IAMJ

INTERNATIONAL AYURVEDIC MEDICAL JOURNAL

Research Article ISSN: 2320-5091 Impact Factor: 6.719

A COMPARATIVE PHYTO-PHARMACOGNOSTICAL STUDY OF SHATAVARI (ASPARAGUS RACEMOSUS WILLD.) IN DIFFERENT ECOLOGICAL CONDITIONS (RAJASTHAN, HIMACHAL, KARNATAKA REGION) ALONG WITH SAVEERYTAAVADHI PARIKSHANA (SHELF LIFE EXAMINATION) AND IN VITRO ASSESSMENT OF ANTIBACTERIAL POTENTIAL OF AQUEOUS EXTRACT ON E.COLI

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https://doi.org/10.46607/iamj05p7052023

(Published Online: July 2023)

Open Access

Article Received: 09/06/2023 - **Peer Reviewed:** 22/06/2023 - **Accepted for Publication:** 18/07/2023.

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ABSTRACT

Shatavari is the most commonly used *Ayurvedic* herb. Its therapeutic properties are described broadly in Traditional medicinal systems such as *Ayurveda*, Siddha, and Unani. It's advised both as a typical tonic or a female reproductive tonic. Medicinal plants are good sources of chemical substances like Terpenoids, Phenols, Steroids, Flavonoids, Tannins, and Aromatic Compounds which are widely used in the pharmaceutical, cosmetics, and food industries. These chemical substances are commonly known as secondary plant metabolites (e.g., specialized products). Opportunity to develop new products presenting few side effects and economic. The name Shatawari means “curer of a hundred diseases” (shat: “hundred”; variety: “curer”). In *Ayurveda*, classics refer to shelf life as *'Saveeryataavadhi',* which is necessary for physiological and biochemical responses, as well as the secondary metabolic process. Secondary metabolites (SMs) are useful for assessing the quality of therapeutic ingredients. In order to authenticate and develop the standards for this popular single drug WHO guidelines will follow; provided by CCRAS. For the standardization of this drug pharmacognostical and phytochemical

parameters will be carried out such as microscopic, extract macroscopy, Protein, sugar, tannins, foreign matter, moisture content, ash value, HPTLC, etc. The standardization of herbal drugs and their bio- constituents are of paramount importance in justifying their acceptability by modern scientific methods.

Key words: *Shatavari, Ayurveda* Anti-microbial, WHO, Ecology

INTRODUCTION

Shatavari (*Asparagus Racemosus*) is an important medicinal plant of tropical and sub-tropical india. The genus of Asparagus includes about 300 species around the world. Out of the 22 species of Asparagus recorded in India and is widely distributed across the globe. Throughout tropical and sub-tropical India and Sri-Lanka upto1000 feet in the Himalayas, Eastward Kashmir, tropical Africa, Java, and Australia, and it is commonly known as *Satawar, Shatamuli, Satavari* found at low altitudes throughout India. The dried roots of the plant are used as a drug. The roots are widely used to be weight gainer and galactgogue all over the world. In *Ayurveda,* classics refer to shelf life as *'Saveeryata avadhi',* which is necessary for physiological and biochemical responses, as well as the secondary metabolic process. Secondary metabolites (SMs) are useful for assessing the quality of therapeutic ingredients. Nowadays, these are used as important natural derived drugs such as Immune Suppressant, Antibiotics, Anti-Diabetic, and Anti cancer. Therefore, it's important to find not only therapeutically but also high yields as well as sustained potency. It is widely used in the *Ayurvedic* system of medicine *Rasayan* to the immune system and the body's resistance against infections. In order to authenticate and develop the standards for this popular single drug WHO guidelines will follow; provided by CCRAS. For the standardization of this drug, Pharmacognostical and Phytochemical parameters will carried out such as microscopic, extract macroscopy, Protein, sugar, tannins, HPTLC, etc. Some of the parameters like Foreign matter, Moisture content, Ash value, etc. The standardization of herbal drugs and their bio- constituents are of paramount importance in justifying their acceptability by modern scientific methods.

Botanical description[1,2]

Habit: Tall climbing deciduous sarmentose shrubs.

Roots: perennial, many fascicled, 30 cms to about one meter or more in length, smooth tapering at both ends, succulent and tuberous.

Stem: scandent twining armed with strong straight or recurved spines at the nodes, woody when mature. The branch lets angular.

Leaves: reduced to minute chaffy scales or spine scent and subtending leaf-like cladodes. Cladodes dark green divaricated faleate triquetrous or very slightly compressed and channeled beneath, 10 to 25 mm. long and about 2 mm. wide, borne in axillaries clusters of 2-6.

Flowers: usually numerous and bractecate, small 3-4 mm. across. They are bisexual whitish and very strongly and sharply scented, permeating the atmosphere with their rather irritating odour. Pedicels - very slender, about 1.2 mm. long, slightly pendulous, and jointed in the middle. Bracts - minute, scarious, cordate, Perianth - six parities, the segments oblong, spreading reflexed 2-3 mm. long and campanulate connivent below.

Fruits: are globular or more often obscurely three-lobed pulpy berries 4 to 6 or 7 mm. in diameter. They are greenish or light rose when young but turn purplish black when ripe and contain 2 or 3 hard seeds.

Seeds: with a thin but hard and brittle black test and horny or cartilaginous endosperm.

Description of Root: The roots arise from the short root-stock adventitiously. They are all generally considerably long - the length varying from 25 cm to about a meter or more and gradually tapering towards the basal and distal ends. The main body of the root is nearly cylindrical or of the same width except for small depressions here and there and varies from one to two cm in diameter. They are cream white or light

cream yellow and have a fairly smooth surface except for the presence of a few rootlets. The surface skin is quite soft and easily scrapable. On dried roots, the surface appears longitudinally wrinkled, and short transverse fissures are found. The transversely cut surface of a fresh root, shows a narrow light yellow peripheral strip, a silvery white fleshy soft middle region that forms the bulk part of the root, and a slightly hard narrow central woody core. In fresh roots, the light yellow coloured outer layer or rind can be easily separated from the silvery white Part but adheres firmly in dried roots.

Flowering and Fruiting season[3,4]

Flowering season: September to December

Fruiting season: July to October

Pharmacological actions are: Antiulcer[5], Central nervous system[6], Respiratory system[7], Cardiovascular system[8]etc.

***Ayurvedic* properties of *Shatavari* root**

Nighantu	***Rasa***	***Guna***	***Virya***	***Vipaka***	***Doshakarma***	***Karma Prayog***
Bhavprakash[9]	*Madhur, Tikta*	*Guru*	*Sheet*	*Madhur*	*Tridoshara*	*Mutrajanna, Vyasthapan , Agnivardhak, Netrarog, Atisaar, Mutrakrich, Raktpitt, Grahni*

Shelf life (*Saveeryatavadhi)*[10]

Acharaya Sharangadhara first mentioned *Saveeryata Avadhi* of various formulations that are known today as Shelf life, expiry date, or stability in the modern system of medicine. *Saveeryta avadhi* of formulations depends upon three main things i.e Ingredients, preparations, and form.

AIM AND OBJECTIVE

- To do a comparative phyto-pharmacognostical study of *Shatavari* Powder (*Asparagus racemosus*) from a recognized pharmacognosy/Pharmacology laboratory.
- To Compare the samples obtained with different ecological conditions; Pharmaceutical preparation i.e. Powder and analysis of the antibacterial activity of the extract.
- To find out the Best shelf life and best sample of *Asparagus racemosus* WSR Of Ecological condition.

MATERIAL & METHODS

- Plant Collection and Authentication; Fresh Roots of *Asparagus racemosus* were collected from the wild from different ecological states i.e. Rajasthan, Himachal, and Karnataka, and identified by the expert of *Dravyaguna vigyan* and botanist; following standard collection methods. All the samples collected in starting of the winter season and tested twice.
- The literature material review for Plant was gathered from books, journals, the library, the internet, classic and contemporary texts, various monographs, and API.
- Pharmacognostical and phyto-chemical methods were used to standardize three Different samples of *Asparagus racemosus* from a recognized pharmacognosy lab and a college Lab.
- An antibacterial study was done by a recognized & affiliated institute of all three Samples.

RESULTS

Organoleptic study

S.no	Sample		Colours	Odour	Taste	Texture
1.	**Sample HP**	Fresh	Brownish, colour rough in texture	Characteristic	Sweetish	Fine in touch
		After 6month	Dark brown in colour	Indistinct and characteristic	Characteristic bitter	Fine in touch
2.	**Sample K**	Fresh	Roots are cylindrical, straight or slightly curved whitish in colour	Characteristic odor	Sweetish, slightly bitter	Fine in touch
		After 6 month	Creamy white	Characteristic odor	bitter in taste With slightly sweet at last	Fine in touch
3.	**Sample R**	Fresh	White	Characteristic odor	Sweet	Fine in touch
		After 6month	Creamy Yellow	Characteristic odor	Bitter in taste	Fine in touch

Physiochemical Screening

S.no	PARAMETERS	HP Fresh/After6 months		Karnataka Fresh/After6 months		Rajasthan Fresh/After6 months		API Values
1.	**Foreign matter**	0.40	0.10	0.24	0.20	0.30	0.25	<1
2.	**Total ash%**	6.15	3.8	5.87	3.26	4.71	2.98	<5
3.	**Acid insoluble ash%**	0.86	0.15	0.73	0.79	0.39	0.51	<0.5
4.	**Water soluble extract%**	50.2	52.14	72.4	58.6	62.4	48.14	>45
5.	**Alcohol soluble extract%**	12.4	14.44	14.6	12.45	10.5	10.17	>10
6.	**Moisture content%**	1.91	2.78	2.85	3.15	2.43	3.55	<3.0
7.	**Ph Values%**	5.63	4.86	5.64	3.26	6.16	5.30	5.50-6.50

Phytochemical Screening

Asperagus Racemosus powder		Fresh sample HP		HP after 6 months		Fresh sample Karnataka		Karnataka after 6 months		Fresh sample Rajasthan		Rajasthan after 6 months	
S.no	Qualitative phytochemical test	Powder drug	Aq. E	Powder drug	Aq. E	Powder drug	Aq. E	Powder drug	Aq. E	Powder drug	Aq. E	Powder drug	Aq. E
1.	**Carbohydrates**	+	+	+	+	+	+	+	+	+	+	+	+
2.	**Glycoside**	+	+	-	-	+	-	+	+	+	+	+	+
3.	**Tannin**	+	+	+	+	+	+	+	+	+	+	+	+
4.	**Phenolic compound**	_	_	_	_	_	_	_	_	_	_	_	_
5.	**Alkaloids**	+	+	+	+	+	+	+	+	+	+	+	+
6.	**Flavonoid**	-	-	-	-	-	-	-	-	-	-	-	-
7.	**Steroids**	+	+	-	-	+	+	-	-	+	+	-	-
8.	**Terpenoids**	_	_	_	_	_	_	_	_	_	_	_	_
9.	**Saponnins**	+	+	-	-	+	+	-	-	+	+	-	-

HPTLC of a sample of Himachal Pradesh

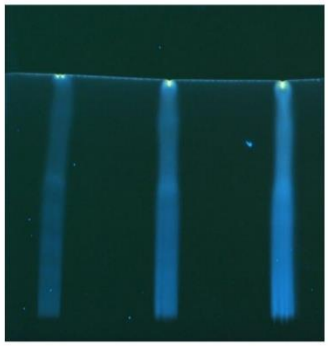
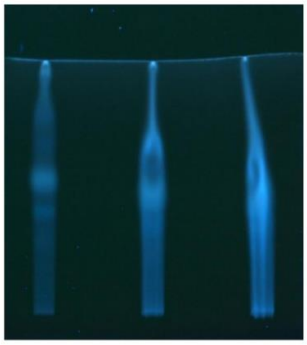
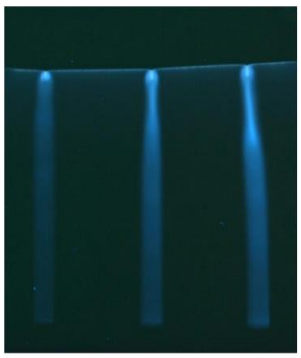
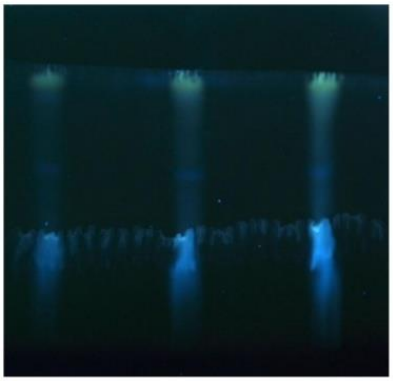
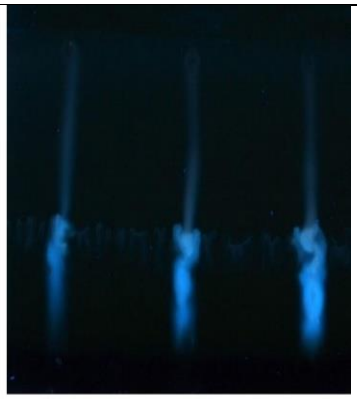
Wavelength	Track	Rf range		No. of Peaks	
		Before	After	Before	After
Wavelength 254	Track I	0.03-0.90	0.32-0.83	1-8	1-5
	Track II	0.04-0.88	0.36-0.84	1-6	1-4
	Track III	0.03-0.86	0.04-0.66	1-7	1-6

HPTLC of sample Karnataka

Wavelength	Track	Rf range		No. of Peaks	
		Before	After	Before	After
Wavelength 254	Track I	0.02-0.89	0.02-0.82	1-6	1-6
	Track II	0.01-0.76	0.29-0.48	1-6	1-4
	Track III	0.02-0.57	0.00-0.69	1-4	1-9

HPTLC of sample Rajasthan

Wavelength	Track	Rf range		No. of Peaks	
		Before	After	Before	After
Wavelength 254	Track I	0.03-0.89	0.01-0.80	1-5	1-6
	Track II	0.02-0.85	0.31-0.81	1-5	1-4
	Track III	0.01-0.70	0.33-0.78	1-5	1-4

HP Fresh sample	Karnataka sample	Rajasthan sample
		
After 6 months	After 6 months	After 6 months
		

Antibacterial study

Sample	O.D. E. Coli	Inhibition (%)	O.D. Bacillus	Inhibition (%)
K(w)	1.243	20.52	2.433	29.58
HP(w)	3.506	No Inhibition	2.507	27.43
R(w)	1.157	26.02	2.645	23.44
R(E)	1.046	33.12	3.769	No Inhibition
HP(E)	0.854	45.39	4.803	No Inhibition
K(E)	0.701	55.17	4.096	No Inhibition
HP(E:W)	1.107	45.70	1.635	52.67
R(E:W)	1.101	29.60	3.659	No Inhibition
K(E:W)	0.704	54.98	1.521	55.97
Control(+)	1.564		3.455	
Kenamycin	0.058		0.041	

DISCUSSION

Foreign matter range- The objective of washing vegetable drugs is to remove all traces of organic matter, which may otherwise interfere with an analytical determination. This should be not more than 1. All the samples show Foreign matter was within the limit. It means there are fewer traces of organic matter.

Ash values- are helpful in determining the quality and purity of crude drugs, especially in powder form. Total ash is a measure of the mineral oxide content of activated carbon on a Wight basis. Here is the study found that the Total Ash range should not be more than 5. according to API. Total Ash was comparatively higher than the range in the fresh sample of hp 6.15 After 6 months 3.8 & the ash value of the Fresh sample of Karnataka was 5.87 and after 6 months 3.29. It means traces of organic matter become less over the time period after 6 months.

Acid insoluble ash- must be less than 0.5 according to API. Value of Fresh sample HP 0.86 After 6 months 0.15. The value of sample Karnataka was 0.73 & after 6 months 0.79. A fresh sample of Rajasthan 0.39 After 6 months 0.51. Only sample R was within limits and Sample HP and K were higher in the fresh sample but reached in limits after 6 months.

Alcohol soluble extractive value- should be higher than 10. A fresh sample of HP 12.4, After 6 months 14.44. The value of sample Karnataka was 14.6 & after 6 months 12.45. A fresh sample of Rajasthan 10.5 after 6 months 10.17. All the Fresh and after 6 months samples Shows in limits. Sample R does not show many changes because there is already a concentrated sample and does not loss of water content.

Water soluble extractive range- should be not less than 45. A fresh sample of HP 50.2 After 6 months 52.14 Value of the sample Karnataka was 72.4 & after 6 months 58.6. A fresh sample of Rajasthan 62.4 After 6 months 48.14. All the samples were in range. The highest was noted in Sample K which means shatavari extract has a higher starch amount which is hygroscopic containing cells in higher amounts.

The moisture of the initial sample is HP 3.99 after 6 months and was tested at 6.78. The initial sample of Karnataka was 2.85 and after 6 months was tested at 5.85.and the initial sample of Rajasthan was 2.43 and after 6 months tested at 5.54. This clearly shows that seasonal changes in the finished of the herbal product impact powder as moisture content plays a major role in the deterioration of the drug. Moisture content increased in powder shows its hydrophilic nature which changes the Sweet Taste into Slightly Acidic Taste. The highest Acidic nature is seen in Sample K. It could be vulnerable to Fungal and Bacterial counts in Samples. Sample R Shows minimum changes in Moisture content and PH value also.

PH Values reached fresh and After 6 months; Sample HP 5.63 to 4.86, Sample K 5.64 to 3.26, Sample R 6.16 to 5.30. It practically means the

quantitative indications of the acidity or base of a solution. Results show that the extract of k is more acidic in nature.

Phytochemical Study: Carbohydrates, Alkaloids, and Tannins were present in all three samples initially. They persisted even after six months. Glycosides were found constant in two samples Karnataka and Rajasthan, Whereas not found in Himachala. Sapnonnins were present in the initial sample but after 6 months were completely absent. Steroidal and saponinns are the main biologically active constituents of Asparagus. The absence of steroidal and saponnins indicates the expiry of the drug.

HPTLC Shows different peaks at different nm wavelengths. The final result after the study of HPTLC found that The Chief constituent Shatavarin IV peak was present in sample K and Remain after 6 months of period. Other samples lost the chief constituent but have shatavarin IX, Apsparacosoide, and Asaranin A which are similarly matached with standard Rf values which have less efficacy and less quantity in the sample.

Antibacterial study shows that Anti bacterial action on E.Coli of the drug (Water extract) showed minimum inhibition zone of sample HP (No inhibition)< Sample K < Sample R. While Anti bacterial action on E.Coli of the drug (Ethanol extract) showed inhibition zone of sample R < Sample HP< Sample K. While Anti bacterial action on E.Coli of the drug (E: W extract) showed inhibition zone of sample R < Sample HP < Sample K. All the Samples Show a less effective against E.coli when used Aquous Extract. Ethanol Extract showed significant results against E.coli Bacteria growth.

CONCLUSION

All the Physiochemical and Phytochemical study Shows that Sample R is Best in the parameters of Standardization of the root of *Asparagus racemosus.* Its fresh Sample also Contain *Shatavarin* IV which indicates in HPTLC. Sample K shows the best shelf life because after six months it reached the standardization limits and also showed No lost of *Shatavarin* IV in HPTLC Reports. All the samples show very less inhibition on healthy bacteria of the colon. Besides Sample K and Sample HP showed significant inhibition of growth against O.D. Bacillus. So the Final Conclusion is that A. racemosus root ethanol extract of the Sample collected from Karnataka showed a significant result against bacteria E.coli. To make a choice, more research and survey are required. *Shatavari* Ethanol extract performs significant activity.

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Source of Support: Nil
Conflict of Interest: None Declared

How to cite this URL:Shikha Thakur et al: A comparative phyto-pharmacognostical study of shatavari (asparagus racemosus willd.) In different ecological conditions (rajasthan, himachal,karnataka region) along with saveerytaavadhi parikshana (shelf life examination) and in vitro assessment of antibacterial potential of aqueous extract on e.coli. International Ayurvedic Medical Journal {online} 2023 {cited July 2023} Available from: http://www.iamj.in/posts/images/upload/434_441.pdf