INTERNATIONAL JOURNAL OF RESEARCH IN PHARMACY AND CHEMISTRY

Available online at www.ijrpc.com

Research Article

PHYTOCHEMICAL EVALUATIONS OF MARKETED SHATAVARI FORMULATIONS AND DEVELOPMENT OF ANALYTICAL METHODS FOR SAPONINS CONTENTS

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ABSTRACT

There is increasing awareness & general acceptability of the use of herbal drugs in today's medical practice. Evaluation is an important for establishment of consistent biological activity, chemical profile or a quality assurance program for production of herbal formulations. *Asparagus racemosus* [*Liliaceae]* traditionally known as Shatavari. In Ayurveda shatavari is considered as female tonic. Major constituent of *Aspargousracemous* are steroidal saponins (Shatavarin I-IV). Three formulations were selected for the study. Which aremanufactured in different regions of India. The selected shatavariformulations were analysedas per official guidelines. For quantitation UV absorption spectroscopic and RP-HPLC method was developed for saponins. Total saponins content and regional variations of saponins content was observed in present study.

Keywords: herbal drugs, standardization, shatavari, saponins.

1. INTRODUCTION

India has a rich heritage of traditional medicines. Ayurveda, Siddha, Unani, Homoeopathy and Naturopathy are various branches of medicines. According to WHO, about 70% of World population extensively use traditional and alternative medicines for the health care.[1]In the Traditional System of Medicine theshatavari is used mainly as galactogauge[2]. It is also used as anticancer, antibacterial, antineoplastic, antihepatotoxic agent.[3]It is sweet and bitter herb which balances Pitta Dosha. In India shatavari is described as a plant, "she who possesses 100 husbands", meaning 100 tubular roots which possess rejuvanitive effect upon the female reproductive organs.[4]

Standardization is defined by American Herbal Product association: "Standardization refers to the body of information and control necessary to product material of reasonable consistency". This achieved through minimizing the inherent variation of natural product composition through quality assurance practices applied to agricultural and manufacturing processes.[5]

Throughout India shatavari is used as general tonic, mostly used in rural areas. So main aim of present study is to select the shatavari preparations from different regions of India and analyzefor saponins content and to develop its quality control aspect.

2. MATERIAL AND METHODS

2.1 Plant material

Raw material - Shatavari roots are collected from botanical garden of Sanjivani College Of Pharmaceutical Education & Research, Kopargaon, Maharashtra, in the month of July 2013. The plant material were identified and authenticated by Dr. Sandanshiv, from Biology Department of S.S.G.M. College of science, Kopargaon. The collected roots were washed

and cut in to the small pieces and dried for 17 days in shed and for 5 to 6 hr. in hot air oven to remove the moisture completely.Dried roots were powdered and passed through mesh16. The fine powder was collected and stored in air tight container for further study.

2.2 Formulations profile

1. Formulation 1

It is in powered form manufactured in Haridwar, [Uttarakhand]. It contents shatavari 100 gm. It is used for galactogauge activity.

2. Formulation 2

It is in granules form manufactured in Gujrat. It contents shatavari 20 % w/w. It is used for galactogauge activity.

3. Formulation 3

It is in granules form manufactured in Pune [Maharashtra]. It contents shatavari 30 gm. It is used for galactogauge activity.

2.3. Methods

2.3.1. Extraction of saponins from raw materialand formulations

Saponins were extracted from roots of shatavari by Soxhlet extraction method. 100 gm of powder was dissolved into the 500 ml of water: methanol [70: 30] mixture. Extraction was carried out for 24 hrs. Then extract was collected and evaporated under reduced pressure condition.

2.3.2. Isolation & identification of saponins

Extracts of all formulations were further extracted with n-butanol. n- butanol fraction of each extract wasprecipitated with di ethyl ether for separation of saponins. White precipitate was obtained. The product was saponins.

2.3.3. Pesticide test

20 gm of sample were powdered and kept in RBF, 100 ml sodium sulphide was added with 100ml n-hexane and refluxed for 1hr. Filtrate from RBF was taken in separating funnel and extraction was done with 50 ml n hexane and 25 ml of acetonitrile. The acetonitrile layer was mixed with 500 ml of demineralized water with 2.5 ml saturated solution of sodium sulphide and again shaken in separating funnel with n-hexane.the n hexene fraction was separated and evaporated on water bath. The residue was used for analysis of organochlorine, organophpsphate and carbamates.[6]

1. Organochlorocolour test Residue + IPA
2. Organophosphate test 1ml residue in 5 ml ethanol +KOH
3. Carbamates test

1ml residue in 5 ml ethanol+ 1 drop furfural + 1 drop HCL.

Colour of test solution was observed and compare with std. solution of each Organochloro, Organophosphate, Carbamates.

3.3.2. Accelerated Stability Study

The accelerated stability study was carried out according to the ICH guidelines with the duration of study for 3 months and 6 months. The conditions of temperature and relative humidity were maintained at 40° C ± 2° C / RH 75% ± 5% respectively in the stability chamber. The parameters studied included physical appearance of formulation, moisture content, pH of the 1%w/v solution o, total ash, water soluble extractive, alcohol soluble extractive, pH, microbial load analysis. The samples were analyzed for all the parameters at 0 month, 3 months 6 months. Percentage of degradation was calculated by UV absorption spectrophotometry.[7]

3. RESULTS AND DISCUSSION

3.1 The shatavari roots were collected authenticated and subjected for macroscopic analysis

Shape - The roots are fleshy, tuberous, tapering towards both ends, swells when soaked in water Size - 10-20 cm in length, 1-2.5 cm in thickness. Colour - Fresh roots are white to buff in colour, dried roots are white to grayish white in colour. Surface- Rough, sign of shrinkage after drying. Texture - Short and Fibrous, Odor- Characteristic, Taste – Sweetish.

3.2. Powdered drug analysis

Some chemical tests were performed for the analysis of powdered drug to confirmed as shatavari. Results are given in **table** 1.

3.3. Preliminary phytochemicals test

The phytochemical analysis showed the presence of alkaloids, glycosides, steroids, carbohydrates, flavonoids. The presence of the glycosides, steroids, alkaloids compound justifies the saponins content in all formulations.

3.4. Physiochemical parameters

Ash value is indicative of contamination, substitution, adulteration or carelessness in preparation drug and drug combinations for marketing.The water soluble extractive values were found to be more than alcohol soluble extractive values indicatemore water soluble constituents. pH of formulation 1 and 2 found little acidic whereas formulation 3 and

shatavari powder found close to neutrality. Ash values, Moisture content Extractive values were found within the I P. limit.Results are given in **table 2**.

3.5. Pesticide test

Chemical color test method was used for detection of pesticide having sensitivity up to 0.6ppm. Standard for each category was used for comparison. Pesticide residue was harmful to the individual consuming the formulation. The major harmful groups likes Organochloro, Organophosphate, Carbamates absent in all formulation indicates all formulations are safe.

3.6. Microbial growth

Nutrient agar media, Nutrient broth, MacConkey agar media were used for the evalution of microbial load. Microbial growth was absent in all formulations. During the stability study, microbial growth was also absent in all formulations and shatavari powder.

3.7.Specific tests for conformations of saponins

Foam test, heam test, forth test – was performed for the conformation of saponins results of all test are found to be positive.

3.8. T L C study

TLC study was carried out by using silica gel as a stationary phase and Chloroform: Methanol: Water [13: 10: 2] as a mobile phase. 10 % v/v sulphuric acid in methanol solution is used as spray solution. The rf value was found to be 0.6.

3.9. FT-IR study

The isolated samples of saponins was analyzed by FT-IR spectrophotometer (8400-s; Shimadzu). Drug sample was placed on the thin film of Eutragid L100 and the IR was performed.2921 – CH, 3440 OH, 1220-1120-C=O ester, 3400-2400 acidic group, 1695 C=C groups found, so saponins are confirmed. Results were shown in **figure** -5.

3.10. Accelerated stability study

The accelerated stability study was done by usingstability chamber [REMI] at temperature - 40° C ± 2° C / relative humidity 75% ± 5%. formulation 2 is more stable than formulation 1 and 3. Results of formulation 1,2,3,and shatavari powder was shown in table 3,4 ,5.6 and figure no. 1,2,3,4.

4. UV method development for quantitative estimation of saponins

By using UV-visible spectrophotometer (Double beam), Shimadzu 1650 PC, the UV spectrum of saponins having concentration of 500µg/ml in methanol: water was recorded at 248 nm. The absorbancefor the different concentrations (100-500µg/ml) was recorded at 248 nm. The total saponins content was found to be 4.2 % 2.86% and 0.70% in formulation 1, 2, 3 respectively. Results was shown in **table 7, figure 6,7.**

5. RP- H P LC method development for quantitative estimation of saponins

The RP-HPLC method was developed for saponins by using RP-HPLC, (Shimadzu,Isocratic), Phenomenex coloum as stationary phase and methanol: water (90:10) mobile phase. At the 1 ml/min flow rate and 210 nm wavelength the saponins content was found 3.8 %, 2.4%and 0.65 % in formulation 1,2,and 3 respectively. Results were shown in **table 7, figure8, 9.**

Table 1: Chemical tests for powdered drug

Test	Observation	Inference
Powder as such	Greyish white	+ ve
Powder with conc. sulphuric acid	Brownish black	+ ve
Powder with ferric chloride solution	Brownish black	+ ve
Powder with conc. hydrochloric acid	Light white	+ ve
Powder with picric acid solution	Greenish yellow	+ ve
Powder with 5% iodine solution	Reddish	+ ve
Powder with antimony trichloride solution	Light brown	+ ve
Powder with acetic acid	Greyish	+ ve
Powder with conc. nitric acid	Reddish	+ ve
Powder with Sodium Hydroxide in water (1 N)	Yellowish	+ ve
Powder with Sodium Hydroxide in Methanol (1 N)	No change	+ ve
Powder with Potassium Hydroxide	Creamish	+ ve
Powder with Ammonia Solution	Creamish	+ ve
Powder with Nitric acid and Ammonia Solution	Orange Yellow	+ ve

Table 2: Phytochemical screening of all formulations and shatavari powder

Parameters	Formulation 1	Formulation 2	Formulation 3	Shatavari powder	I.P. Standards[8]
Total ash (%)	9	8	9	10	Not More Than - 15%
Acid insoluble ash value (%)	2	3	2	2	Not More Than 3%
Water insoluble ash value (%)	5	4	4	4	Not More Than 20%
Water soluble extractive value (%)	13.4	13.8	12	12	Not More Than 20%.
Alcohol soluble extractive value (%)	6.2	2.4	4.2	4	Not More Than15 %.
Moisture content [%]	10	5	4.5	10	Not More Than 15%
pH 1 % w/v	5.1	5.4	6.0	6.3	-
pH 10 % w/v	5.1	5.4	6.2	6.36	-

Table 3: Accelerated stability study of Formulation 1

Parameters	0 Month	3 Month	6 Month
Ash value	11	11	11
Acid soluble	4	4	5
Water soluble	4	7	7
Moisture content	4.5	7	76.2
pH	6.0	6.2	6.2
Water soluble extractive value	12	14	14
Alcohol soluble extractive value	12	12	13
Microbial growth	No growth	No growth	No growth

Table 4: Accelerated stability study of Formulation 2

Parameters	0 Month	3 Month	6 Month
Ash value	9	8	8
Acid soluble ash value	4	5	5
Water soluble ash value	5	4	5
Moisture content	10	18	1.8
pH	5.1	5.1	5.1
Water soluble extractive value	13.4	14	15.2
Alcohol soluble extractive value	6.2	7.5	8
Microbial load	No growth	No growth	No growth

Table 5: Accelerated stability study of Formulation 3

Parameters	0 Month	3 Month	6 Month
Ash value	11	11	11
Acid soluble	4	4	5
Water soluble	4	5	5
Moisture content	10	15	15
pH	6.3	6.3	6.3
Water soluble extractive value	12	13	15
Alcohol soluble extractive value	4	4	5
Microbial load	No growth	No growth	No growth

Table 6: Accelerated stability study of Formulation shatavari powder

Parameters	0 Month	3 Month	6 Month
Ash value	8	8	8
Acid soluble	5	4	5
Water soluble	4	5	5
Moisture content	5	8	8
pH	5.4	5.2	5.2
Water soluble extractive value	13.8	13	13
Alcohol soluble extractive value	2.4	2.5	3
Microbial growth	No growth	No growth	No growth

Table 7: Results of U V method development

S.No.	Parameter	UV- Observations	RP-HPLC- Observations
1.	Linearity i. Range ii. Regression iii. Line equation	 100- 500 µg/ml 0.995 Y= 0.002± 0.00675	 0.1 -0.6 0.9989` 948x+ 176.2
2.	Intraday Precision i. SD ii. %RSD	 0.0057 0.96 %	 1.16 0.260 %
3.	Interday Precision i. SD ii. %RSD	 0.0048 0.885%	 1.15 0.268
4.	Robustness i. SD ii. %RSD	 0.0052 0.959 %	1.82 0.682
5.	Ruggedness i. SD ii. %RSD	 0.00523 0.96%	 1.96 0.682 %
6.	LOD	3.66 µg/ml	0.017µg/ml
7.	LOQ	11.11 µg/ml	0.0528µg/ml
8.	Accuracy	96.56 %	97.66%

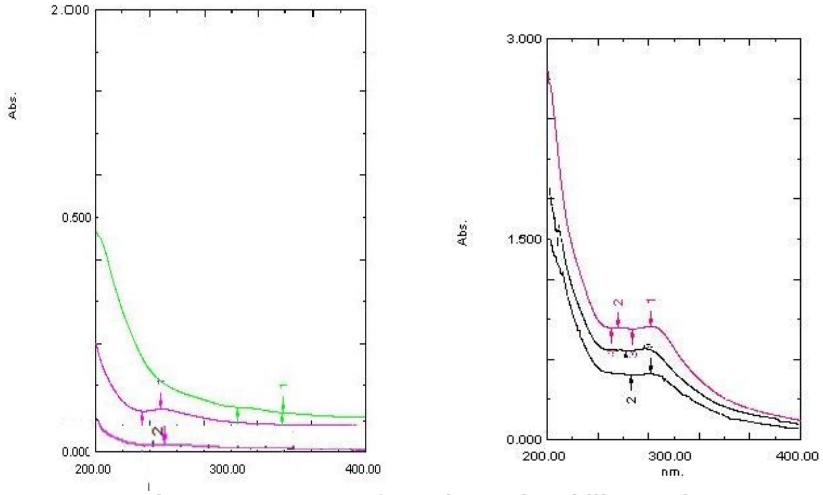


Fig. 1: UV spectra of accelerated stability study
Fig 2 : UV spectra of of Formulation 2
accelerated stability study of Formulation 1

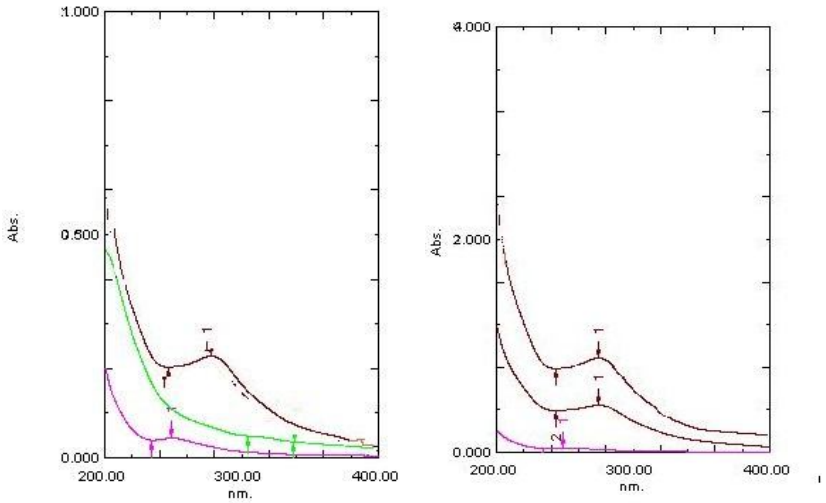


Fig. 3: UV spectra of accelerated stability
Fig. 4: UV spectra of accelerated stability study of Formulation 3.study of shatavari powder

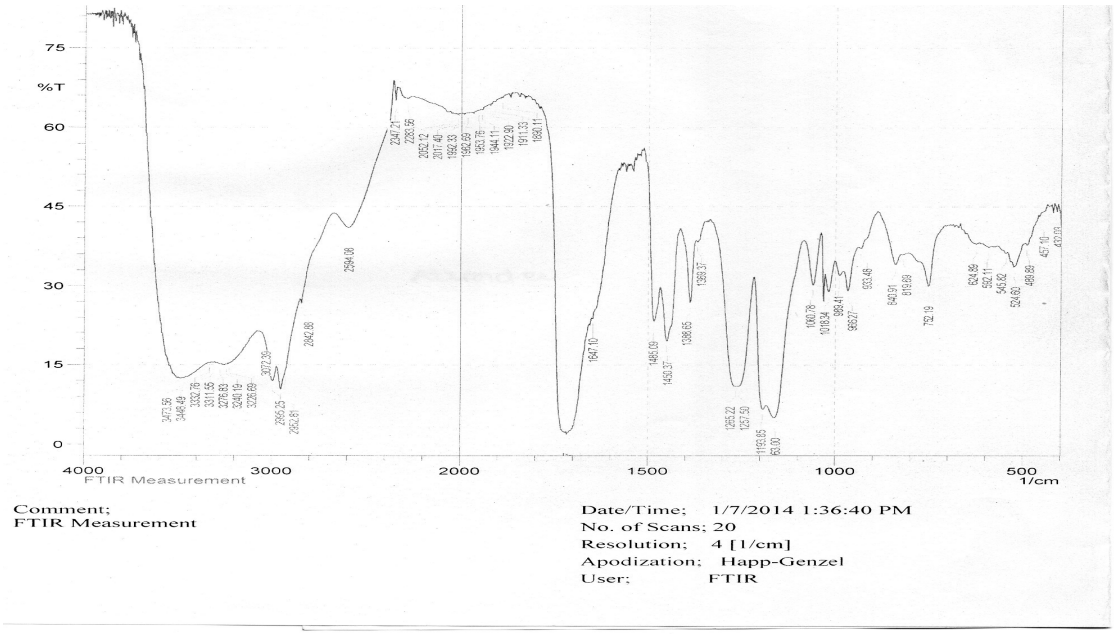
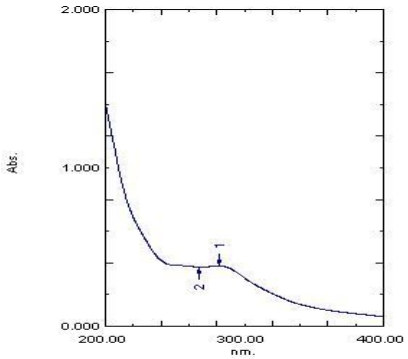


Fig. 5: FT-IR spectra



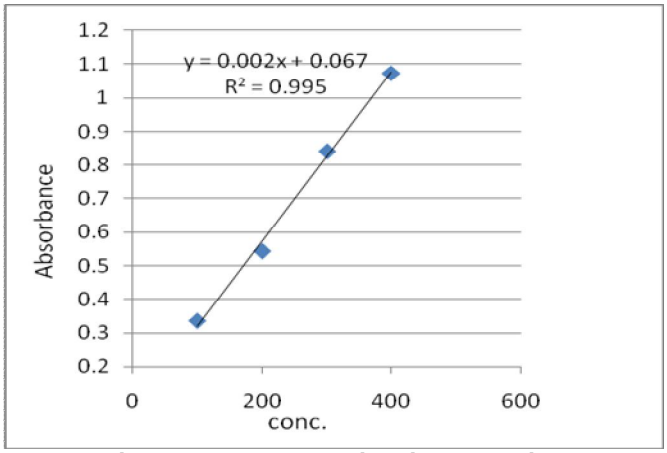


Fig. 6: UV spectrum showing saponins

Fig. 7: Calibration graph of at 248 nm saponins

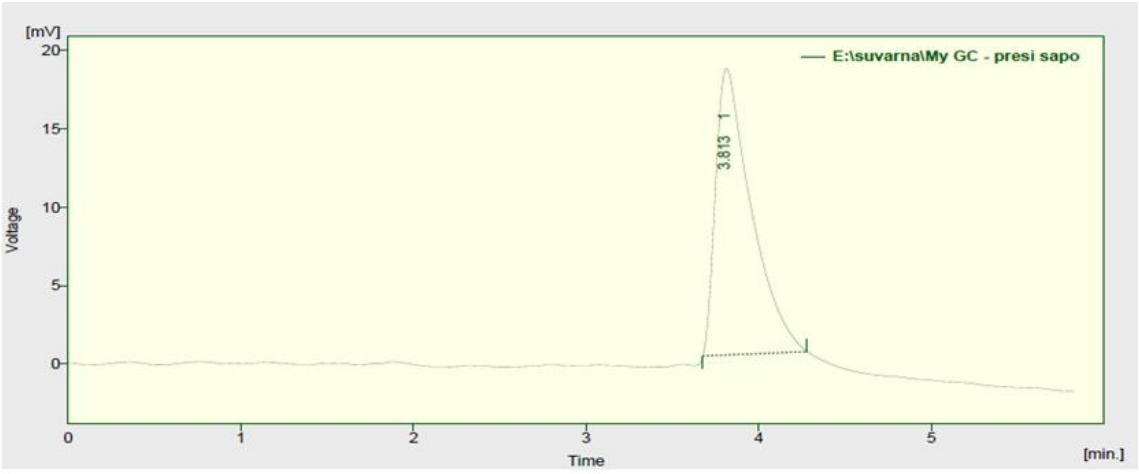


Fig. 8: Chromatogram of saponins

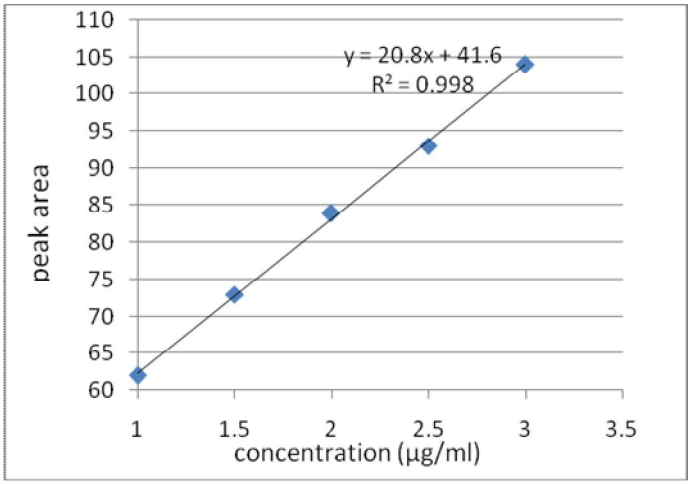


Fig. 9: Calibration graph of Saponins by HPLC

6. CONCLUSION

The marketed herbal formulations were evaluated as per official guidelines. All formulation satisfied most of the requirements. The study discovered that formulation 2 is more stable than formulation 1 and 3. The quantitative estimation of total saponins content in various formulations by modern method of analysis proves formulation 1 contains more saponins than others.

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