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ANALYTICAL STUDY OF SHATAVARI ROOT COLLECTED IN DIFFERENT SEASONS

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Abstract : Aim- Analytical study of Shatavari root collected in two different seasons and comparison their free radical scavenging activity**.** Materials and methods- Macroscopic as well as microscopic study, physicochemical analysis of both the samples was done. Antioxidant activity was evaluated by DPPH method. Antioxidant activity of both the sample was compared. Results- There is no major change is observed in the analytical study of Shatavari root collected in Sharad ritu and Shishir ritu. However, free radical scavenging activity of Shatavari root collected in Shishir ritu is better than that of Shatavari root collected in Sharad ritu. Further more detail study is required to come to the final conclusion.

IndexTerms **- Shatawari, Free radical scavenging activity, DPPH, Sharad ritu,Shishir ritu**

Introduction

Ayurveda is a life science in which Ayuşya & Anayuşya dravyas (useful to the life and harmful drug to the life) and their guna as well as Karma are described.[1] Ayurveda, a life science, developed in India based on Indian philosophy. It is a life style close to the disciplines of nature. Its history is quite ancient. Ayurveda a holistic medical science is dedicated for the human welfare. It provides longevity and positive health. It covers the preventive and curative aspects of health. Its ultimate goal is to attend salvation. This is only possible through the healthy status of body and mind. And to maintain the healthy status of body and mind Acharya Charaka has mentioned four pada of cikitsā (i.e.four basic factors of treatment) & Dravya is one of them.[2]

IMPORTANCE OF DRAVYA:

The Acharya says the Vaidya, the Auşadhas or dravya, the Paricarak and the Rogi are the chatuspada of Chikitsa. In the Catuspada, the Vaidya gets prime importance as it is the most unavoidable factor of Cikitsä. After vaidya, it is the Auşadha or Dravya which is important.

When Acharyas are insisting on the importance of the Auşadha, they are very conscious about the choice of appropriate one. They repeatedly insisted about it.

तदेव युक्तं भैषज्यं यदारोग्याय कल्पते।

स चैव भिषजां श्रेष्ठो रोगेभ्यः यः प्रमोचयेत् च. सू. 1.135[3]

It is the proper medicine which leads to the health, and he is the great physician or Vaidya who clinches off the Rogi from the diseases. (Rogas) When the Vaidya doesn't give a proper Auşadh or saying himself as Vaidya fails to give a proper medicine he is criticized.

The Acaryas stress a lot about the perfection of the knowledge of a particular drug, they stress about dynamic knowledge by Nama, Rupa, and Guna i.e. Name, external features and its properties. If it is not known by all the three dimensions, it may be very destructive. Even if it used in the improper way, while knowing everything about it, results in destruction.

CONDITIONS TO COLLECT AUSDHAS:

In Ayurveda, the drug is used in its natural forms as well as nominally processed and specially processed forms. For this purpose the drug is said to be collected, preserved, & stored in airy environmental and technical condition. There are certain rules to collect preserve and to store the drug which is to be used singly or in combination of after being processed. Acharya are very elaborative on this process. They guide to collect plant drugs as follows.

तत्र यानिकालजानान्युपागतसंपूर्णप्रमाण-रसवीर्यगन्धानिकालातपाग्निसलिलपवनजन्तुभिरनुपहतगन्ध-वर्ण-रस- स्पर्श - प्रभावाणि प्रत्यग्राण्युदीच्यां दिशि स्थितानि तेषां शाखापलाशमचिरप्ररूढं वर्षावसन्तयोर्ग्राह्यं, ग्रीष्मे मूलानि शिशिरे वा शीर्णप्ररूढपर्णानां, शरदि त्वक्कन्दक्षीराणि, हेमन्ते साराणि यथर्तु पुष्पफलमिति मंगलाचारः कल्याणवृत्तः शुचिः शुक्लवासाः संपूज्य देवता अश्विनौ गोब्राम्हणांश्च

कृतोपवासः प्रांमुख उदंमुखो वा गृ हीयात् तेषां शाखापलाशमचिरप्ररूढं वर्षावसन्तयोर्ग्राहियं, ग्रीष्मे मूलानि शिशिरे वा शीर्णप्ररूढपर्णानां शरदि त्वक्कन्दक्षीराणि, हेमन्ते साराणि यथर्तु पुष्पफलमिति ।। च. क. 1.10[4]

Acharya lay down the guidelines, about the quality of their drug as it should be –

- कालानि जातानि- should be evolved during proper environment & period of the year.
- उपागतसंपूर्णप्रमाण रसवीर्यगन्धानि- Acquiring its proper quantity and built in the taste, smell & virya.
- कालातपाग्निसलिलपवनजन्तुभिरनुपहतगन्ध-वर्ण-रस-स्पर्श-प्रभावाणि- Its Gandha, Rasa, Sparşa & Prabhāvani should be unhurt or unchanged by the effect of Kala, Agni, Salila, Pavana & Jantus.
- प्रत्यग्राण्युदीच्यां दिशि स्थितानि The parts to be collected should be on the good direction and towards the North.

After these rules they give us preferential list when to collect the respective plant part throughout the year.

शरधखिलकार्यार्थ ग्राहथं सरसौषधम् विरेकवमनार्थे च वसन्तान्ते समाहरेत् ।। शा.पू.खं. 1. 59 [5]

Acharya Sarangdhara told that all herbs should be collected in Sarad ritu and the drug for Vaman and Virecana should be collected at the end of Vasant ritu.

कन्द हिमत शिशिरे च मूलं पुष्पं वसन्ते गुणदं वदन्ति । प्रवालपत्राणि निदाघकाले स्यु पंकजातानि शरत्प्रयोगे ।। रा.नि. 2/57

According to Raj Nighantu, Kanda should be collected in Hemant ritu, mula in Sisir tu, Puspa (flowers) in Vasant rtu. [6] And lastly Acarya Suśruta has told to collect the 'Saumya Auşadhi in Saumya rtu (Visarga Kala) and Agneya auşadhi in Agneya rtu. (Adän Kala).[7]

The demand for medicinal plant is increasing day by day as now various pharmacies are entering in the field of Ayurveda. The pharmacies have demand for raw material & the effort should be done for supplying the standard and quality raw material which is being cultivated collected & preserved in a proper way. Standardization is the process of standardizing especially that of determining the strength or scale value of a substance by comparing with some standards established by law, custom or authority. Again standard means to affix parameters or specifications of raw drug material.

Shatavari

'Shatavari' means one 'who possesses a hundred husbands'. It is a versatile traditional plant used for a variety of serious diseases as also impotency of both the sexes. It is considered both a general tonic and a female reproductive tonic. It is considered both a general tonic and a female reproductive tonic. 'Shatavari' is the main Ayurvedic rejuvenative tonic for females. 'Shatavari' is however also used for sexual debility and infertility in both sexes. It is also used for the menopausal symptoms and to increase the lactation.

Asparagus racemosus is a woody climber growing to 1-2 m in height. The leaves are like pine needles, small and uniform. Flowers are white and have small spikes. This plant belongs to Liliaceae family, is common at low attitude shade and in tropical climates throughout India, Asia, Australia, and Africa.[8]

Locally this plant is called 'Satavari' in Hindi. In Sanskrt it is called 'Satmuli'. Śata means hundred and 'muli' means root.

The genus Asparagus has been recently moved from the subfamily Asparagae in the family Liliaceae to a newly created family Asparagaceae. 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.

Asparagus is a Greek word for 'stalk' or 'shoot'. About 300 species of Asparagus are known to occur in the world. Some of the Europian species to be mentioned are A. officialis, A. sprengeri and A. acutifolis.

Out of several species of 'Asparagus' grown in India A. racemasus, A. gonaclades and A. adsendens are most commonly used in indigenous medicine.

A. racemosus is commonly mentioned as a 'Rasayana' in Ayurveda.

Rasayana are those plant drugs which promotes general well being of an individual by increasing cellular vitality or Resistance.

NOMANCLATURE OF 'SHATAVARI':

Botanical name - Asparagus racemosus willd.

English/Common name - Asparagus

BOTANICAL DESCRIPTION OF THE PLANT:

The plant is tall climbing undershrub with annual woody terete stems growing to 1-2 m in length.

.Branchlets-triquetrous, Spines 5-13 mm long, recurved or rarely straight. Cladodes 1.3-2.5 cm long, in tufts of 2-6, curved, Roots stunt root stock bearing numerous, long, fingerlike and clustered fleshy and tuberous. Roots are tapering towards the base and swollen in the middle.

Leaves pine needle like, small, uniform reduced to minute spinescent structure subtending the leaf like cladodes which are falcate. Slightly compressed and channeled beneath, born in axillary cluster or 2-6. Flowers-white fragrant, in solitary or fascicled, simple or branched recemes 2.5-5 cm long. Pedicles - 5 mm long, joined in the middle slender. Perianth 6, about 3 mm long, partite, segments, oblong refluxed and connivent below. Stamens Filaments free, opposite to the perianth segments, as long as perianth. Anther-2 celled. Ovary Trigonous, three chambered, style short, columnar ending, in three recurved stigmatic lobes, fruits globose berry. Berry-5-6 mm diameter, red Color-Silver white or light ash in color of roots Size of Roots-5-15 cm Maximum diameter of Root-1-2 cm. Surface max or less smooth in fresh samples and fine longitudinal wrinkles in dry roots. Fracture -complete .[9]

AYURVEDIC PROPERTIES:

Rasa-Madhura, Tikta

Guna-Snigdha, Guru

Virya - Sita

Vipäka – Madhura[10]

Karma-

Rasayana,vrushya, Balya Medhya, Netrya, Sukrala, Agnipuştikara, Vranaropana, NariVaidyaka, Grähi, Stanyajanana.[10]

Doshaghnata- Vätapittashamaka [10]

Active constituents

Recent chemical analysis indicate that the following active constituents are present in Shatavari plants-

Steroidal saponins, known as shatavarins - I - IV. Shatavarin I is the major glycoside with 3 glucose and rhamnose attached to sarsasapogenin. Isoflavones including 8 methoxy 5,6,4" -trihydroxyisoflavone 7-0-beta-D-glucopyranoside ,Asparagamine, a polycyclic alkaloid.Racemosol,a cyclic hydrocarbon (9,10 -dihydrophenantherene), Polysaccharides, mucilage.[11]

Materials and Methods

In the present study the material which were studied were Shatavari roots (Asparagus racemosus - Willd) collected in Sharad ritu and Shishir ritu from same soil, same area, same place, under similar environmental conditions.

Before actual study the roots of the plant were collected as per the texts, and observed externally purified and preserved to facilitate the proper study.

INCLUSION CRITERIA:

1. The roots of Shatavari (Asparagus racemosus - Willd) family - Liliaceae/Asparageceae, genus - Asparagus, species -racemosus were collected and taken for the study.
2. The present study material were collected from the same place, same soil, and under same environmental condition.
3. These roots were collected in Sharada ritu and Shishira ritu as per text references.

EXCLUSION CRITERIA:

1. The species other than Asparagus racemosus Willd were not taken for the study..
2. Plant Parts other than root were not taken for the study.

SHATAVARI ROOT COLLECTION:

The roots of Shatavari collected according to the direction or reference from the texts in two different seasons i.e. Sharad ritu and Shishir ritu.

They were collected from the same place.

It was seen that collection was not from the places like temple, Pradaksina märg, cremation places, grave yards, caitanya, Yadnyaghat, valmik, subha sthāna and should be placed having Kuša, Rohişna Snigdha and Krynu soil.

The plant should be such that whose growth is not affected by any other bigger tree.

Those were collected on good day which was having auspicious constellation and method of collection of drug given in the text.

Period of collection of plant in Sharad ritu -mid of November month

Period of collection of plant in Shishira ritu-. First week of February month .

AUTHENTIFICAION OF SHATAVARI:

Preparation of herbarium sheet

Plant specimen was collected in the month of November in flowering season very carefully and patiently moisture was removed from the plant by light pressure by keeping it between filter paper.

This is then tied on paper board and card sheet paper by stitching with thread and stapler.

The paper board was used to record the name of the collector, date, place and season of the collection. Then the prepared specimen was sent to the experts for their examination and opinion.

Voucher specimen

The sent specimen sample was vouchered by the experts in the department of botany and voucher specimen number was taken.

External purification:

Before the purification, Shatavari roots were observed as to confirm the proper state. The roots were examined for the effect of both soil and environment or any malformation of them. The roots in a good condition were taken for the tests and study.

These roots were washed thoroughly with pure water as to eliminate any foreign matter and soil.

Preservation of sample:

The roots collected in Sharada ritu, after cleaning they were dried externally by using clean tissue paper then with the help of sterilized knife, the wet roots were cut into small pieces with average length 4 to 5 cm. then they were taken into large size plate and dept where proper ventilation was there. The roots were observed twice a day to facilitate proper drying and was achieved on 34th day. The dried roots were preserved in air tight container covered with polythene bag named as sample A. The same procedure was carried out for the preservation of the roots collected in Shishir ritu.

Here the roots were dried on 22nd day and the sample was preserved named as sample B.

STANDARDIZATION:

External morphology/Macroscopic study:

The plant and the plant parts were identified according to its character, external features and typical features as described in texts with the help of naked eye and by using stereo microscope. The characters were as for law of taxonomy-computer print outs.

Taxonomy:

Systematic Position:-

Kingdom: Plantae
Phylum: Magnoliophyta
Class: Liliopsida
Order: Asparagales
Family: asparagaceae also placed in family liliaceae
Genus: Asparagus
Species Racemosus

EXTERNAL MORPHOLOGY OF ROOT (MACROSCOPIC STUDY)

Sample A:

The roots were silver white or light ash in color & 179 in number.
They were tuberous, swollen in the middle and tapering at both ends.
They were fresh, fleshy and fasciculated, adventitious They had longitudinal wrinkles on them

Sample B:

The roots were silver white or light ash in color & 157 in number. They were tuberous, swollen in the middle and tapering at both ends. They were fresh, fleshy and fasciculated , adventitious, externally more or less smooth without longitudinal wrinkles on them.

MICROSCOPIC STUDY OF ROOT:

Internal morphology (Microscopic study):

For microscopic studies compound microscope was used. The transverse section of Shatavari root (sample A and B) were taken. These were stained and viewed under microscope and compared with standard ones.
Microscopy of Churna (Shatavari Powder): For Microscopic study, Shatavari Powder of sample A and B were mounted in water and shape of the granules was sketched. And confirmed by staining with iodine . And observations were made.

Preparation of Shatavari powder (sample A and B):

Preserved dried roots of sample A and B were taken and with the help of mixer grinder fine as well as coarse powder was prepared as per the requirement & preserved in a air tight container so as to avoid moisture and contamination named as sample A and B organoleptic tests were carried out.

PHYSICO-CHEMICAL ANALYSIS:

1. Determination of foreign matter-

50 gm of dug sample was taken. It was spread on white paper in a thin layer. The foreign mater was detected with the help of unaided eye and also by using (6 x) lens, and then foreign matter were separated. Again the powder is collected and weighed and percentage of foreign matter was calculated.

2. Determination of loss on drying-

10 gm accurately weighed sample was taken in a dry dish heating at 110°c for 2 hours in oven. Then dish was removed from the oven and cooled. And wt. was taken. Again kept in oven for half an hour and wt. was noted after cooling. And this procedure was repeated till two consecutive weights were equal.

$$\text{Percentage of moisture} = \frac{B-C}{B-A} \times 100$$

Here, A is wt. of dry evaporated dish.

B is wt. of dry dish + sample.

C is constant wt. after heating.

3. Determination of total ash-

5 gm accurately weighed drug powder was taken in weighed crucible. Then it was ignited at 450° C in a muffle furnace until become free from carbon and allowed it for cooling and weighed again. Thus the weight of ash (residual) was calculated. Then by applying the formula the percentage of total ash was calculated.
Formula:

$$\text{Total Ash content} = \frac{\text{Weight of residual}}{\text{Weight of sample}} \times 100$$

4. Determination of acid insoluble ash:

Ash calculated above was dissolved in dil.HCI, boiled, called and filtered through Watmann's 41 ashless filter paper washed with distilled water to remove chlorides then filter paper was ignited in weighed crucible in muffle furnace to 450° C and weight of the residual was noted and percentage of acid insoluble ash was calculated by applying following formula:

Formula:

$$\text{Acid insoluble ash} = \frac{\text{Weight of residual}}{\text{Weight of sample}} \times 100$$

5. Determination of alcohol soluble extractive:

Accurately weighed 2 gm sample was taken in 100 ml and 90 % ethyl alcohol kept it for 24 hours with occasional shaking. Then it was filtered with filter paper by using 90% ethyl alcohol volume the solution was again made to 100 ml From that 25 ml of solution was taken in weighed beaker and evaporated and the weight of the residual was calculated. By applying formula % of alcohol soluble extractive was calculated.

Formula:

$$\% \text{ of alcohol soluble extractive} = \frac{\text{Weight of residual} \times 100 \times 4}{\text{Weight of sample}}$$

6. Determination of water soluble extractive:

Accurately weighed 2 gm sample was taken in 100 ml of chloroform water (1 liter water + 2 drops of chloroform) kept it for 24 hours with occasional shaking. Then it was filtered with filter paper by using chloroform water volume the solution was again made to 100 ml From that 25 ml of solution was taken in weighed beaker and evaporated and the weight of the residual was calculated. By applying formula % of alcohol soluble extractive was calculated

Formula:

$$\% \text{ of water soluble extractive} = \frac{\text{Weight of residual x 100 x 4}}{\text{Weight of sample}}$$

Determination of Swelling Index :-

1 gm of sample was taken in 50 ml or stoppered cylinder and moistened with 205 ml of alcohol. Then 50 ml of water, water was added and it was shaken well.

The volume occupied by the swollen sample was measured after 3 hrs. This procedure was run 3 times and average was taken. Same procedure was done for sample B.

Determination of foaming index:

1 gm. of sample was taken in 100 ml. stoppered cylinder and then50ml of water was added. soaked for 3 hrs. It was shaken vigorously for 3 min. and immediately measured the volume of of foam above, the liquid meniscus. This procedure was run 3 times and average was taken.

Same procedure was done for sample B.

T.L.C. (Thin Layer Chromatography)

1 gm of sample and 50 ml of 95% of alcohol was taken. This solution was kept over I night with occasional shaking. Then this mixture was filtered and evaporated to 10 ml, on water bath.

Mobile phase:

N. Butanol 40%, Acetic acid 10%, water 50%These together was taken vigorously and organic layer was used.Thin plates, silica get g-2, 54 Alluminium plates were taken. Out of 10 ml extract, sample spot was made by using 20 to 50 micro lit.It is developed in a chamber till solvent front rises to 10 cm. Then plates was removed dried in an air. Then one plate was exposed to iodine vapour for half an hour. Plates exposed in iodine vapour showed for sample A & B.3 spots at 0.9 cm, 7.5 cm, 9.9 cm. Solvent front 14.6 cm. Same procedure was done for sample B. From this RF value of each spot was calculated and compared with the standard one.

H.P.L.C.:-

For HPLC analysis of sample A & B, JASCO, HPLC machine was used for the analytical purpose acetonitrate 30% and distilled water 70% was used as a mobile phase.

Sample Preparation:

100 mg of sample was dissolved in 100 ml. of distill water and was shaken & filtered. From the above mixture 5 ml of the sample was again mixed in 50 ml of distilled water to achieve a desired concentration.

20 micro litre (20 micro lit.) of the sample prepared was pipetted with as electronic pipette and the same was the injection volume. For the purpose of analysis UV detector was used and the column C18 4.6 mm x 25 cm column was used and the reports were noted.

Free radical & scavenging activity (DPPH Method)

For the analysis of free radical scavenging activity DPPH (Sigma Aldrich chemical Ltd. Germany) reagent is used & its certificate of purity was obtained. DPPH method was analyzed by using UV.VLS - JASCO V630 double beam spectrophotometer.

Procedure:

3 ml of ethanolic solution of DPPH, 0.05 ml of test extracts / ascorbic acid dissolved in ethanol were added. Test extracts of sample A & B were prepared in diff. event concentration. The solutions were incubated as 37°c for 30 min. Absorbance was measured at 516

nm. The % of inhibition of DPPH radical was calculated by comparing the results of test with those. Or the control and results were calculated.

MICROSCOPIC STUDY OF ROOT:

Transverse Section (T. S.) of Root

T.S. of root shows presence of piliferous layer often ruptured at some places, composed of small, thin walled, rectangular, asymmetrical cells. The number of cells elongated to form outer cortex is of 7 to 10 layer thick walled cells. Inner cortex is of 16 to 22 layers in upper level and raphides are present. The cells are oval to polygonal, thin walled, and tangentially elongated cells with intercellular spaces. The endodermis composed of thin walled, parenchymatous cells. Pericycle present below the endodermis. The pericycle followed by the vascular bundle. It is radial in position. The xylem consists of vessels and trachides. The vessels have pitted thickening.

Microscopy of Curna (Shatavari Powder):

Sample A

Sample of powder dark yellowish, fragment of lignified, thick Walled cells, vessels with simple pits

Sample B

Powder yellowish cream, fragment of lignified, thick walled cells, vessels with simple pits.

ANALYTICAL STUDY;

1) Determination of foreign matter

Name of sample	Standard	% of foreign material
A	NMT 1%	0.10%
B	NMT 1%	0.02%

As the values are same the moisture content in the sample may be same.

2) Determination of total ash:

Formula

Total Ash content = Weight of residual / Weight of sample× 100

Sample	**calculations**	**Total ash content**
Sample A	0.1505/5× 100	3.01%W/w
Sample B	0.1300/5× 100	2.60% W/w

3) Determination of acid insoluble ash:

Formula

Acid insoluble ash = Weight of residual / Weight of sample× 100

Sample	**calculations**	**Acid insoluble ash**
Sample A	0.008 /5× 100	0.16%w/w
Sample B	0.006/5× 100	0.12% w/w

4) Determination of alcohol soluble extractive

Formula

% of alcohol soluble extractive = Weight of residual × 100 × 4/ Weight of sample

Sample	**calculations**	**% of alcohol soluble extractive**
Sample A	0.0543× 100 × 4/5	10.86 % w/w
Sample B	0.0506× 100 × 4/5	10.12% w/w

5) Determination of water soluble extractive

Formula:

% of water soluble extractive =Weight of residual x 100 x 4/ Weight of sample

Sample	**calculations**	**% of water soluble extractive**
Sample A	0.227 x 100 x 4/2	45.42%w/w
Sample B	0.263 x 100 x 4/2	52.60%w/w

6) Swelling index

Sample A- 1.35ml/gm
Sample B- 1.3ml/gm

Foaming index
Sample A - 250
Sample B - 250

Note: All above tests and observations shows difference in between sample A and B, but as per the standards set by A.P.I. they are in standard limit.

T.L.C. (Thin Layer Chromatorgraphy)

Plate exposed in iodine vapour showed 3 spots at 0.9 cm, 7.5 cm, 9.9 cm solvent front was at 14.6 cm.

Calculations:

$$\text{Rf value} = \frac{\text{Distance traveled by solute}}{\text{Distance traveled by solvent front}}$$

		Colour of Sample A	Colour of Sample B
Rf value of Spot 1	$\frac{0.9}{14.6} = 0.62$	Cream	Cream
Rf value of spot 2	$\frac{7.5}{14.6} = 0.51$	Yellowish	Dark yellow
Rf value of spot 3	$\frac{9.9}{14.6} = 0.68$	Yellowish	Yellowish

Free Radical Scavenging Activity (DPPH Method) -
Absorbance was measured at 516 nm and the % or inhibition or DPPH. Radical was calculated by comparing the result or test with those or control which were as follows;

For sample A:

DPPH Pure	0.059
Ascorbic acid	0.0045
Satavari A	0.00903

Sample B:

DPPH Pure	0.059
Ascorbic acid	0.0045
Satavari B	0.109

From the above data

Free radical scavenging activity of both the samples is as follows.

Sample	Free radical scavenging activity EC 50 (legal)
Sample A	5.5
Sample B	6.250
Ascorbic acid	2.95

Discussion

Ayurveda a holistic medical science is dedicated for human welfare. It provides longevity and positive health. Acharyas has described the importance of dravya and details regarding its collection time & condition of dravya. Ayurveda is globally accepted ancient system of medicine throughout the world and world is looking towards great expectation. For this fulfillment basic raw drug material should be of standard quality. Keeping this view of mind the various Acāryas has different opinions regarding the collection time of root. To evaluate the controversies as per the texts Satavari was collected in two seasons as per. Carak, Vägbhata, Raj Nighantu in sharad ritu and as per Sushruta, Sharangdhara, in Shishir ritu

The aim of the present study was to collect Shatavari roots in two different season that is Sharad ritu & Shishir ritu. For its analytical study, as well as free radical scavenging activity, Shatavari root were studied and observes which were as follows:

The macroscopic & Microscopic study of the sample (Root & r powder) is concern. It shows following facts:

1. The average length and wt. of the roots collected in Sharad ritu more than the roots collected in Shishir ritu. This may be because of seasonal variation.
2. The average width of root collected in Shishir ritu is more than the root collected Sharad ritu .This may be also because of seasonal variation.
3. The roots collected in Sharad ritu was more in number than roots collected in Shishir ritu.
4. Both roots were Fleshy and fasciculate tapering at both ends, swollen in the middle but roots collected in Sharad ritu had longitudinal wrinkles on the surface while roots collected in Shisir ritu had more or less smooth surface.
5. In the organoleptic analysis, according to Ayurveda for the powders of both sample A & B, the sample A shows prominent pitvarna while sample B had shwetabh pitvarna.
6. Microscopic study of the root of both the sample (T.S) does not show any differences.
7. Powder microscopy of both the samples A & B did not show any differences.

Physico-chemical study (Analytical study) reveals;

Sr.No.	Analytical tests	Sample A	Sample B
1	% of foreign matter	0.10% w/w	0.02% w/w
2	% of total ash	3.01% w/w	2.60% w/w
3	% of Acid insoluble ash	016% w/w	0.12% w/w
4	% of water soluble extractive	45.42% w/w	52.60% w/w
5	% of alcohol soluble extractive	10.86% w/w	10.12% w/w
6	Swelling Index	1.35% w/w	1.3% w/w
7	Foaming index	250	250
8	Free radical scavenging activity by DPPH method	5.5 Micro gm/ml	**6.25 Micro gm/ml**

Free radical scavenging activity by DPPH method.

Free Radical Scavenging activity of Sample A and B was compared to each other. Sample A showed 5.5 Micro gm/ml of free radical scavenging activity & Sample B showed 6.25 Micro gm / ml of free radical scavenging activity. This result indicates that Sample B is having better free radical scavenging against sample A. This change can be attributed to the seasonal variation of the plant.

It has been mentioned in Ayurvedic Texts that herbs have are to be collected at particular time & season. In the context of free radical scavenging activity of Shatavari it can be said that Shatavari collected in Shishir ritu exerts better antioxidant activity (Free radical scavenging activity) as compare Sharad ritu.

As Rasayana is very broad concept, it has many aspects. To assess Rasayan Karma as it is, we have to study each and every aspect in vivo as well as vitro. The antioxidant property or free radical scavenging activity is one of the aspects of assessing the Rasayan karma.

The present study reveals that the two samples collected in two different seasons does not show any kind of major difference in constitute primarily. But quantitative analytical study reveals that the two sample show quantitative difference.

Conclusion

By observing the results we can conclude that,

1] There is no major change is observed in the analytical study of Shatavari root collected in Sharad ritu and Shishir ritu.

2] However, free radical scavenging activity of Shatavari root collected in Shishir ritu is better than that of Shatavari root collected in Sharad ritu. Further more detail study is required to come to the final conclusion.

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