 0.31, 0.35, 0.45, 0.55, 0.59, 0.64 and 0.70.
- In track-1, 17 spots were seen under 366nm at R_f 0.01, 0.04, 0.11, 0.13, 0.22, 0.27, 0.30, 0.38, 0.42, 0.44, 0.47, 0.57, 0.59, 0.62, 0.67, 0.70 and 0.72. While in track-2 15 spots were seen under 366 nm at R_f 0.08, 0.11, 0.14, 0.17, 0.27, 0.31, 0.35, 0.38, 0.40, 0.44, 0.49, 0.55, 0.59, 0.64 and 0.68.
- After spraying with vanillin sulphuric reagent, 4 yellowish and brownish colored spots were seen in track-1 while 3 spots were seen in track-2 respectively.
- In Short UV, 3 spots were seen in both sample at R_f 0.27,0.45, 0.59 and in Long UV 5 spots were seen in both samples at R_f 0.27, 0.11, 0.38, 0.44 and 0.59

Table 4: Area percentage of active constituents at same R_f value under Short UV

S. No	R_f value	Area percentage Under Short UV	
		Track-1	Track-2
1	0.27	20.15	12.20
2	0.47	37.17	13.21
3	0.59	2.43	3.79

- This table shows that, at 0.27 R_f value, track-1 covers 20.15 % area, while at the same R_f value, track-2 covers 12.20 % area under Short UV.
- At 0.47 R_f value, track-1 covers 37.17 % area, while at the same R_f value, track-2 covers 13.21 % area under Short UV.
- At 0.59 R_f value, track-1 covers 2.43 % area, while at the same R_f value, track-2 covers 3.79 % area under Short UV.

Table 5: Area percentage of active constituents at same R_f value under Long UV

S. No	R_f value	Area percentage Under Long UV	
		Track-1	Track-2
1	0.11	7.21	12.16
2	0.27	5.75	10.17
3	0.38	4.97	3.76
4	0.44	4.96	10.98
5	0.59	6.09	3.83

- This table shows that at 0.11 R_f value, track 1 covers 7.21 % area under long uv, while at the same R_f value, track 2 covers 12.16 % area.
- At 0.27 R_f value, track 1 covers 5.75 % area under Long UV, while at the same R_f value, track 2 covers 10.17 % area.
- At 0.38 R_f value, track 1 covers 4.97 % area under Long UV, while at the same R_f value, track 2 covers 3.76 % area.
- At 0.44 R_f value, track 1 covers 4.96 % area under Long UV, while at the same R_f value, track 2 covers 10.98 % area.
- At 0.59 R_f value, track 1 covers 6.09 % area under Long UV, while at the same R_f value, track 2 covers 3.83 % area.

DISCUSSION

Physico-chemical parameters show total mass difference during drying process. But the change in result may be useful to predict the state of raw drug used.

Qualitative tests showed the presence of different functional groups in both the samples.

Quantitative estimation of saponin, tannin and sugar shows greater value in dry form as compared to fresh sample.

HPTLC study showed that moisture content affects the behaviour of drug. Due to the presence of the moisture content, the behaviour pattern of the molecule changes and show independent growth. This type of behaviour changes among dry and fresh sample can be observed through increase in no. of spot and simultaneously increasing the common spot.

At 254nm wavelength, fresh sample shows 7 spot while dry sample shows 11. UV spectra show that 3 spot are common in both the sample at the R_f 0.27, 0.47 and 0.59.

At 366nm wavelength, fresh sample shows 17 spots while dry shows 15 spots. UV spectra show 5 spots are common in both the samples at the R_f 0.11, 0.27, 0.38, 0.44 and 0.59

HPTLC study showed that the components are more susceptible to long UV as compared to short UV. When a superimposable UV spectrum was observed, it showed that due to the process of drying, the component becomes more concentrated within the drug. This can be seen by

increasing the area percentage of the active constituent at the same R_f in the common spot.

CONCLUSION

This study shows that there is a vast difference in the physical state of the plant *Shatavari*. Classics say that this drug should be used in wet state. But in the dry form, the drug shows more concentration of the active constituents as compared to the wet form. So we can also use the dry form of *Shatavari* in the absence of wet drug to get good results.

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