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Original Article

ISOLATION AND CHARACTERIZATION OF SHATAVARIN IV FROM ROOT OF ASPARAGUS RACEMOSUS WILLD

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ABSTRACT

Objective: To develop a method for the isolation of the steroidal saponin Shatavarin IV from the root of *Asparagus racemosus Willd*.

Methods: Powder of *Asparagus racemosus* dried roots were defatted with hexane and extracted with methanol. The methanolic extract was used for isolation of pure compound by column chromatography. Isolated compound was identified by melting point analysis, IR and Mass spectroscopic analysis. Purity was checked by High-performance thin layer chromatography (HPTLC).

Results: The highest yield of Shatavarin IV was obtained 401.1 mg from 250 g crude powder and highest purity was achieved 66% of lower atmospheric temperature (17-22 °C).

Conclusion: Shatavarin IV isolation using column chromatography was affected with the change in atmospheric temperature. Maximum yield was obtained at the lower temperature i.e. 17-22 °C, whereas at higher temperature the Shatavarin IV showed degradation in purity and yield.

Keywords: *Asparagus racemosus Willd*, Column Chromatography, Shatavarin IV, HPTLC, Spectroscopy.

INTRODUCTION

Shatavari (*Asparagus racemosus Willd.*) is a well known Ayurvedic drug [1] belonging to family Liliaceace [2]. Fasciculate tuberous roots of Shatavari are considered as one of the Rasayana (adaptogenic) drugs, having cooling, diuretic, and emollient, galactogogue, nervine tonic, rejuvenating and stomachic properties [3]. As a part of ongoing investigations on saponins from Indian medicinal plants, phytochemical study of *Asparagus racemosus* root was initiated. Chemical constituents reported from the plant material include steroidal glycosides (Shatavarin I-IV), a novel polycyclic cage type pyrrolizidine alkaloid, asparaginine and a 9, 10-dihydrophenanthrene derivative [4].

The Shatavari contains four steroidal saponins. The roots of the plant show antioxytocic activity (Shatavarin IV), anti-abortifacient activity (Shatavarin I) and also used to treat infertility [5], arising the need to develop an easy method for isolation of phyto constituents from the roots of plants. Earlier few studies have been developed for isolation of two important saponins Shatavarin I and Shatavarin IV from the roots of *Asparagus racemosus*. The isolation and extraction methods described in previous studies had long isolation process with fewer yields. The present study aimed to provide a new method for isolation of Shatavarin IV with minimum steps of column chromatography with better yield and easy identification.

The phytochemical evaluation of the isolated compound was also performed for better identification. The purity of same was calculated with the help of HPTLC method. The structure of isolated Shatavarin IV was characterized using IR and Mass Spectroscopy.

The effect of temperature on the isolation of Shatavarin IV by column chromatography has been discussed. Inadequacy of details regarding structural identification of Shatavarin IV made our study more useful and relevant.

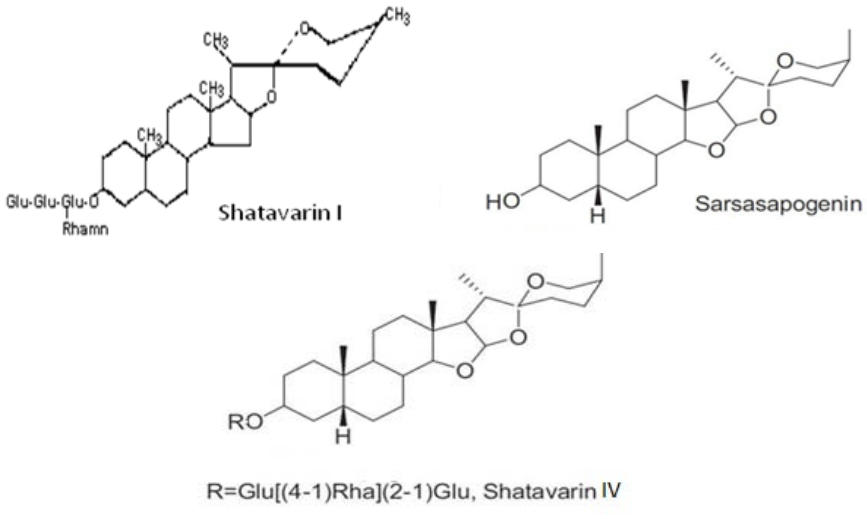


Fig. 1: Chemical structure of Shatavarin I, Sarsasapogenin and Shatavarin IV

MATERIALS AND METHODS

Apparatus

Soxhlet apparatus (Make: Merck) was used for the extraction process. Other apparatus used were Water Bath (Make: Cintex), Hot air oven (Make: Cintex), Sonicator (Make: Toshniwal), Weighing balance (Make: Shimadzu).

For HPTLC analysis CAMAG Linomat 5 sample applicator, CAMAG TLC Scanner 4, and CAMAG UV Chamber were used. Shimadzu IR affinity 1 FTIR-8400S was used for IR analysis.

Reagents and materials

The reference standard of Shatavarin IV was purchased from Natural Remedies Pvt. Ltd., Bangalore. Methanol, n-Hexane, Ethyl acetate, Chloroform, n-Butanol were procured from Merck specialties Pvt. Ltd. *Asparagus racemosus* dried root was supplied by Vasu Healthcare Pvt. Ltd., Vadodara. The identification of the plant material was done by Dr. P. S. Nagar, Asst. Prof, Faculty of Sciences, Dept. of Botany, M. S. University of Baroda, Vadodara.

Extraction and isolation

Tuberous roots of *Asparagus racemosus* (250 g) were powdered, defatted with Hexane and repeatedly extracted by maceration with 90% methanol at room temperature for 24 h. The total methanolic extract was evaporated on a water bath to obtain a concentrated liquid syrupy mass (100 mL) which was dissolved in 10% methanol. The resulting solution was partitioned with Chloroform, Ethyl acetate and n-Butanol, successively. The n-Butanol extract was dried on a water bath and the dried fraction was dissolved in minimum quantity of 90% methanol to load on the column as the sample. Silica gel G (60-120) was used for packing the column and eluted with Ethyl acetate: Methanol: Water (8:1:1 v/v) as mobile phase. Multiple fractions, each of 27 mL were collected and out of which eluted fraction 10 to 22 showed presence of Shatavarin IV. Shatavarin IV containing fractions was confirmed by TLC using Ethyl acetate: Methanol: Water (7.5:1.5:1 v/v) as developing solvent and the spots were visualized by dipping the plate with Vanillin-Sulfuric acid reagent. All the fractions (10-22) were mixed and dried at room temperature. Out of this, isolated compounds were obtained in a form of beige color shiny powder. It was further characterized by using HPTLC, melting point, FTIR and MASS Spectroscopy compared to the reference standard.

Identification and estimation of shatavarin IV by HPTLC [6]

All the fractions individual and final dried mixture was subjected to identification and estimation of shatavarin IV by HPTLC. Shatavarin IV standard and isolated compound was dissolved in methanol individually and prepared 100 µg/ml solutions. 16 µl of sample and standards were applied to a precoated Silica gel 60 F_{254} plate of 0.25 mm thickness with the help of CAMAG Linomat V. The plates were then developed with the mobile phase consisting of Ethyl acetate: Methanol: Water (7.5:1.5:1 v/v). The spots were visualized by dipping the plate in Vanillin Sulphuric acid reagent and heating at 105˚C for 5 min. The plates were scanned using CAMAG TLC Scanner IV at 425 nm to estimate the concentration of Shatavarin IV. Peak purity also checked using spectrum mode of the scanner.

RESULTS AND DISCUSSION

Asparagus racemosus root contains the combination of various types of saponin with different polarities; their separation still remains a big challenge for the process of identification and characterization. In present communication, isolation of Shatavarin IV was performed by proposing a column chromatographic method using mobile phase Ethyl acetate: Methanol: Water (8:1:1 v/v). All the fractions were analyzed using TLC and fractions 10 to 22 showed spot at the R_f of standard Shatavarin IV (fig. 2a). Combined fractions also showed the same spot which confirmed the presence of the Shatavarin IV (fig. 2b). In few research works the polarity of the mobile phase was changed and recolumn was performed which lead to a tedious process and the decrease in yield of isolated compound. The physical characteristics of the isolated compound were observed which indicates isolated compound as Shatavarin IV (table 1). The finalized isolation method gives 66% pure Shatavarin IV, which was determined against a reference standard using HPTLC (table 2, fig. 3). Peak purity was established through spectral detection of peaks at 0.45±0.05 R_f (fig. 4). The yield of isolated Shatavarin IV was 401.1 mg from 250 g of root powder which is more compared to other methods of isolation.

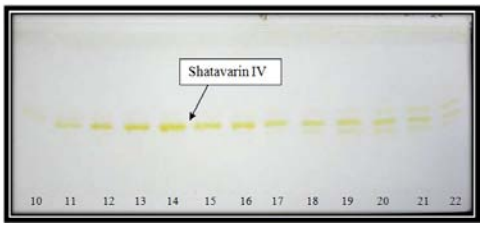
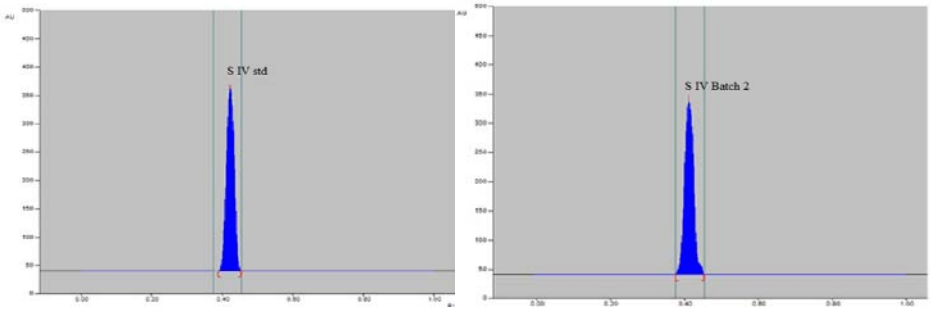


Fig. 2(a): TLC of the individual fractions showing presence of Shatavarin IV

Fig. 2(b): It shows TLC of the Shatavarin IV Standard and Isolated

Table 1: Physical Characters of isolated compound

Colour	Beige colour shiny powder
State	Solid
Solubility	Water and Methanol
Melting point	250-255˚C[7]
Yield	401.1 mg from 250g crude drug
% Purity	66%



(a) **(b)**

Fig. 3: Chromatogram of (a) Shatavarin IV Standard (b) Isolated Shatavarin IV

Table 2: % Purity of isolated Shatavarin IV

Compound	R_f	Peak area	% purity
Shatavarin IV Standard	0.49	8585.00	90.0%
Shatavarin IV isolated	0.41	6291.90	66.0%

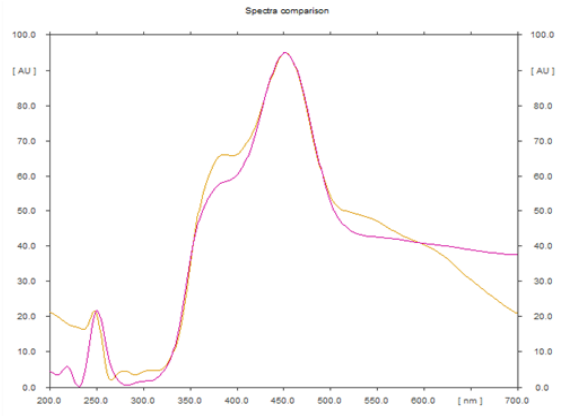


Fig. 4: Comparison spectra of Shatavarin IV Standard and Isolated Shatavarin IV

The spectral analysis showed that the isolated compound was not detectable under UV-Visible range because of absence of chromophore group [8]. The IR spectra of isolated compound showed the band at 2935 cm^{-1} may be due to alkyl C-H stretching, 1448 $cm^{-1}$and 1375 cm^{-1}due to CH_3 of ketone (CH_3-O-R), 1120 cm^{-1} and 1066 cm^{-1} band corresponds to C-O stretching matched with standard IR spectra [9] (fig. 5, 6). The ESIMS [10] gave an ion at 909.61 ([M+Na^+]) indicating a molecular formula is $C_{45}H_{74}O_{17}$ (fig. 7).

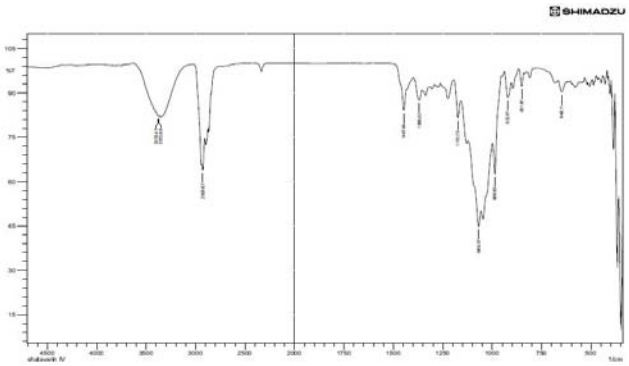


Fig. 5: IR spectra of Shatavarin IV Standard

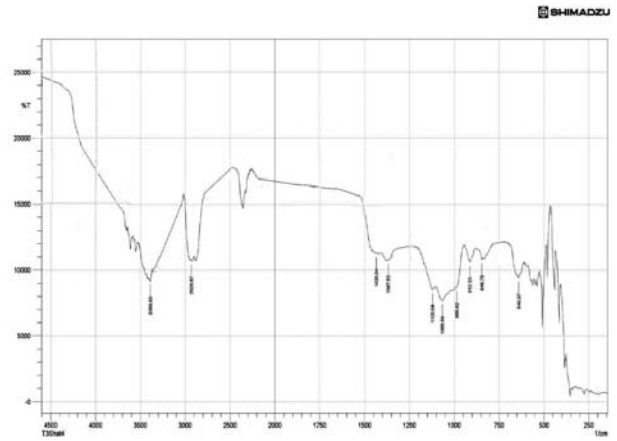


Fig. 6: IR spectra of Shatavarin IV isolated

Table 3: IR spectra Interpretation of isolated Shatavarin IV

S. No.	Peak	Interpretation
1	3398 cm^{-1}	O-H typically from alcohol
2	2935 cm^{-1}	Alkyl C-H stretching
3	1448 cm^{-1} and 1375 cm^{-1}	CH_3 of Ketone (CH3-O-R)
4	1120 cm^{-1} and 1066 cm^{-1}	C-O stretching
5	985 cm^{-1}	Alkenes (=C-H bending)
6	912 cm^{-1}	Alkenes (=C-H bending)
7	846 cm^{-1}	C-H bending of Aromatics
8	640 cm^{-1}	C-H bend

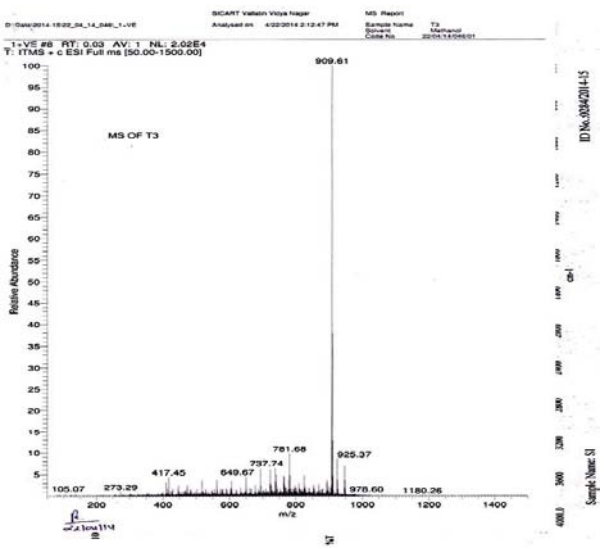


Fig. 7: Mass spectra of isolated Shatavarin IV

Table 4: Mass spectra Interpretation of isolated Shatavarin IV

S. No.	Mass to charge ratio (m/e)	Intensity
1	41745	738.4
2	649.67	682.3
3	737.74	1256.1
4	781.68	1508.0
5	909.61	14770.0
6	925.37	1447.3
7	978.60	320.2

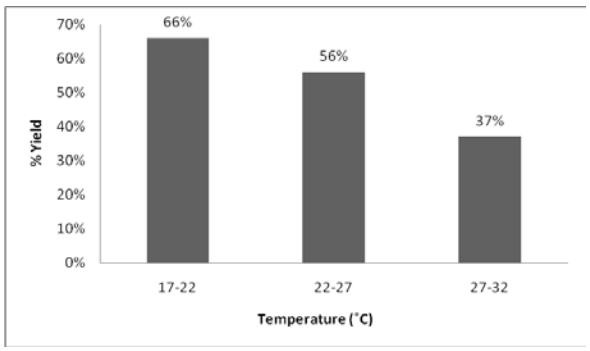


Fig. 8: Graph of % purity of Shatavarin IV vs. Temperature

Temperature effect on isolation

During the isolation process, the effect of temperature on column and in turn on yield and purity of Shatavarin IV was observed.

Column chromatography performed at cold atmospheric temperature, i.e., 17-22°C yielded the highest by 66% purity, followed by isolation conducted at normal room temperature i.e., 22-27°C yielded 56% pure compound. The yield was along with purity decreased to 37% when the atmospheric temperature was slightly warm i.e. 27-32°C. Decreasing temperature enhances both the yield as well as purity of the isolated compound (fig. 8). The decreasing yield could be due to the decomposition of some compounds at high temperatures or the evaporation of some volatile compounds from the crude extracts. The scarcity of data giving information regarding temperature effect on column chromatography made this research work useful.

CONCLUSION

On the basis of the revealed data of this study it can be concluded that isolation method of Shatavarin IV presented is simple and less time consuming as compared to earlier developed methods. It has also been found that higher temperature affects the isolation for Shatavarin IV through Column chromatography. For higher purity and better yield lower temperature was found more appropriate.

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CONFLICT OF INTERESTS

Declared None

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