Published online in http://ijam.co.in ISSN No: 0976-5921

International Journal of Ayurvedic Medicine, Vol 15 (3), 2024; 744-748

Impact of season on phytochemistry with special reference to *β*-Asarone content, extract yield and Pharmacognostic parameter in rhizome of *Acorus calamus* L.

Research Article

Venkat Narsimhaji Ch[1], Kanakrajan Vijayakumari Rakhesh[2], Ilavarasan Raju[2], Anupam K Mangal[3], Ravinder Singh[4], Srikanth N[4], Shyam Baboo Prasad[1,3*]

1. Central Ayurveda Research Institute, Jhansi. India.
2. Captain Srinivasa Murthy Central Ayurveda Research Institute, Chennai. India.
3. Central Ayurveda Research Institute, Kolkata. India.
4. Central Council for Research in Ayurvedic Sciences (CCRAS), New Delhi. India.

Abstract

Demand of medicinal plants is growing exponentially due to its extensive use in nutraceutical, cosmeceutical and pharmaceutical industries. The major problem is the variation in quality and quantity of phytoconstituents with the collection of medicinal plants in each batch. Though, the quality and quantity of phytoconstituents in plants are influenced by various factors, chief among them is seasonal variation. Rhizome of *Acorus calamus* L. is used in treatment of neurological disorder specially in children. The Ayurvedic system of medicine believes blends of constituent imparts therapeutic activities. The main chemical constituent responsible for cognition enhancement is Asarone. An attempt has been taken to record change in Pharmacognostic parameter with season and evaluate the best collection time of rhizome of *Acaorus calamus* L. with special reference to extractive value and asarone content.

Keywords: Asarone, *Acorus calamus, Vacha,* Seasonal variation, Flag root.

Introduction

Acorus calamus L. is a perennial monocot herb belongs to the family Acoraceae. The plants were cultivated throughout India in for its medicinal properties and seen grown in wetland and marshy places in the wild. In Ayurveda, formulations were prepared from the rhizome of *Acorus calamus* L. (*A. calamus*) and it has been used as substitute for ginger due to the characteristic odour. It has been known to have various vernacular names such as cinnamon sedge, flag root, myrtle flag, myrtle grass, myrtle sedge, sweet cane, sweet myrtle, sweet root, sweet rush, sweet sedge, etc (1). In Ayurveda texts, V*acha* has been described to enhance the vocal capacity and is the intellect promoting agent. In traditional medicinal systems, the medicinal properties of *A. calmus* was well documented and have been used for treatment of various ailments as individual drug or as an ingredient of formulation. It has been used as memory enhancing drug and have been given to babies in milk for improving the development of their brain (2). Classical Ayurvedic texts mention the different activities and properties of *A. calamus* and its uses in various therapies and medications. It was used as a *vantikrut* (inducing vomiting) in *Vamana* therapy, *vanhikrut* (appetiser) in dyspepsia, *vibandhhara* (carminative), *Shulaghni* (antispasmodic), *Shakrut Vishodini* (removes stool from body), *Mathrubhum*i (diuretic), *Bhodhaneeya* (arousing consciousness), *Karshini* (weight loss), *Bhutaharet or Jantuharet* (antimicrobial or antihelminthic properties), *Anilhara or Vatanasaka* (anti-inflammatory, analgesic, pain, reducing), *Vednasthapaka* (analgesic, anti-inflammatory, arthritis), Lekhana (lipid lowering), *Swaralu* (improving speech or voice), *Smarani* (memory promoter), *Shleshmaghni* (pacifies kapha), *Vijaya* (victory over diseases), *Mangalya* (helps to keep healthy) (3).

Apart from classical Ayurvedic validation *A. calamus* have well documented history in folklore and ethnobotanical medications. It has been used for the treatment of cough, cold, throat infection, fever, diarrhoea, etc. Various tribals peoples and ethnic groups used these plants against tumours, dental disorders, respiratory and gastrointestinal tract diseases, jaundice, ringworm infections, lice and flee infections, snake bites, etc. Apart from Ayurvedic and folklore claims *A. calamus* extracts were scientifically validated for its neuroprotective and memory enhancing properties. Studies in rat model shows the dried rhizomes shown decrease in brain damage and the antioxidant property of the plant increase the memory in animals (4).

The secondary metabolites such as Acorone, Acorenone, Asaraldehyde, Asarone , Isocalamendiol, 2-hydroxyacorenone, 2-acetoxyacorenone, Epiacorone, 1-Hydroxyepiacorone, epiacoronene, isocalamendiol, asaraldehyde, Acorusdiol, Acorusnol, 1-(2,4,5-tri-

*** Corresponding Author:**
Shyam B Prasad
Research officer (Pharmacognosy).
Central Ayurveda Research Institute
Kolkata. West Bengal.
India.
Email Id: b.shyam2@gmail.com

metoxyphenyl)- propane-l,2-dione and 1-(2,4, 5-tri-methoxyphenyl) - 1-methoxy-propane-2-ol (Nawamaki and Kuroyanagi, 1996), Calamusin A – I, Calamusin L – Q, hedytriol, (−)-1β,4β,7α-trihydroxyeudesmane, oplodiol (9), 36 6-eudesmene-1β,4β-diol, 4β,5α,10β-trihydroxycadinan, tatarinowin A, bullatantriol, homalomenol A, 4′-dihydrophaseic acid, blumenol C, (6R,9S)-9-hydroxy-4-megastigmen-3-one, (+)-dehydrovomifoliol and 1,10-seco-4ξ-hydroxy-muurol-5-ene-1,10-diketone (5,6) were isolated from the tubers of *A. calamus.*

These pharmacological properties increased the demand of *A. calamus* and the quality of the supplied material must be accessed before procurement. According to Ayurveda studies quality of raw material depends with collection time. Studies conducted in the determination of antioxidant potential in *A. calamus* plants shown difference in concentration of phenolic compounds and activity in plants collected during various seasons (7).

Many research works are being carried out to explore the influence of different season on phytoconstituents and/or its biological activities, but still there is slight or no awareness among local harvesters for choosing suitable season for harvesting to get potential yield of maximum raw material causing minimum harm to the plant . Modern science mainly focusses on active phytoconstituents of plants and biological activity.

So, the aim of the study is analysing the seasonal variation in *A. calamus* rhizome and thus to determine the correct harvesting period on the basis of *β*-Asarone content and yield of extracts. To evaluate the seasonal variation in Pharmacognostic parameter. The present study will be helpful to get rhizome of *A. calamus* with best quality and efficacy.

Material and Methods

Collection of Plant Material and Authentication:

Plant was collected season wise from the CARI garden (Fig. 1) and authenticated at CARI, Jhansi. The raw plant materials that were collected were washed under running tap water and were subjected to shade dry. The dried material was powdered to pass through the mesh 22 and stored in air tight container for anatomical and phytochemical studies. The reference material is kept for reference (CARI/JHS/AC01 to 06)

Pharmacognostic evaluation

Macroscopic features such as colour, shape, size were studied and photographs were taken with Nikon DSLR Camera in for the samples collected in each season. The measurement of the sample was taken by scale through naked eye. Microscopic examinations were carried out and described in table-1and image is shown in figure 1. Transverse sections of plant parts were depicted in figure 2 and table 2. powder microscopic characters were depicted in figure 3 and table 3 (8-10).

Extraction

The dried powdered rhizome material (5 g) was extracted with 200 ml of methanol by using soxhlet for 24 h. The extracts were evaporated to dryness under reduced pressure. The same procedure was followed for ethanol and hydro-alcoholic extraction. The obtained residue weights for the above extractions given in the table 2 (11).

HPLC estimation of *β*-Asarone

Test solution

The residues obtained from methanol, ethanol and hydro alcohol extracts of each six seasons were weighed in triplicate and dissolved in methanol using 10 ml volumetric flask, filtered through 0.22 µ membrane filter and used for HPLC analysis (12).

Standard solution

Dissolve 3.9 mg of *β*-Asarone in HPLC grade methanol in a 10 ml volumetric flask and make up the volume.

Chromatographic conditions

- Instruments-Agilent 1200 series with manual sampler
- Column-C_{18} Eclipse, XBD, 4.6 mm x 150 mm
- Detection-VWD Detector at 254 nm
- Mobile phase-Acetonitrile: Water (80: 20)
- Flow rate -1.5 ml/min
- Injection volume-10 µl
- Retention time-1.540

Estimation of *β*–Asarone in the drug

Inject 10 µl each of the test solution to HPLC system. Record the chromatogram and determine the area of the peak of the test solution corresponding to that of *β*-Asarone as described above from the calibration curve. Calculated the amount of *β*-Asarone present in the residues extracted in various solvents such as Hydro-alcohol, ethanol and methanol each test samples obtained from the various seasons of *Acorus calamus* given in the table 3.

Results and Discussion

Pharmacological characters of rhizome

Macroscopic analysis

Dried rhizome pieces are subcylindrical, occasionally bent at places, rarely straight, dorsiventrally slightly compressed 5 to 15 cm in length and 1 to 2 cm in thickness. The rhizomes were covered with thin cork skin and adherent triangular shriveled scaly withered leaf bases encircles the upper surface. The lower surface show of tubers shows circular tubular root scars. The cut portions of tubers show fracture which extend towards the endodermis (Figure 1).

Microscopic analysis

Transverse section (Figure 2) of the *A. calamus* tubers shows an outer layer of epidermis covered with thin cuticle or few thin-walled cells of cork developed underneath it, followed by 3 to 4 rows of collenchymatous hypodermis. A wider zone of parenchymatous cortex embedded with starch grains, isolated with oleoresin cells were seen in sections during all season. Vascular bundles are oval to spherical in shape, showing centrally located phloem encircled by

a ring of xylem vessels and pericycle fibres. A layer of endodermis separating the cortex and stelar region is distinct. Ground tissue of the stelar region is very wide and embedded with similar type of vascular bundles but those lying underneath the endodermis are smaller in size and are devoid of fibres. Parenchymatous cells of the stellar tissue are also embedded with oleo resin cells and starch grains.

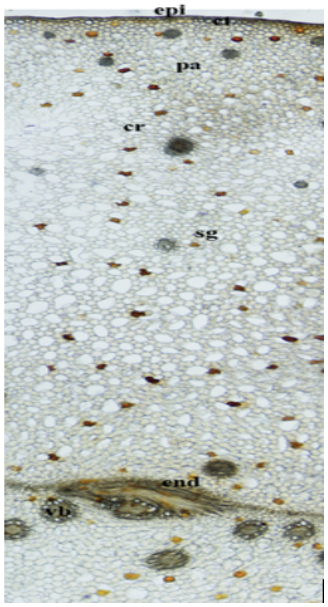
Powder analysis

Rhizome powder of *A. calamus* (Figure 3) contains transversely cut fragments of cortical aernchymatous tissue embedded with oleoresin cells. Plenty of simple and compound, small sized starch grains scattered as such or embedded in parenchymatous cells were seen on powder. Starch grains are mostly round, rarely oval and irregular, simple, single or in aggregation, provided with several concentric rings and central faintly stained hilum. Large thin walled spheroidal oil cells with yellowish content scattered in the powder.

The detail characters of Pharmacognostic parameter in different season is depicted in table 1.

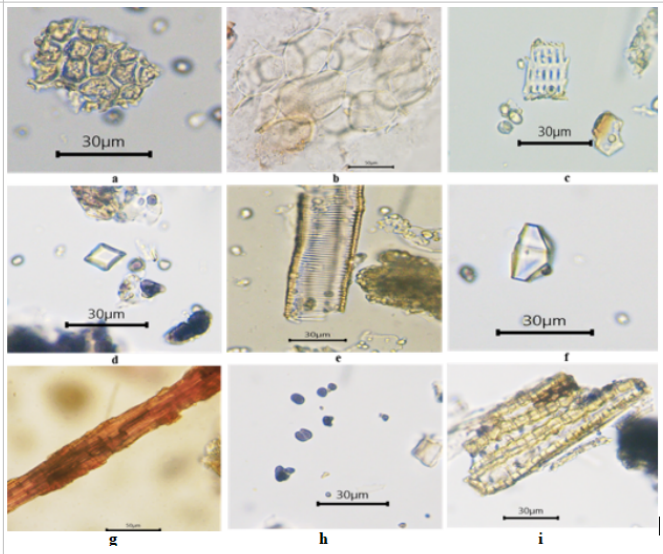
Figure 1: Macroscopy of *Acarus calamus* Linn rhizome

Figure 2: TS of Rhizome of *Acarus calamus* Linn at 4X.



(**Abbreviation: epi,** epidermis; **ct,** cortex; **pa,** parenchyma; **cr,** crystals; **sg,** starch grains; **end,** endodermis; **vb,** vascular bundles)

Figure 3: Powder microscopy



a, polygonal epidermis cells in surface view; b, oval shaped parenchymatous cells; c, simple pitted vessels; d, prismatic crystals; e, elements of scalariform vessels; f, rhomboidal crystals of calcium oxalate; g, lignified fibres; h, iodine stained round to oval shaped simple and compound starch grains; i, thickened inner and radial walls sclereids cells

Table 1: Macro & Microscopical features of *A. calamus* in different seasons
(+ Represents presence of the character without significant changes)

Macroscopic features of rhizome						
	Shishir ritu	**Vasant ritu**	**Grishm ritu**	**Varsha ritu**	**Sharad ritu**	**Hemant ritu**
Vegetative character based on Fig1						
Flowering and fruiting	+	+	-	-	-	+
Macroscopic characteristics of Root (Fig 1)						
Subcylindrical, Dorsiventral tubers	+	+	+	+	+	+
5 to 15 cm in length	+	+	+	+	+	+
1 to 2 cm in thickness	+	+	+	+	+	+
Presence of scale leaves and root scars	+	+	+	+	+	+
Fractures on surface extend towards the endodermis	+	+	+	+	+	+
Microscopic characteristics of rhizome (Fig 2)						
Presence of cork cells	+	+	+	+	+	+
4 to 5 layers of inner parenchymatous cortex	+	+	+	+	+	+

Presence of oil cells	+ (Lower in abundance than all other season)	+	+	+	+	+
Presence of aerenchymatous cells	+ (Lower in abundance than all other season)	+	+	+	+	+
Vascular bundles were scattered in cortex and stelar region	+ (Lower in abundance than all other season)	+	+	+	+	+
Lumen size of xylem vessels	+ (Smaller in size than all other season)	+	+	+	+	+
Powder microscopic characters of rhizome (Fig 3)						
Presence of oleoresin cells	+	+	+	+	+	+
Presence of starch grains	+	+	+	+	+	+
Presence of oil cells	+	+	+	+	+	+

Extraction

Table: 2. Yield of Methanol, ethanol and hydroalcoholic extract in different season

S.No.	Seasonal name	Residue weight		
		Methanol extract	Ethanol extract	Hydro-alcohol extract
1	Shishir ritu S_1 (5g)	0.7055 g	0.7764 g	0.9684 g
2	Basant ritu S_2 (5g)	1.3228 g	0.8816 g	1.1410 g
3	Grishma ritu S_3 (5g)	1.3684 g	1.1778 g	1.1999 g
4	Varsha ritu S_4 (5g)	1.3368 g	0.9863 g	1.0850 g
5	Shared ritu S_5 (5g)	0.7920 g	0.4868 g	0.6351 g
6	Hement ritu S_6 (5g)	0.8202 g	0.5378 g	0.7085 g

Selected chemical constituent: *β*-Asarone (Retention time-1.540)

Figure 4: *β*-Asarone

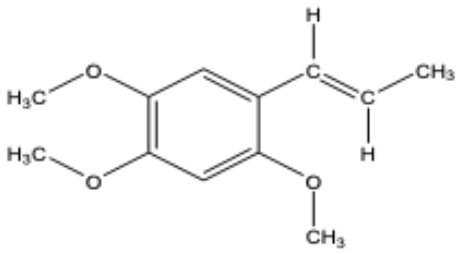


Figure 5: HPLC Chromatogram of *Acorus calamus* rhizomes

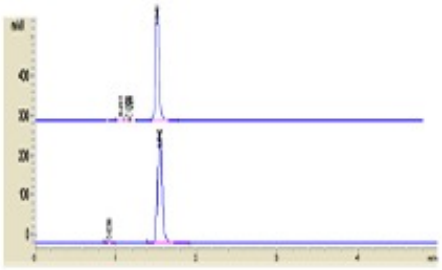


Figure 6: Calibration curve

Calibration curve

3.9 mg of *β*-Asarone was accurately weighed and added to a 10 ml volumetric flask, dissolved in HPLC grade methanol and the volume was made up to 10 ml with HPLC grade methanol to obtain 0.39 mg/ml. This solution was appropriately diluted further to get a concentration of 0.39, 0.195, and 0.0975 mg/ml of *β*-Asarone. Each of the standard solution run through the HPLC and recorded the respective peak areas figure 5. Calibration curve was established for peak area vs concentration of *β*-Asarone applied. figure 6.

Table 3: *β*-Asarone content in Methanol, ethanol and hydroalcoholic extract in different season

S.No	Seasonal name	* *β*-Asarone (% w/w)
1	Shishir ritu	1.3415 - 4.0153
2	Basant ritu	3.5327 - 8.0135
3	Grishma ritu	8.1613 - 10.8332
4	Varsha ritu	7.1750 - 11.848
5	Shared ritu	3.6773 - 6.8031
6	Hament ritu	2.0365 - 7.8083

*Range of results was given for all seasons from the means of triplicates of optimised three solvent extracts of hydro-alcohol, ethanol and methanol.

From the above table it was observed the abundance of *β*-Asarone is more in **Varsha ritu** samples received from National Vrkshayurveda Research Institute, Gwalior road, Jhansi.

Discussion

According to world health organisation over 10 lakhs registered traditional practitioners were there in India and in recent years there was an exponential increase usage of traditional medicines(World Health Organization (WHO), 2004). Since the number of persons depending on traditional medication increases, the demand for plant drugs tend to increase rapidly. Quality of raw drug will improve the efficacy of formulations and this quality depends on the phytochemical contents inside the plant material. As the quantity of phytochemical differ from various season the proper collection time will be standardised for the collection of high quality drugs.

The similarities in macroscopic and microscopic results were not enough to discriminate the quality of the tubers (Table 1). Since in *A. calamus* the difference in macroscopic and microscopic character were negligible the pharmacological activities were found to be high on samples collected from specific season irrespective of their morphological and anatomical similarities. These studies justify the rational of selecting such work on *A. calamus* and phytochemical evaluation could only be able to determine the seasonal variation.

Conclusion

There is negligible change in Pharmacognostic parameter. Developmental variation and dimension variation only observed. The best procurement time for medicinal preparation using rhizome as powder or extracts are *Grism ritu*. However, the rhizome can be also collected in vasant ritu and varsha ritu. Rhizome collection must be avoided in Hemant, Shishir and vasant ritu. The best procurement time for *β*-Asarone is grism ritu. The rhizome can be also collected in varsha ritu. However, in Hemant, Shishir vasant and vasant ritu the rhizome collection must be avoided.

Acknowledgements

We are thankful to CCRAS, Ministry of AYUSH for providing IMR project and financial support.

Compliance with ethical standards
Conflict of interest: Nil
Ethical issues: None.

References

1. Balakumbahan, R., Rajamani, K., and Kumanan, K. (2010). Acorus calamus: An overview. *J. Med. Plants Res*. 4, 2740–2745. doi:10.7439/ijbr.v2i10.174.
2. Jadhav, T. (1994). *Sushrut Samhita*. 1st Ed. Chaukhambha Surbharati Acadamy, India.
3. Rajput, S. B., Tonge, M. B., and Karuppayil, S. M. (2014). An overview on traditional uses and pharmacological profile of Acorus calamus Linn. (Sweet flag) and other Acorus species. *Phytomedicine* 21, 268–276. doi:10.1016/j.phymed.2013.09.020
4. Davoudi-Kiakalayeh, A., Mohammadi, R., Pourfathollah, A. A., Siery, Z., and Davoudi-Kiakalayeh, S. (2017). Alloimmunization in thalassemia patients: New insight for healthcare. *Int. J. Prev. Med*. 8, 1–8. https:doi:10.4103/ijpvm.IJPVM.
5. Hao, Z. Y., Liang, D., Luo, H., Liu, Y. F., Ni, G., Zhang, Q. J., et al. (2012). Bioactive sesquiterpenoids from the rhizomes of Acorus calamus. *J. Nat. Prod*. 75, 1083–1089. https:doi:10.1021/np300095c.
6. Hao, Z. Y., Liu, Y. F., Cao, Y. G., Liang, D., Luo, H., Zhang, C. L., et al. (2021). Sesquiterpenoids of diverse types from the rhizomes of Acorus calamus. *RSC Adv.* 11, 14229–14236. https:doi:10.1039/d1ra00350j.
7. Bahukhandi, A., Rawat, S., Jugran, A. K., Bhatt, I. D., and Rawal, R. S. (2021). Seasonal Variation in Phenolics and Antioxidant Activity of Acorus calamus Linn.: An Important Medicinal Plant of Himalaya. *Natl. Acad. Sci. Lett.* 44, 13–15. https:doi:10.1007/s40009-020-00959-3.
8. Mangal AK, Tomer R, Jindal H, Dahiya J, Prasad SB, Singh H, Sharma BS, Srikanth N. Development of quality control parameters for classical ayurvedic formulation: Vyoshadi Churna. Journal of Ayurveda. 2023 Jan 1;17(1):38-44. *DOI:* 10.4103/joa.joa_318_21
9. Prasad SB, Sharma A. Standardisation of convolvulus pluricaulis choisy herbs collected from Jalandhar, Punjab. Int. J. Pharmacogn. Phytochem. Res. 2016;8:1412-6.
10. Sharma M, Prasad SB. Standardisation of Guazuma tomentosa leaf. Int J Pharmacogn Phytochem Res. 2014 Dec;6(4):1010-4.
11. Gandhi Y, Rawat H, Kumar V, Thakur A, Mishra SK, Charde V, Soni H, Singh G, Tandon S, Kumar R, Shakya SK, Prasad SB. Identification of two kinds of Colchicum corms by DNA barcoding, physicochemical, chromatographic and chemometric analyses along with pharmacognostic parameters. Microchemical Journal. 2023 Dec 1;195:109399.
12. Gandhi MY, Prasad SB, Kumar V, Soni H, Rawat H, Mishra SK, Grewal J, Singh S, Charde V, Gupta A, Jha SK. Quantification of Phytochemicals and Metal Ions as well as the Determination of Volatile Compounds, Antioxidant, Antimicrobial and Antacid Activities of the Mimosa pudica L. Leaf: Exploration of Neglected and Under-Utilized Part. Chemistry & Biodiversity. 2023 Oct;20(10):e202301049.

*****