IAMJ

INTERNATIONAL AYURVEDIC MEDICAL JOURNAL

Research Article ISSN: 2320-5091 Impact Factor: 6.719

α – AMYLASE AND α -GLUCOSIDASE INHIBITORY ACTIVITY OF *SHATAVARI* (ASPARAGUS RACEMOSUS WILD) *MOOLA SWARASA* WITH *GODUGDHA* AND *SHATAVARI* EXTRACTS VIS- A- VIS *PRAMEHA HARA KARMA*- INVITRO – STUDY

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https://doi.org/10.46607/iamj1213032025

(Published Online: March 2025)

Open Access

Article Received: 06/02/2025 - **Peer Reviewed:** 27/02/2025 - **Accepted for Publication:** 08/03/2025.

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ABSTRACT

Diabetes mellitus is a chronic, metabolic syndrome with hyperglycemia secondary to an absolute relative deficiency of insulin or reduction in biological effectiveness of insulin or both1. Considering the higher incidence of the disease, management with long-term, safer and minimal side-effect drugs is the better choice. Inhibition of enzyme α – amylase and α –glucosidase delays the glucose breakdown, thus slowing down the glucose uptake in the blood. This is effective in controlling Type 2 diabetes. Control of PPHG (postprandial hyperglycemia) can be achieved by inhibiting both α-amylase and α-glucosidase with a higher percentage of inhibition. Diabetes mellitus is co-related to prameha in classical texts. As *Shatavari swarasa* with *godugdha* is said to be beneficial in all twenty types of *prameha*, an attempt is made to evaluate its efficacy through an in vitro study.

The aim of the study is to evaluate the inhibitory activity of *Shatavari swarasa* with *godugdha* and *Shatavari* extracts on α- amylase and α –glucosidase enzymes.

Materials and methods: Shatavari roots were collected from Chengalpet. An analytical study of *Shatavari* was carried out using a standard protocol. Enzyme – α-amylase and –α-glucosidase inhibitory activity was carried out by the in vitro method. Different concentrations (0.1,0.2,0.3,0.4and 0.5mg/ml) of trial drug and fractions were subjected to: α – Amylase, α –Glucosidase inhibitory activity. The absorbance was measured at 540 and 405 nm using multiple readers, and the percentage of inhibition and the IC50 values of each trial group were calculated

and compared with the standard drug Acarbose. Classical dosage forms and extracts were compared to inhibiting the enzyme activity. Results: *Shatavari swarasa and Shatavari swarasa with godugdha* showed significant results on enzyme inhibition, which was the potent inhibitor compared to the standard drug.

Keywords: α – Amylase, α –Glucosidase, Prameha, Shatavari, Diabetes mellitus.

INTRODUCTION

The burden of diabetes is high and increasing globally and in developing economies like India, mainly fueled by the increasing prevalence of overweight/obesity and unhealthy lifestyles. The estimates in 2019 showed that 77 million individuals had diabetes in India, which is expected to rise to over 134 million by 2045. Approximately 57% of these individuals remain undiagnosed. Type 2 diabetes, which accounts for the majority of the cases, can lead to multiorgan complications, broadly divided into microvascular and macrovascular complications. These complications are a significant cause of increased premature morbidity and mortality among individuals with diabetes, leading to reduced life expectancy.2

Treatment in conventional systems includes insulin, glibenclamide, Tolbutamide, a-glucosidase inhibitors, etc., with certain limitations and side effects; however, at the same time, the use of Alternative and complementary medicine along with these antidiabetic medicines is also reported. Bhaishajya ratnavali mentions the practical usage of *Shatavari* swarasa and *Godugdha* in managing all 20 types of *prameha3*.

Objectives of the study: To carry out phytochemical and pharmacognostic evaluation of *Shatavari* moola

To perform an in vitro evaluation of amylase and glucosidase inhibitory activity using Shatavari Moola swarasa with godugdha and shatavari extracts.

Materials and methods

Drug source

Fresh Shatavari Swarasa was extracted using the khalwa, and Godugdha (A2milk) was added to the swarasa.

"शतावर्या रसं नीत्वा क्षीरेण सह यः पिबेत् । प्रमेहा विंशतिस्तस्य क्षयं यान्ति न संशयः" ॥१०॥5 (B.R Pramehadhikara)

A mixture of this dosage form in different concentrations was used as the first trial sample. The second sample was *swarasa*, and the third and fourth samples were aqueous and alcoholic extracts, respectively. The Trial drug was extracted with Distilled water for aqueous and Methanol for alcoholic extractions. The percentages of the extracts were calculated.

PLACE OF WORK

COLLECTION, AUTHENTIFICATION AND PREPARATION OF TRIAL DRUG *Shatavari* roots (Asparagus racemosus willd) was collected in greeshma –pravrut rutu as mentioned in classics. About 5kgs of roots were collected. The Drying and powdering of Shatavari root were carried out at the postgraduate Department of Dravyaguna Vignana, Government Ayurveda Medical College, Bengaluru.

The Aqueous and Alcoholic extraction of shatavari root, HPLC analysis and Invitro analysis was carried out at Azymes bioscience, Bengaluru.

The drug was analysed physicochemically and phytochemically at the Drug Testing laboratory Ashoka Pillar, Jayanagar, Bengaluru.

PHYSICO-CHEMICAL EVALUATION

Moisture content (Loss on Drying), Total ash, acid-insoluble ash, alcohol-insoluble ash, water-soluble extractive values, alcohol-soluble extractive values, and pH Values were carried out per standard protocol. The standard value for the trial drug is available in the API Test, which was carried out by standard operating procedures.

HPLC ANALYSIS

To estimate the content of Saponin and Quercitin of shatavari moola extracts by HPLC. Chromatographic system: HPLC system equipped with dual Quarter nary pump, manual/auto-injector and photodiode array detector or UV detector supported by suitable software.

HPLC–SAPONIN Chromatographic condition

Column- RP-HPLC, C-18 column

Size- 4.6 X250 nm

Mobile phase- A common mobile phase mixture of water and acetonitrile in the ratio of 4 6 is adjusted.

Stationary phase-silica

Injection volume- 20µl

Flow rate – 1ml/ min

Wavelength- 203nm

Instrument used- shimad ZU –Ic 10A

Sample preparation

Aqueous and Alcoholic extracts, each 10 mg/ml, were dissolved in the mobile phase. The above procedure was carried out for the extraction of Shatavari moola.

Procedure: The mobile phase was forced through the packed column with a flow rate of 10ml/min and under pressure gradient elution 203 nm; a chromatograph of standard saponin with a concentration of 1mg/ml was done by injecting 20 µl of standard saponin solution. The chromatography of Aqueous and Alcoholic extract of Shatavari (10 mg /ml) concentration is used for injecting. 20 µl of the sample solution is used for the same. The computer recorded the peaks of the substance's absorbance. The report contains the retention time (RT), and the amount of saponin in the sample was estimated.

HPLC QUERCETIN

Materials and methods-

Column- C18

Size-4.6x 250nm

Mobile phase- Acetonitrile and water 7:3 (272nm)

Stationary phase –column

Injection volume-20 µl

Flow rate-1ml/min

Wavelength-272nm

Run time-10min

The instrument used- shimmed ZU SPD-101.

Aqueous and Alcohol extract of 10mg/ml were dissolved in the mobile phase. The above procedure was carried out for the extraction of all the samples of *Shatavari*

Procedure: The mobile phase was forced through the packed column with a flow rate of 10ml/min and under pressure gradient elusion 272nm.

The chromatography of the standard Quercetin with the concentration 1mg/ml is done by injecting 20µl of standard Quercetin solution. The chromatography of Aqueous extract with a concentration of 10mg/ml of *Shatavari*, all the samples are also done by injecting 20µl of sample solution. The computer recorded the peaks of the substance's absorbance. The report contains the retention time (RT). The amount of Quercetin present in the sample was estimated.

IN- VITRO STUDY

An enzyme inhibition assay was performed using spectrometric analysis4.

1. Method of preparation of enzyme stock solution

To prepare an amylase enzyme stock, the following requirements:

A) Preparation of buffer solution

Ultrapure water prepared a solution containing 2.4 mg/mL of sodium phosphate, monobasic, and 0.39 mg/mL of sodium chloride. Adjust the pH to 6.9 at 20 °C using 1 M NaOH/1 M HCl (1 mole of sodium hydroxide and hydrochloric acid in every litre of solution.)

B) Preparation of starch solution

Dissolve 1.0 gm of soluble starch in 100 ml of the buffer solution. Bring to a gentle boil to dissolve, then cool and bring the volume to 100 ml with water. Incubate at 25°C for 4-5 minutes before assay.

C) α-Amylase Sample solution – Prepare a solution containing 0.75-1.5 units/mL of α-Amylase in 20 °C ultrapure water before use.

2. METHOD OF PREPARATION OF DNSA SOLUTION (100ML)

*1gm of DNSA (3, 5- Dinitro salicylic acid) (MW = 228.1g/mol) is dissolved in 20ml of 2M NaOH (sodium hydroxide) at 700c.

*Add slowly 30gms of sodium potassium tartrate to stabilise the DNSA.

*The final volume is diluted and made up to 100ml using distilled water.

3. Sample preparation

Reagents and Equipment required:

*DNS colour reagent solution

*Enzyme solution

* Soluble starch

*Cuvettes

*Testing drug of swarasa with godugdha and AQ, AL Extracts

CENTRIFUGED SAMPLE AND ENZYME STOCK SOLUTION

ENZYME-Assay-amylase inhibitory activity

In a clean test tube, 20 µl of varying concentrations of extractions and fractions of the test samples are taken and added distilled water accordingly to make 1ml

Pipette into 15 ml cap-covered containers 1 ml of the substrate (solution of potato starch), mix by swirling and incubate at 20°C for 3-4 minutes to achieve temperature equilibration.

Test: Then add 1 ml of enzyme solution, mix by swirling, and incubate at 20°C for exactly 3 minutes. After 3 minutes, stop the solution by adding 1 ml of colour reagent, place it in the boiling water bath, boil for exactly 15 minutes, and cool it on ice for a few minutes. Add 9 ml of purified water. Mix by inversion. Pipette 3 ml of the reaction mixture in a spectrophotometer cuvette and record the absorbance at 540 nm, noted A540 Test.

Blank: The protocol is similar for blank assays, but no enzyme is added before the 3-minute incubation. Only after the addition of the colour reagent and after putting the reaction vessel in the boiling bath is 1 ml of enzyme solution added. After the blank procedure, the absorbance, A540 Blank, is similarly recorded. The results were expressed as percentage inhibition, which was calculated using this formula.

$$\text{Inhibitory activity (\%)} = (1 - As/Ac) \times 100$$

where "As" is the absorbance in the presence of the test substance and Ac is the absorbance of the control. Keeping these values, a standard calibration curve is drawn.

Table no: showing α – amylase inhibition assay procedure

SAMPLE	TEST DRUG µL	Distilled water Ml	Starch (0.1%) µL		α-amylase µL	Incubation	DNSA ML		DISTILLED WATER µL	ABS AT 540 Nm
BLANC	-	1	1	20^0c	1	20^0c	1	Water bath	9	
IA	0.2	0.8 µL	1	For	1	3min	1	15min	9	
IB	0.4	0.6 µL	1	10 Min	1		1		9	
IC	0.6	0.4 µL	1		1		1		9	
ID	0.8	0.2	1		1		1		9	
IE	1	-	1		1		1		9	

α- glucosidase Inhibitory activity

α - Glucosidase Inhibitory activity of the fresh *swarasa* with milk and different extracts of the trial drug was carried out according to the standard method with minor modifications.

Substrate: The substrate used is often 4-nitrophenyl-α-D-glucopyranoside (pNPG)

Buffer: The buffer is usually a sodium phosphate buffer at pH 6.8

Temperature: The reaction is usually carried out at 37°C

Measurement wavelength: The absorbance is generally measured at 405 or 410 nm

Preparation of buffer: K_2HPO_4 (mol wt 136.09) and KH_2PO_4 (MOL WT 174.18) are made into a solution with 30ml of distilled water with distilled water, and both are mixed until they attain H 6.8.

Procedure

In clean test tubes, 50 µl phosphate buffer (100 mM, pH = 6. 8), 10 µl alpha glucosidase (1 U/ml), and 20 µl of varying concentrations of extract and fractions of *Shatavari swarasa* with *Godugdha* (0.1, 0.2, 0.3, 0.4, and 0.5 mg/ml) was preincubated at 37°C for 15 min. Then, 20 µl P-NPG (5 mM) p-nitrophenyl α-D- glucopyranoside was added as a substrate and incubated further at 37°C for 20 min.

Table no: showing α – glucosidase inhibition assay

SAMPLE	PO_4buffer	PNPG(5Mm)in water	37^0c	Enzyme	37^0c	$Na2CO_3$	400nm
BLANC	500µl	250 µl	In		In	2	
10Ml	490 µl		CU	250µl	CU	Ml	
20 µl	480 µl		Ba		BA		
30 µl	470 µl		TI		TI		
40 µl	460 µl		ON		ON		
50 µl	450 µl		5min		5min		

The reaction was stopped by adding 50 µl Na2 CO3 (0.1 M). The absorbance of the released p-nitrophenol was measured at 405 nm using a Multiplate Reader.

SPECTROPHOTOMETRIC ANALYSIS

The different concentrations of trial samples were run through UVSPECTROPHOTOMETER with indicated nanometer and values of absorbance are displayed on the system connected to the spectrophotometer is noted. These values are converted into percentages of inhibition by the formula stated below. The absorbance released was measured at 405nm using a UV spectrophotometer and was considered directly proportional to the enzyme's activity.

STANDARD

Acarbose at various concentrations (0.1-0.5mg/ml) was used as a standard. The results were expressed as percentage inhibition, which was calculated using this formula.

Inhibitory activity (%) = (1- As/Ac) x 100

PERCENTAGE OF INHIBITION

Results were expressed as percentage inhibition, which was calculated using the formula: Inhibitory activity (%) = (C-T/C) x 100, where c is the control and t is the concentration of the trial sample.

IC50 VALUE

The IC50 is determined by Y= mx + c

OBSERVATION AND RESULTS

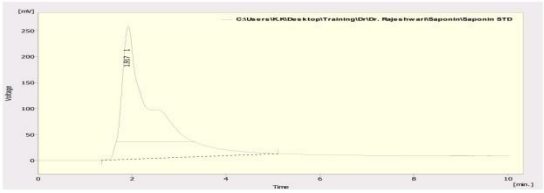
The observation of the study was done under two headings:

1. Analytical observation study

2. In-vitro Activity.

HPLC (High-performance liquid chromatography)

Saponin - standard

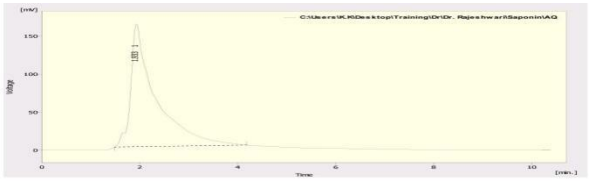


	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]
1	1.917	11252.000	256.104	100.0
	Total	11252.000	256.104	100.0

The HPLC Chromatograph of standard saponin at an optimum wavelength of 203nm showed a retention time of 1.917min and a mean area of 11252.00

GRAPH NO 1: SHOWING HPLC OF STANDARD SAPONIN

SAPONIN – AQUEOUS EXTRACT

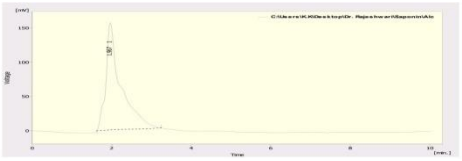


	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]
1	1.933	5690.033	161.734	100.0
	Total	5690.033	161.734	100.0

The HPLC Chromatograph of the Aqueous extract of Shatavari shows that saponin at an optimum wavelength of 203nm showed a retention time of 1.933 min and a mean area of 5690.033.

GRAPH NO 2 SHOWS THE HPLC OF AQUEOUS EXTRACT FOR SAPONIN

SAPONIN – ALCOHOLIC EXTRACT



	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]
1	1.967	4069.516	157.103	100.0
	Total	4069.516	157.103	100.0

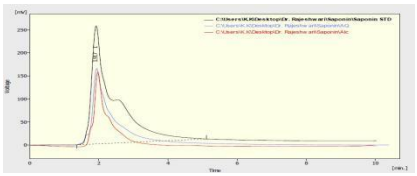
The HPLC Chromatograph of the Alcoholic extract of Shatavari shows that saponin at an optimum wavelength of 203nm showed a retention time of 1.967 min and a mean area of 4069.516.

GRAPH NO: 3 SHOWS THE HPLC OF ALCOHOLIC EXTRACT FOR SAPONIN

The HPLC Chromatograph of overlaid of AL, AQ, and Std saponin at an optimum wavelength of 203nm showed a retention time of 1.967 min and a mean area of 4069.516

	Reten.Time(min)	Area(Mv.s)	Height (Mv)	Area (%)
1	1.917	11252.000	256.104	100.0
	Total	11252.000	256.104	100.0

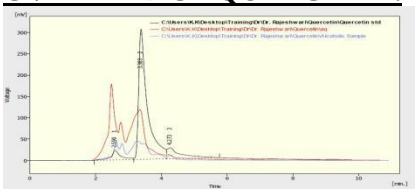
OVERLAID - SAPONIN



GRAPH NO 4: SHOWING HPLC OF OVERLAID OF SAPONINS

HPLC ANALYSIS OF QUERCETIN PRESENT IN THE SAMPLE

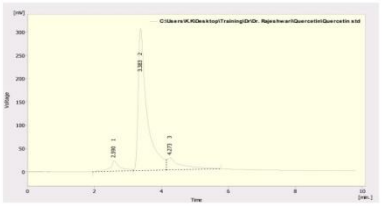
OVERLAID OF QUERCETIN.



The HPLC Chromatograph of AL, AQ, and Std Quercitin overlaid at an optimum wavelength of 272nm showed a retention time of 2.590 min and a mean area of 461.636.

GRAPH NO 5: SHOWING HPLC OF OVERLAID OF QUERCETIN

QUERCETIN - STANDARD

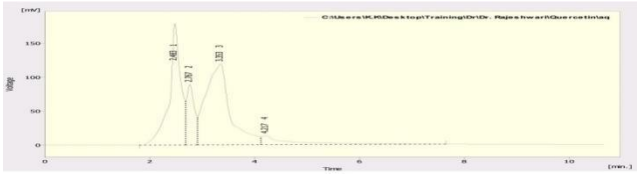


	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]
1	2.590	461.636	22.719	6.7
2	3.383	5610.170	304.776	82.0
3	4.273	770.425	25.735	11.3
	Total	6842.231	353.229	100.0

The HPLC Chromatograph of standards Quercetin at an optimum wavelength of 272 nm showed a retention time of 2.590 min and a mean area of 461.636.

GRAPH NO 6: SHOWING HPLC OF STANDARD QUERCETIN

QUERCETIN – AQUEOUS EXTRACT

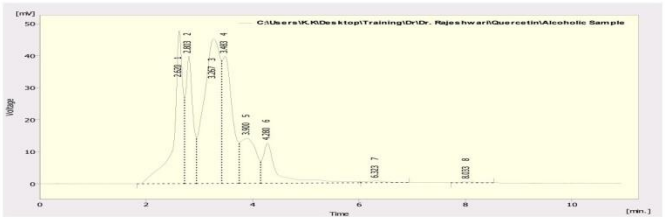


	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]
1	2.483	2715.740	178.850	33.6
2	2.767	925.407	89.201	11.5
3	3.353	3894.352	119.208	48.2
4	4.217	535.859	13.239	6.6
	Total	8071.358	400.498	100.0

The HPLC Chromatograph of the Aqueous extract of Shatavari shows Quercetin at an optimum wavelength of 272 nm, which showed a retention time of 2.483 min and a mean area of 2715.740.

GRAPH NO 7: SHOWING HPLC OF AQUEOUS EXTRACT FOR QUERCETIN.

QUERCETIN – ALCOHOLIC EXTRACT

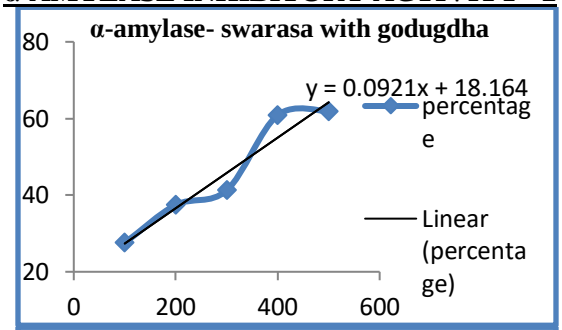


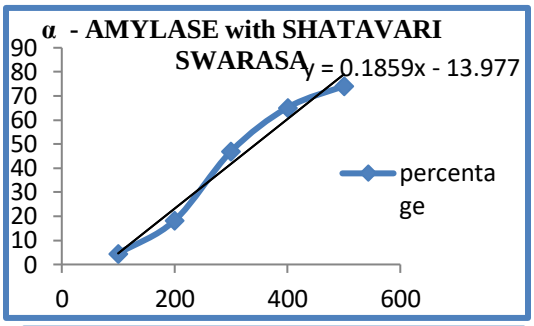
	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]
1	2.620	567.647	47.728	19.0
2	2.803	378.587	39.714	12.7
3	3.267	952.521	45.062	31.8
4	3.483	539.534	39.640	18.0
5	3.900	290.898	14.098	9.7
6	4.280	241.509	12.521	8.1
7	6.323	14.836	0.544	0.5
8	8.033	5.830	0.298	0.2
	Total	2991.362	199.605	100.0

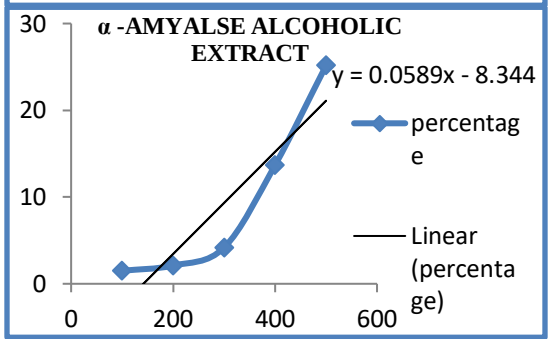
The HPLC Chromatograph of the Alcoholic extract of Shatavari shows Quercetin at an optimum wavelength of 272 nm, which showed a retention time of 2.620 min and a mean area of 567.647.

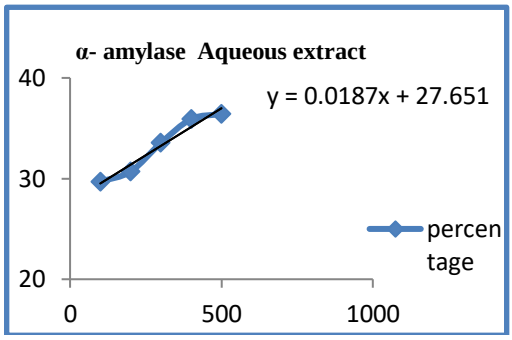
GRAPH NO 8: SHOWING HPLC OF ALCOHOLIC EXTRACT FOR QUERCETIN

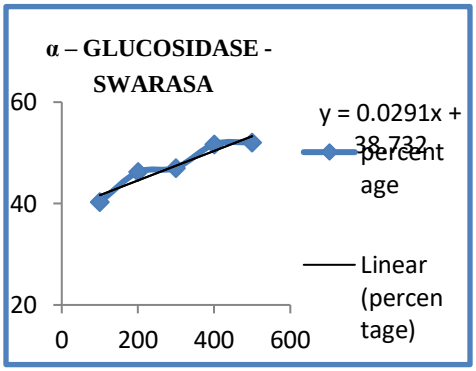
1. α-AMYLASE INHIBITORY ACTIVITY 2

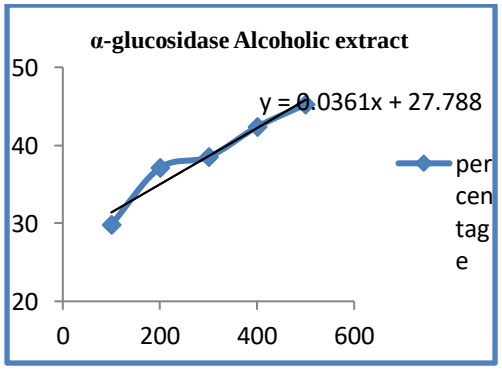


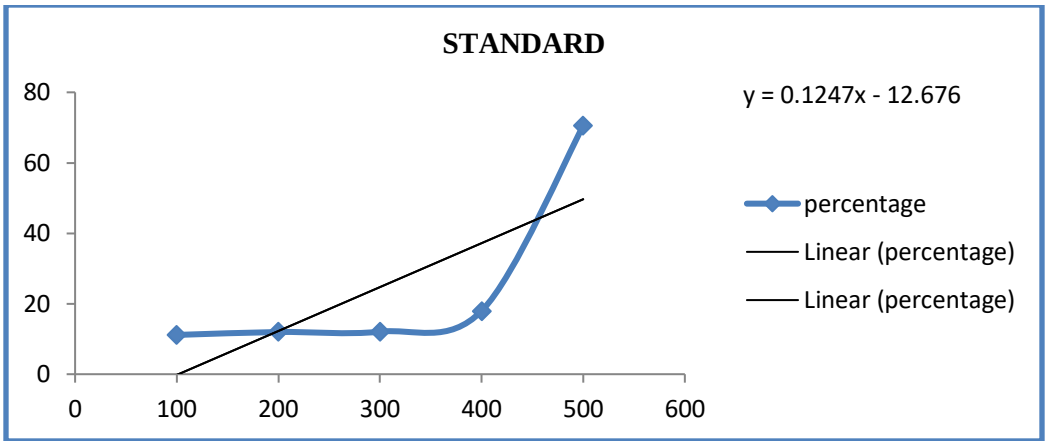






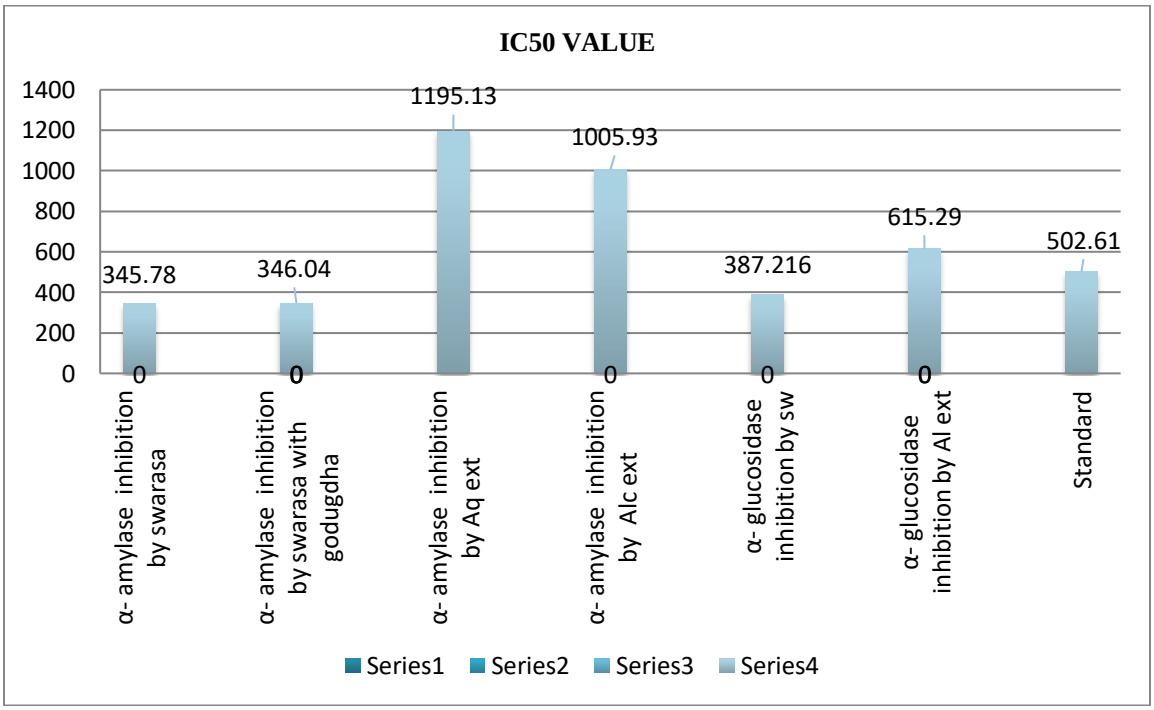






RESULTS OF IC50 VALUE OF DIFFERENT TEST SAMPLES

IC 50 VALUE OF DIFFERENT TEST SAMPLE RESULTS REPRESENTING IN BAR GRAPH.



GRAPH NO 16: SHOWING IC 50 VALUES OF TRIAL SAMPLES

TABLE NO 55: SHOWING THE IC50 VALUES OF TRIAL SAMPLES

SL	**Inhibition enzyme- Dosage form**	**IC 50 VALUE**
1	α- amylase using *swarasa*	345.78
2	α- amylase using *swarasa and godugdha*	346.04
3	α- amylase using AL ext	1005.94
4	α- amylase using AQ ext	1195.13
5	α- glucosidase sw	387.216
6	α- glucosidase AL ext	615.29
7	Standard	502.61

DISCUSSION

Various α-glucosidase inhibitors like Acarbose, miglitol and voglibose have been found adequate by inhibiting the absorption of carbohydrates from the gut. Hence, they are used in the treatment of Diabetes mellitus6 Due to the high cost of the currently available synthetic α-glucosidase inhibitors and their undesirable side effects like flatulence, bloating abdominal discomfort (Kaur et. al.2021) drives the need for natural inhibitors with lesser side effects.

. In Bhaishajya ratnavali prameha adhikara, we find a reference of Shatavari swarasa with godugdha in managing 20 types of prameha.

Discussion on drug7

Shatavari, botanically identified as Asparagus racemosus, belongs to the Liliaceae family. It is known for its properties in *Stanyajanana, Vrishya, Rasayana, Balya, and Bruhmana*. It possesses *Guru Snigdha guna, Madhura, Tikta rasa, Sheeta veerya Madhura vipaka, and tridoshahara* properties. The root of Asparagus racemosus wild contains various phytochemicals, such as flavonoids, saponins, quercetin, triterpenoids, tannins, and phenols.

Discussion on Dosage form selection

Swarasa is the most potent (Balavaha) among the *panchavidha kashaya kalpana* due to its *guru guna*

Some water-soluble phytoconstituents, like flavonoids and glycosides, may have a greater bioavailability profile than conventional plant extracts. Swarasa extraction is a preferable method for certain drugs that can yield a good quantity of juice. *Shatavari* is a drug that yields a good quantity of *juice*. *Swarsa,* along with *godugdha,* has a synergistic action, which improves the drug's bioavailability.

In this study, *Shatavari swarasa* and *godugdha* were used as a trial sample for group 1, as we have classical reference. An additional analysis was carried out with swarasa alone as a trial sample for group 2.

However, the fresh juice form is practically difficult for daily administration in chronic diseases like *prameha,* and the new dosage forms that are available in the market use extracts of the drugs. Thus, alcoholic and aqueous extracts were also chosen for trial groups 3 and 4, respectively.

Discussion on selection of standard drug - ACARBOSE

Acarbose, Miglitol, and Voglibose are the enzyme inhibitors that are currently used for controlling PPHG

Acarbose inhibits both α-amylase and α-glucosidase.

In Type 2 Diabetes mellitus, Acarbose is used alone or in combination with metformin or insulin; hence, it is chosen as the standard drug.

4. Discussion on Analytical study

Powder microscopy of the root powder of Shatavari shows the presence of raphides, starch grains, and pitted vessel elements with scalariform thickenings, confirming its identity.

Physicochemical analysis

Asparagus racemosus wild was devoid of foreign matter, indicating the absence of adulteration.

Loss on drying - The moisture content of the drug was 7.2%, indicating no contamination from moulds.

The total Ash value of the drug was 3.5%, which is as per the API standard value

The acid-insoluble Ash value of Shatavari root powder was 0.28%.

The water-soluble extractive values of the drug were 46.87% as per the reference of API. Alcohol soluble extractive values were 11.675%.

The pH value of the root is 4.85, indicating that the drug is acidic. The standard reference was not found, so it was done using the triplicate method. Discussion on Preliminary Phytochemical Analysis

Phytochemical analysis was carried out for the Aqueous and Alcoholic extracts of Shatavari. Primary metabolites like carbohydrates, starch, and mucilage were found in both extracts.

Secondary metabolites like alkaloids, glycosides, flavonoids, polyphenols, triterpenoids, steroidal sodium, and phenolic compounds were found in both extracts.

INORGANIC CONSTITUENTS:

The presence of Iron, calcium, potassium, magnesium, and phosphate was found to be in trace in aqueous and alcohol extracts of Shatavari

DISCUSSION ON INVITRO STUDY

The invitro study was carried out to evaluate the inhibitory activity of Shatavari moola swarasa with

godugdha and Shatavari moola extracts for α- amylase and α-glucosidase enzymes as per the standard protocol of enzyme assay.

ACARBOSE was taken as the standard drug.

The evaluation was carried out for the enzyme inhibition using five concentrations of each test sample and a blank as a control, and results were estimated using the UV spectrophotometric analysis method.

Dose-Dependent Effects*: The degree of inhibition may vary with the concentration of the test sample, and then the IC50 value was calculated

IC50 values, which represent the half-maximal inhibitory concentration, indicate the drug's efficacy. Lower IC50 values suggest the drug is effective at a lower concentration, indicating lower systemic toxicity.

Discussion on the results of the in vitro study

α- amylase inhibition results

The final results showed shatavari moola swarasa is a highly significant inhibitor with IC50 value- 345.78 µg/ml compared to other test samples, including the standard drug Acarbose.

As per classics, *Swarasa* is the most potent (*Balavaha)* among *pancha vidha kashaya kalpan* because of its *guruguna*. This dosage form is found to be more effective at lower concentrations.

Swarasa with godugdha showed an IC 50 value of 346.04 µg/ml, which is a highly significant result compared to the standard drug. This may be due to the potency and synergetic action of the classical dosage form.

The Aqueous and Alcoholic extracts showed 1195.13 µg/ml and 1005.94 µg/ml inhibition ofα amylase, respectively. This indicates that extracts will be effective at higher concentrations than classical dosage forms and standard drugs.

α- glucosidase inhibition results

Shatavari swarasa showed a value of - 387.216 µg/ml, which is lesser than the standard drug and Alcoholic extract of Shatavari. This may probably be due to the potency of the classical dosage form because of its *Guru guna.*

The IC50 value of Alcoholic extracts was 615.29µg/ml, which shows its effectiveness in lower concentrations but a little higher when compared to the standard drug.

Swarasa with *godugdha* and Aqueous extract did not have inhibitory activity. As casein, peptides from milk may compete with plant-based inhibitors. (Wang et .al 2019)

However, the Aqueous extractive values were higher than the alcoholic extractive values, which need to be revalidated with an in vivo study.

As enzyme inhibition assay is done in a controlled environment, in a sterile lab, while there are always chances of difference like inhibition, in In vivo trials due to other various metabolic factors

Probable Mode of action of phytoconstituents.

Shatavari is loaded with primary and secondary metabolites.

All extracts contain primary metabolites like Carbohydrates, starch, proteins, and mucilage, which denotes their nutritive nature.

Important Secondary metabolites are Alkaloids, Glycosides, Flavonoids, Tritrerpinoids, Saponins, Tannins, Steroids and Phenols.

Quercetin8

A previous in-vitro and in-vivo experiment suggests that bioactive compounds can be potential inhibitors of the enzymes.

Quercetin prevents oxidative stress, which results in the regeneration of pancreatic β-cells islets and the subsequent release of insulin.

Studies suggest that antioxidants decrease triglycerides, total cholesterol, liver glucose content, and oxidative stress, contributing to insulin deficiency114.

Quercetin prevents oxidative stress, which results in the regeneration of pancreatic β-cells islets and the subsequent release of insulin.

Saponin: steroidal Saponin, shatavarin IV, can reduce the increment of blood glucose by inhibiting the enzymes that breakdown disaccharides into monosaccharides

Glycosides are responsible for pharmacological activities like Cardiotonic action.

Tannins and Flavonoids10, polyphenols, have pharmacological actions like Antioxidants, which reduce

oxidative stress and may correlate with rasayana karma.

Triterpenoids12 has pharmacological activities like antihyperlipidemic and Immunomodulatory activity, which is helpful in diabetes.

Probable Mode of action based on Rasapanchaka9

Prameha is a tridoshaja vyadhi which manifests as *'prabhuta avila Murata and Dhatushaithilya* due to the involvement of kleda and dushyas

Shatavari, by its *sowmya guna* due to *madhura* and tikta rasa, reduces the *Dhatupaka*, further reducing *dhatu Shaithilya.*

Tikta and kashaya rasa help in decreasing *madhura bhava* in the body

Madhura rasa and *madhura vipaka* might be helpful in reducing *pipasa, karapada daha, and angaparidaha*.

Madhava Dravyaguna, says *Shatavari* possesses *vata pittahara*, and *tridoshahara* property helps to pacify the aggravated *tridoshas*, making it a better *shamanoushadhi.*

Sheeta virya and kashaya rasa, due to its sthambana guna, reduce prabhuta mutrata.

By its nature, Dravya possesses the qualities of Karma, like *Deepana, Pachana, Vayasthapana, Balya, Hridya, and Pushtida.*

Prameha is associated with a deranged metabolism. *Deepana and pachana* help with *amapachana*, thus correcting the metabolism.

HRUDYA karma helps in preventing cardiovascular diseases, which are the complications of chronic Diabetes

Shatavari is a balya, *Rasayana dravya* this quality is attributed as *a Prabhava* of the drug. This will also affect *dhatushaithilya*, one of the cardinal signs of prameha.

Vyadhi Kshamatva by Oja Vriddhi and *dourbalyata* is corrected by the *balya guna*

Shatavari appears to be a potent inhibitor of α - amylase and α -glucosidase enzymes, implying that glucose release into the blood can be delayed.

Classical dosage forms, i.e swarasa and swarasa with godugdha, were found to be highly effective in enzyme inhibition compared to the standard drug.

Swarasa of shatavari and *swarasa with godugdha* showed significant results on α- amylase inhibition, which was the potent inhibitor compared to the standard Acarbose. *Swarasa* on α- glucosidase also shows a good inhibitory value11. At the same time, *swarasa with Goksheera* and aqueous extract showed no inhibitory values on α - glucosidase.

Aqueous and Alcoholic extracts showed significant inhibition on α -amylase but were inadequate compared to the standard. This concludes that the swarasa form is better compared to the extracts. The alcoholic extract showed considerable inhibition on α - glucosidase.

The study provides valuable insights into the effects of Shatavari on inhibiting carbohydrate-metabolizing enzymes. The effectiveness of *Swarasa and swarasa with godugdha* justifies the importance of classical dosage forms. It is also necessary to carefully consider choosing specific dosage forms for different therapeutic goals.

CONCLUSION

The study provides valuable insights into the differential effects of Shatavari preparations on carbohydrate-metabolizing enzymes. The superior activity of *Swarasa* preparations and the unique effects of *Godugdha* addition highlights the importance of traditional preparation methods while suggesting careful consideration when choosing specific preparations for different therapeutic goals.

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Source of Support: Nil
Conflict of Interest: None Declared

How to cite this URL: : A – amylase and A -glucosidase inhibitory activity of shatavari (asparagus RACEMOSUS WILD) moola swarasa with godugdha and shatavari extracts vis- a- vis prameha hara karma- invitro - study. International Ayurvedic Medical Journal {online} 2025 {cited March 2025}