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Formulation and evaluation of milk-based spray dried carrier system to enhance stability of Steroidal Saponin from Shatavari (Asparagus Racemosus Root)

Maheswari S[1], Venkatesh[2*], P K Kulkarni[3], Hanumanthachar K Joshi[4]
[1] Department of Pharmaceutics, Sarada Vilas College of Pharmacy, Mysuru, Karnataka, India
[2] Professor & Head, Department of Pharmaceutics, Sarada Vilas College of Pharmacy, Mysuru, Karnataka, India
[3] Dean Research, Department of Pharmaceutics, Sarada Vilas College of Pharmacy, Mysuru, Karnataka, India
[4] Principal, Department of Pharmacognosy, Sarada Vilas College, Mysuru, Karnataka, India

Abstract
Asparagus racemosus, commonly known as Shatavari, is a significant medicinal plant traditionally used in Ayurveda, particularly recognized for its benefits as a female tonic. This plant, belonging to the family Asparagaceae (previously classified under Liliaceae), is native to tropical and subtropical regions of India and has been utilized for centuries for its therapeutic properties. The natural bioactive compound or marker shatavarin IV which is a well-known steroidal saponin gycosides present in the roots of asparagus racemosus. Milk is one such carrier that has been effectively used to deliver phytochemical for targeted health benefits in the traditional Indian system of medicine. Aim of the present research work is to formulate and evaluate the steroidal saponin glygosides from shatavary loaded into spray dried milk powder by using spray drying technology and to improve the stability and efficacy. The study on Shatavarin IV evaluated through the HPTLC method indicates that the spray-dried milk decoction powder remains stable for a minimum of three months. This conclusion was drawn from the analysis of physicochemical parameters, which showed no significant changes during this period.

Keywords: Shatavarin IV, milk, spray drier, HPTLC, stability enhancement

Introduction

Ayurvedic Therapeutics

"Ayurveda' means 'science of life' It is a holistic system of healthcare with the concept that hu-man body is a matrix of seven basic tissues (Sapta Dhatu), three types of energies (Tridoshes) and waste product of body (Mala) Any imbalance or disturbances in these basic principles of body cause disease. The ancient history of India is very rich in herbal medicine and one of the oldest surviving systems of health care in the world and known as Ayurveda derived from its ancient Sanskrit roots 'Ayur' (life) and 'ved' (knowledge).

Milk is a nearly complete food for neonates, providing essential nutrients for growth. Shatavari (Asparagus racemosus) is recognized in Ayurveda for its galactagogue properties, enhancing breast milk production by increasing prolactin levels due to steroidal saponins. It balances hormones, supports reproductive health, and is often used in formulations like Shatavaryadi Ksheerapaka, which combines Shatavari with other rejuvenating herbs in cow's milk. This herb, known as the "queen of herbs," promotes both reproductive and digestive health, making it valuable for nursing mothers. Milk serves as an effective carrier for delivering these nutraceuticals due to its ability to solubilize both polar and non-polar compounds, enhancing stability and bioavailability. Recent advancements in spray-drying techniques have improved the formulation of milk-based systems, ensuring the effective encapsulation and delivery of Shatavari's active ingredients. This innovative approach holds promise for developing functional products that promote hormonal balance and overall female health. [1, 3]

Materials and methods

Procurement and Authentication of Asparagus racemosus

Procurement: Roots of Asparagus racemosus were sourced from local markets in Mysuru, India.

Authentication: The roots were verified by a pharmacognosist from Sarada Vilas College of Pharmacy, ensuring they were genuine A. racemosus.[2]

Organoleptic Parameters of Shatavari Roots

Characterized by a silver white or light ash color observed under natural light, slightly sweet taste, the roots are tuberous, typically 10 to 24 cm long and 0.5 to 2.5 cm in diameter, with a smooth surface when fresh and potential wrinkles when dried. These sensory attributes aid in the identification and quality assessment of the herb. [1, 5]

Microscopic Examination

To examine Asparagus racemosus roots microscopically, soak dried roots in water until softened, then cut thin transverse sections. Clear the sections in chloral hydrate solution, stain with a mixture of phloroglucinol and concentrated hydrochloric acid, and mount using dilute hydrochloric acid for stained sections or glycerol for unstained ones. This method reveals key anatomical features, such as vascular structures and calcium oxalate crystals, essential for studying the plant's medicinal properties. [4, 5]

Physicochemical Parameters in Ayurvedic Pharmacology

The analysis of physicochemical parameters in herbal drugs, particularly those referenced in the Ayurvedic Pharmacopoeia of India and WHO guidelines, is critical for assessing the quality, purity, and efficacy of crude drugs.

This includes the determination of ash values, extractable matter, and moisture content. [1, 2]

Ash Values
Ash values serve as indicators of contamination, substitution, adulteration, or carelessness in the preparation of crude drugs.

They are determined through several methods
Total Ash Value
To determine the total ash value, the following steps are taken
The formula used is:
Total Ash w /w = (Weight of Ash/Weight of Sample) ×100

The calculation is as follows:
Water Soluble Ash w/w = (Weight of Total Ash−Weight of Insoluble Ash/ Weight of Sample) ×100

Extractive Values:
The extractive values are calculated as:
Extractive Value w /w =(Weight of Extract/Weight of Sample)×100

Moisture Content:
Calculate moisture content using:
Moisture Content= (W1+W2−W3/W2) ×100
Where W1 is the weight of the petri dish, W 2 is the weight of the sample, and W 3 is the weight after drying.[5,9]

Milk
Milk was collected from dairy farm, Mysuru Karnataka. It was assured for quality before further utilization.

Lactometer Reading of Milk
Lactometer readings are crucial for detecting water adulteration in milk, as added water lowers the specific gravity, above or below 60 °F. Higher readings indicate purer milk, while lower readings suggest dilution or adulteration. [6]

Preparation Methods
Milk Decoction Method
To prepare and optimize a milk decoction of Asparagus racemosus, two distinct methods were utilized, focusing on the evaluation of pH, total solid content, and total saponin content to identify the most effective preparation technique.[8,14]

Formula for milk decoction preparation

Table 1

Incredients	Quantity	Quantity
	Astang Hardaya	Sharngdhar Samhita
A. racemosus root powder	1 Part	1 Part
Milk	4 Part	4 Part
Water	8 Part	32 Part

The milk decoction was prepared by heating milk with A. racemosus and then straining the mixture through a nylon sieve into a jug.
After straining, the extract was allowed to cool to room temperature and concentrated under reduced pressure. [14]

Optimization and Validation of Milk Decoction
To optimize milk decoction for extracting total saponin content from Asparagus racemosus, key factors include decoction time, temperature, solvent choice (milk), and plant material preparation. Analytical techniques like HPTLC and spectrophotometry can validate the process by quantifying saponin levels. This optimization enhances the therapeutic potential of the decoction while ensuring scientific validation of its efficacy. [10]

Drying of Milk Decoction
The drying of milk decoction from Asparagus racemosus is a crucial food preservation method that reduces moisture, inhibiting microbial growth and extending shelf life. Various techniques, including spray drying, freeze drying, and drum drying, are employed. Spray drying is particularly effective due to its cost efficiency and ability to retain nutritional quality. It produces a fine powder with excellent solubility and rehydration properties. Overall, spray drying offers significant advantages over other methods for preserving milk decoction. [6,10]

Optimization of Spray Dryer for Drying of Milk Decoction of A. Racemosus.
To optimize spray drying of milk decoction from A. racemosus, key parameters include inlet temperature (80°C to 200°C), outlet temperature, feed rate (4 to 40 ml/min), air volume, and atomization speed (25,000 rpm). Utilizing Response Surface Methodology (RSM) can systematically explore interactions between these factors to identify optimal conditions for maximum yield and quality. [5, 9]

Experimental conditions during the spray drying of milk decoction of A.racemosus root extract

Table 2

Experimental condi-tions	Inlet temperature ^{O}C	Outlet temperature ^{O}C	Feed Rate %
I	140	110	10
II	140	110	8
III	140	110	5
IV	120	90	10
V	120	90	8
VI	120	90	5
VII	100	70	10
VIII	100	70	8
IX	100	70	5

Feed rate 100% means 1000ml/hour.

Preparation of Powder Dosage Form from Milk Decoction of A.racemosus.
Two thousand ml milk decoction of A. racemosus was prepared with optimized formula and total solid content, specific gravity, pH, total fat and total saponin were determined. A 2000 ml milk decoction was dehydrated under reduced pressure.

Spray Dried Powders Preparation
Two batches of spray-dried powder were prepared from milk decoction using a Labultima mini-spray dryer under co-current flow conditions. The operational parameters included an inlet temperature of 120 °C, outlet temperature of 90 °C, feed rate of 4 ml/min, and spraying pressure of 2 bars. The resulting powders were stored in tight, light-resistant containers within glass desiccators over silica gel to maintain quality. This method effectively preserves heat-

sensitive components and enhances shelf life for various applications in food, pharmaceuticals, and cosmetics. [6]

Evaluation of Spray Dried Powder of Milk Decoction
Spray dried milk decoction were evaluated for its Shatavarin-IV and total saponins content, solubility, tapped density, angle of repose, moisture content and color.

Determination of Shatavarin IV with HPTLC
Shatavarin IV was determined with HPTLC Method (ICMR, 2003):[4]

Sample Preparation
Accurately weigh each sample on a Mettler Toledo balance, transfer to a test tube, and solubilize in methanol. Sonicate for extraction, filter the solution, and adjust the volume to 10 mL in a volumetric flask.

Standard Preparation
Weigh 1.0 mg of shatavarin-IV, dissolve in a small volume of methanol in a 10 mL volumetric flask, sonicate for one minute, and make up to volume with methanol.

TLC Application
Apply aliquots of the prepared samples and standard solutions onto silica gel GF254 precoated TLC plates. [9, 15]

Chromatographic parameters and conditions:

Table 3

Plate size (X x Y)	20.0 x 10.0 cm
Material	HPTLC plates silica gel 60 F 254
Manufacturer	E. MERCK KGaA
Pre-washing	YES
Solvent name	Methanol
Drying device	Oven
Temperature	120 °C
Time	20 Minutes

Calibration parameters
Calibration mode- multi level
Statistics mode- CV
Evaluation mode- Peak Heat and area

Total Saponin Content
The total saponin content in Asparagus racemosus was extracted from dried milk and aqueous decoctions using A-butanol and diethyl ether, followed by drying and weighing the precipitates. Insoluble solids were measured by dissolving 10 grams of powder in water. Bulk and tapped densities were determined by filling a measuring cylinder with powder, tapping it, and measuring the volume. The angle of repose was calculated using the height and radius of a powder heap formed from a funnel. Moisture content was assessed with an IR moisture balance, while recovery yield from drying was calculated based on mass, volume, and concentration. Stability studies were conducted under controlled conditions for three months. [4]

Stability Study of Spray Dried Powder of Milk Decoction
International Conference on Harmonisation (ICH) stability guidelines were followed to carry out stability studies. Milk decoction A. racemosus was prepared as per optimized and validated method; it was dried with spray drying technique. Three batches of spray dried milk decoction were kept in a screw capped amber coloured glass bottles. All the glass bottles were packed in a black canvas bag and placed in the stability chamber at 40 ± 2 °C and 75% RH for 3 months. Samples were withdrawn at periodic time intervals i.e. 0, 1, 3 months and tested for parameters mentioned above. [9,15]

Result and discussion
Physicochemical Properties of Raw Material
A.racemosus
Macroscopic Study
Root tuberous, 5 to 10 cm in length and 0.1 to 0.5 cm thick, tapering at both ends with longitudi-nal wrinkles. Fracture complete, internal appearance white.

Organoleptic parameters of powder
Color: creamish white or light ash
Taste: slightly bitter sweetish
Odour: not well marked

Microscopic Examination
The transverse section of Asparagus racemosus root reveals a circular structure with an outer layer of piliferous cells, forming unicellular root hairs for absorption. The cortex is divided into an outer layer of thick-walled, lignified cells and an inner layer of thin-walled, elongated cells with intercellular spaces. Stone cells are present in the upper cortex, while the endodermis, composed of thin-walled parenchyma, regulates nutrient uptake. The exarch stele contains xylem with pitted vessels and phloem, surrounded by central parenchymatous pith. Raphides of calcium oxalate are also present, contributing to the root's adaptive functions.

Physicochemical Parameters
The physicochemical parameters of Asparagus racemosus (Shatavari) indicate its suitability for medicinal use, with results as follows:
Moisture Content: 5.91%
Total Ash: 3.98%
Acid Insoluble Ash: 0.55%
Ethanol Soluble Extractive Value: 8.99%
Water Soluble Extractive Value: 40%
These values align with the Ayurvedic Pharmacopoeia of India.
The qualitative chemical examination of Asparagus racemosus (Shatavari) revealed the presence of several bioactive compounds in its aqueous extract:
All results comply with the standards outlined in the Ayurvedic Pharmacopoeia of India, confirming the extract's quality and supporting its traditional medicinal uses.

Qualitative chemical examination of A.racemosus

Table 4

S. No	Chemical tests	Inferences
1.	Alkaloids: Mayer's Test Dragendorff's Test Hager's Test Wagner's Test	 Absent Absent Absent Absent

2.	Carbohydrates: Molich's Test Fehling's Test Benedict's Test	 Present Present Present
3.	Fat and Oil: Solubility Test Paper Test	 Absent Absent
4.	Saponins: Foam Test	 Present
5.	Protein: Biuret Test Precipitation Test	 Absent Absent
6.	Steroids:	Present
7.	Amino acid: Ninhydrin Test	 Absent
8.	Glycosides: General Test	 Present
9.	Tannins: FeCl, Test	 Absent

Milk

The results for pH, CLR, Fat % and SNF % were found to be 6.6, 28, 4.6 and 9.0 respectively. All results are within the standard limit.

Evaluation of Shatavari Ksheerpaka Prepared as Per Formula given in table

Ksheerpaka prepared according to Astang Hridaya curdled due to a low pH of 3.8, below the isoelectric point of milk proteins (pH 4.0). In contrast, the method from Sharangdhar Samhita, using a ratio of 4 parts milk to 32 parts water, resulted in a higher pH of 6.1, preventing curdling. This highlights the importance of pH and formulation ratios in ensuring the quality and stability of milk-based Ayurvedic preparations.

Drying of Milk Decoction

Optimization of Spray Dryer for Drying of Milk Decoction of A. racemosus

With the primary data, spray drying of milk decoction was performed at three steps. At first concentrated milk decoction of A. racemosus was used. Secondly as the tests continued a hard glass film was shaped on walls. Thirdly as the operating condition was changed such inlet air temperature and feed flow rate the powder yield and production was improved. At different dryer operating condition, physical properties of powder were measured as given in Table**.**

Physical properties of spray dried powder of milk decoction of A. racemosus

Table 5

Experimental Conditions code	Moisture content (% w/w)	Bulk density	Insoluble solids (%)	Yield (%)
I	2.2	0.27	38	35
II	2.5	0.28	36	35
III	2.5	0.31	35	41
IV	2.6	0.34	33	48
V	2.7	0.39	29	57
VI	3.0	0.43	26	60
VII	3.2	0.47	25	62
VIII	3.4	0.49	24	59
IX	3.5	0.50	25	63

Spray Dried Powders Preparation

Spray-dried powder were prepared from the remaining milk decoction using a Labultima mini-spray dryer provided with a pneumatic nuzzle and functioning by co-current flow.

The operational conditions were

Inlet temperature - 120 °C
Outlet temperature - 90 °C
Feed rate - 4 ml/min
Spraying pressure - 2 bar

The spray-dried powders were preserved in tight, light-resistant containers and stored in glass Desiccators over silica.

Information

Application Position-14.0 mm
Solvent front position-80.0 mm

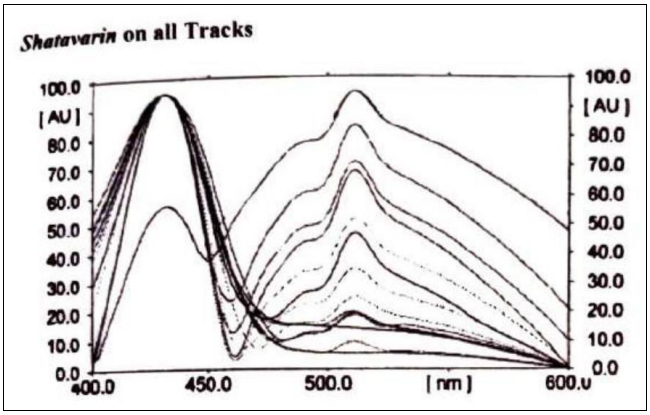

Fig 1

Table 6

Track	Rf	Substance	Max. @
1	0.47	Shatavarin	511 nm
2	0.43	Shatavarin	511 nm
3	0.40	Shatavarin	432 nm
4	0.45	Shatavarin	432 nm
5	0.44	Shatavarin	432 nm
6	0.44	Shatavarin	432 nm
7	0.46	Shatavarin	431 nm
8	0.47	Shatavarin	431 nm
9	0.48	Shatavarin	431 nm
10	0.43	Shatavarin	431 nm
11	0.44	Shatavarin	431 nm
12	0.49	Shatavarin	431 nm
13	0.46	Shatavarin	431 nm
14	0.48	Shatavarin	431 nm

Results per track

Substance: Shatavarin@430 nm
Regression via height: linear = 1.7%
Regression via area: linear = 1.2%
Y=82.97+5.322*X
r=0.9849
r=0.9995

Shatavarin-IV content in all samples after 3 months storage time during the Stability study

Calibration Curves

The standard solutions (1-120 ng per respective spot) were applied on TLC plate, HPTLC chromatograms are as shown in Figure-2. The plate was developed and scanned as per the

chromatographic conditions mentioned above.Calibration curve of Shatavarin-IV was prepared by plotting peak areas vs.concentrations of Shatavarin IV. The linear regression analysis of calibration plots of Shatavarin IV exhibited linear relationship in the range of 5-80 ng and with the correlation coefficient of 0.998 and thus exhibits good linearity between concentration and area.

Caliberation curve of Shatavarin-IV: stability study for 3 months stored samples

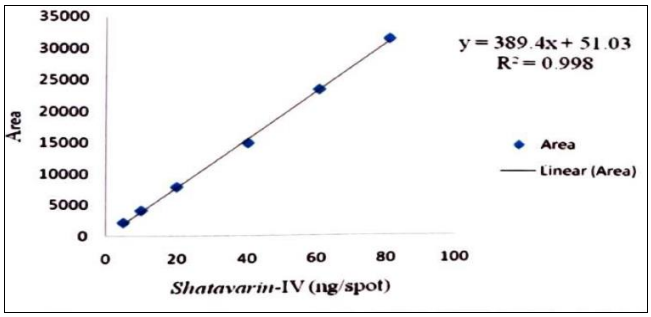

Fig 2

Table 7

Track	Sample ID	Area	Shatavarin-IV(ng spot)
1	Month III (B1)	7729.29	19.72
2	Month III (B2)	7909.12	20.18
3	Month III (B3)	7745.57	19.76

Estimation of shatavarin iv content after 3 months

The spot at Rf: 0.46 corresponding to Shatavarin-IV was observed in the respective chromatograms of the various samples of batch-1, batch-2 and batch-3 at 3 month of spray dried milk decoction There was no interference from other components present in the formulation. The data from Table- revealed that the Shatavarin-IV contents found to be consistent in all batches of 3 months stored samples of spray dried milk decoction powder.

The present study reveals that the spray dried milk decoction powder is stable for at least 3 months period as there was no major change in the values when calculated by applicable physiochemical parameters.

Summary and conclusion

The study formulated and characterized a Ksheerapaka milk decoction from Asparagus racemosus roots using spray drying technology. The roots were authenticated and found to contain various bioactive compounds, including flavonoids and steroid glycosides. The decoction was prepared traditionally and spray dried at specific parameters, resulting in a product that retained essential nutrients and met micromeritic properties. Stability studies over three months showed no changes in physical characteristics or chemical composition, indicating a stable formulation. This research demonstrates the effectiveness of spray drying in preserving the properties of herbal formulations**.**

References

1. Sivanandan A, Dei L, Harisha CR, Shukla VJ. pharmacognostical and physico-chemical analysis of shatavaryadi ksheerapaka-a polyherbal milk-based formulation for the management of menopausal syndrome.
2. Wagh DS, Kasture VS, Pawar SS. Phytochemical evaluations of marketed Shatavari formulations and development of analytical methods for saponins contents.
3. Pasha S, Khanam S, Afsar Z. Isolation and characterization of chemical constituents of Asparagus racemosus as markers. Int. J. Res. Dev. Pharm Life Sci,2016:5:2255-63.
4. Hayes PY, Jahidin AH, Lehmann R, Penman K, Kitching W, De Voss JJ. Steroidal saponins from the roots of Asparagus racemosus. Phytochemistry,2008:69(3):796-804.
5. Alok S, Jain SK, Verma A, Kumar M, Mahor A, Sabharwal M. Plant profile, phytochemistry and pharmacology of Asparagus racemosus (Shatavari): A review. Asian Pacific journal of tropical disease,2013:3(3):242-51.
6. Nijdam JJ, Langrish TA. An investigation of milk powders produced by a laboratory-scale spray dryer. Drying technology,2005:23(5):1043-56.
7. Zuppa AA, Sindico P, Orchi C, Carducci C, Cardiello V, Catenazzi P, Romagnoli C. Safety and efficacy of galactogogues: substances that induce maintain and increase breast milk production. Journal of Pharmacy & Pharmaceutical Sciences,2010:13(2):162-74.
8. Talmale S, Rathi B, Wanjari A, Rajput DS. Modification of Drug dosage form of Arjuna Ksheerapaka by using Spray Drying Technology. Journal of Research in Traditional Medicine,2017:3(3):72-.
9. Selvarajan S, Devi VG, John AS, Jeyakannan J, Balakrishnan D, Raaman N. Pharmacognostical identification of Asparagus racemosusWilld.(root) with the help of HPTLC method. World Journal of Pharmaceutical Research,2014:3(6):486-98.
10. Veena N, Arora S, Singh RR, Katara A, Rastogi S, Rawat AK. Effect of Asparagus racemosus (shatavari) extract on physicochemical and functional properties of milk and its interaction with milk proteins. Journal of food science and technology,2015:52:1176-81.
11. Sawale PD, Rani R, Veena N, Mesharam BD, Deosarkar SS, Wasnik PG. Technical Interventions in Development of Herbal Fortified Milk Products.
12. Rajni VR, Sindhu SC. Influence of Shatavari (Asparagus racemosus) Root Powder in Increasing Mothers Milk Output and Infants Weight Gain.
13. Hayes PY, Jahidin AH, Lehmann R, Penman K, Kitching W, De Voss JJ. Asparinins, asparosides, curillins, curillosides and shavatarins: structural clarification with the isolation of shatavarin V, a new steroidal saponin from the root of Asparagus racemosus. Tetrahedron letters,2006:47(49):8683-7.
14. Suprabha K, Dei LP, Harisha CR, Shukla VJ. pharmacognostical and physico-chemical evaluation of Shatavaryadi ksheerapaka in IUGR. Global Journal of Research on Medicinal Plants & Indigenous Medicine,2016:5(9):244.
15. Pandiyan GD, Leela V, Eswari S, Ramachandran M, Ranganathan V, Visha P. Evaluation of Shatavarin IV Compound from Methanolic Extract of Asparagus racemosus by High Performance Thin Layer Chromatography. J. Phytopharmacol,2022:11:89-91.