Journal of Advanced Zoology

ISSN: 0253-7214

*Volume **45** Issue **1 Year 2024** Page **680-687***

In vitro Alpha-Amylase and Alpha-Glucosidase Inhibitory Activity of *Solanum Nigrum L* Crude Extracts

Virendra Prasad Tiwari[1*], Anjani Kumar[1], Nilesh Dwivedi[2], Vandna Pathak[1] and Indra Prasad Tripathi[1]

[1]*Department of Physical Sciences , Mahatma Gandhi Chitrakoot Gramodaya Vishwavidyalaya, Chitrakoot, Satna (Madhya Pradesh) 485334*

[2]*Deendyal Shodh Sansthan, Chitrakoot, Satna (Madhya Pradesh) 485334*

***Corresponding Author**: *Virendra Prasad Tiwari*

**E-mail - virendratiwari6686@gmail.com*

Article History

Received- 03.11.2023
Revised- 27.11.2023
Accepted- 15.12.2023

CC License
CC-BY-NC-SA 4.0

Abstract

Solanum nigrum has traditionally been used to treat diabetic person as it contains various phytochemicals which are widely used to treat various ailments. In the present work Total Phenolic Content (TPC), Total Flavonoid Content (TFC), antioxidant and antidiabetic activities of 80% (v/v) methanolic crude extract of *S. nigrum* was done. Total amount of phenols in methanolic extract of fruit, leaves, stem and root of *S. nigrum* were found to be 63.31, 102.70, 47.96 and 62.17 µg/ml respectively. The flavonoid content was found 23.72, 128.11, 13.54 and 25.12 µg/ml in fruit, leaves, stem and root respectively. The IC_{50} values in µg/ml for DPPH scavenging activity of various parts of *S. nigrum* plant was found to be 38.77 for fruit, 51.45 for leaves, 56.35 for stem and 114.57 for root. The IC_{50} values for ABTS radical scavenging activity of different parts of *S. nigrum* plant were found to be fruit (46.93) leaves (51.26), stem (55.96) and root (101.35) µg/ml. The IC_{50} values in µg/ml for Alpha-amylase activity of different parts of *S. nigrum* plant was found to be fruit (49.47) leaves (62.83), stem (84.42) and root (178.75).The IC_{50} values in µg/ml for Alpha-glucosidase activity of different parts of *S. nigrum* plant was (154.36) for fruit, (163.93) for leaves, (180.10) for stem and (194.17) for root. This study provides a valuable insight into the therapeutic potential of *S. nigrum* plant constituents for managing diabetes and warrants further research and clinical investigation for practical applications in diabetic patients.

Keywords: Antioxidant, Diabetes mellitus, Alpha-glucosidase, Alpha-amylase, Solanum nigrum,

Introduction

Diabetes mellitus (DM) is a major public health constraint, the prevalence of diabetes is increasing, particularly in middle-income countries, with India projected to become the diabetic capital of the world by 2030[1]. Diabetes mellitus affects over 422 million adults globally, with prevalence rising from 4.7 to 8.5% compared to 1980[2]. Hyperglycemia and its associated metabolic complications lead to various biochemical changes resulting in complications such as nephropathy, retinopathy, and peripheral neuropathy. Type 2 diabetes mellitus (non-insulin-dependent diabetes mellitus) is primarily treated with oral antidiabetic drugs and gliptins, which may lead to various complications including hypoglycemia, weight gain, water retention, diarrhea, malabsorption, and necrotizing pancreatitis[3–5]. Individuals with T2DM experience both insulin resistance and inadequate insulin production. Insulin resistance develops initially, followed by changes in pancreatic cell mass and function (insulin secretory failure). T2DM patients exhibit insulin resistance and impaired insulin

internalization due to genetic mutations in insulin receptors, resulting in prolonged circulation of insulin and hyperinsulinemia[6]. In type 2 diabetes management, α-glucosidase inhibition therapy proves highly effective in regulating postprandial glucose levels. Under normal circumstances, α-glucosidase enzymes convert carbohydrates into glucose, maintaining healthy glucose levels within the body. Antioxidants derived from plants prevent the destruction of β cells by inhibiting the peroxidation chain reaction, thereby reducing oxidative stress in diabetes[4]. Given the limitations of currently available drugs for diabetes treatment, numerous herbal medicines have been utilized and recommended. Various phytoconstituents present in medicinal plants act on different targets through various mechanisms, contributing to their therapeutic effects[7]. This necessitates the search for newer antidiabetic drugs with minimal adverse effects from plant materials. *Solanum nigrum Linn*, also known as black nightshade, a member of Solanaceae family traditionally used for liver disorders, skin conditions such as psoriasis and ringworm, inflammatory conditions, painful menstruation, fevers, diarrhea, eye diseases, and hydrophobia[8]. Different parts of the plant exhibit pharmacological activity against diabetes[9].

Materials and methods

Plant materials and extraction

The different plant parts (leaves, bark, fruit, and stem) of *Solanum nigrum* were collected, washed with water, and then rinsed with methanol before being dried in shade at room temperature. Once dried, the plant parts were ground and sieved through a 120-mesh sieve. A quantity of 20 g of the sieved powder of *S. nigrum* was taken and mixed with 100 ml of 80% methanol using the cold maceration method for extraction. Maceration method was adapted from Tiwari *et al*[10]. The extract was filtered through Whatman No.1 filter paper, and the filtrate was concentrated under room temperature for dryness.

Phytochemical Screening:

Phytochemical analysis of different parts *of S. nigrum* was conducted following standardized procedures.

Quantitative analysis

Total Polyphenolic Content

The total polyphenolic content of the plant extract was determined by using Folin-Ciocaiteu reagent. Specifically, 50 µl of the plant extract was diluted with 50 µl water, followed by the addition of 150 µl of Folin-Ciocalteu (1N) and 25 µl of Na_2CO_3 (20% w/v). After incubation at 45°C for 60 minutes, absorbance was measured spectrophotometrically at 765 nm on the UV-Vis spectrophotometer. Quantification was performed with respect to the standard curve of Catechol (y= 0.00521x+0.05537, R^2 =0.99939). The results were expressed as milligrams of Catechol equivalent per ml of extract[11].

Total Flavonoid Content

The total flavonoid content in the plant extracts was determined by adding 50 µl of the sample to 50 µl of $AlCl_3.6H_2O$ in ethanol and 25 µl Sodium acetate solution. After incubation at 20°C for 2.5 hours, absorbance at 430nm was measured. Total flavonoids contents were calculated with respect to the standard curve of the flavonoids querecrtin dehydrate (Y= 0.0057x+0.08682, R^2=0.99697). The results were expressed as micrograms of quercetin dehydrate equivalents (QE) per ml of the extract[12].

DPPH Method

The DPPH assay was performed using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method. Various dilutions (10-400 µg/ml) of the methanolic extract were added along with tris–HCl buffer (0.1M, pH 7.4) and DPPH solution (0.004% w/v in methanol). After shaking well, the DPPH decolorization was recorded at 517 nm on a UV-Vis spectrophotometer (UV-1601 SHIMADZU) after 30 minutes of incubation in the dark. The results were expressed as milligrams of ascorbic acid equivalent per ml of extract.

ABTS Free Radical Scavenging Assay

The ABTS (2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt) radical cation decolorization test is a widely used spectrophotometric method for evaluating the antioxidant activity of various substances. The experiments were carried out using an improved ABTS decolourisation assay[13] with slight modification. This assay can be applied to both lipophilic and hydrophilic compounds. For the assay, ABTS radicals were generated by oxidizing ABTS with potassium per sulfate. Then, 1 ml of the ABTS cation solution was mixed with 200 µl of test samples at varying concentrations (25 µg/ml to 200 µg/ml). The mixture was

incubated for 5 minutes, and the absorbance was measured at 734 nm using a UV-Vis spectrophotometer (UV-1601 SHIMADZU). Ascorbic acid served as the standard.
The ABTS radical scavenging activity was calculated using the formula:

ABTS radical scavenging activity (%) = $\frac{(A_0 - A_1)}{A_0} \times 100$

Where A_0 = Absorbance of control
A_1 = Absorbance of sample
All the determinations were performed in triplicates (n=3).

α- Glucosidase Inhibition Activity:
The α-glucosidase inhibitory activity was assessed using the method described by Tripathi *et al*[11]. with slight modification. In brief, rat intestinal acetone powder was dissolved in 4 ml of 50 mM ice-cold phosphate buffer and sonicated for 6 minutes at 4°C. After vortexing for 20 minutes, the suspension was centrifuged at 10,000 rpm for 30 minutes at 4°C, and the resulting supernatant was used for the assay.
A reaction mixture containing 50 µl of 50 mM phosphate buffer (pH 6.8), 20 µl of rat α-glucosidase, and 200 µl of the sample at varying concentrations was pre-incubated for 5 minutes at 37°C. Then, 50 µl of 3 mM PNPG was added as the substrate. After incubation at 37°C for 30 minutes, the enzymatic activity was quantified by measuring the absorbance at 405 nm using a UV-Vis spectrophotometer (UV-1601 SHIMADZU). Acarbose was used as a positive control, and water was used as a negative control.
All experiments were performed in triplicate. The IC_{50} value, which represents the concentration of an inhibitor required to inhibit 50% of enzyme activity, was determined using the formula ($y = 0.193x + 19.68$, $R^2 = 0.965$).
The percentage of enzyme inhibition by the sample was calculated using the following formula:

Percentage of enzyme inhibition% Inhibition = $\frac{(A_0 - A_1)}{A_0} \times 100$

Where: -
$A0$ = Absorbance of the control
$A1$= Absorbance of the sample

α- Amylase Inhibition Method
The α-amylase inhibition assay was conducted using the 3, 5-dinitrosalicylic acid (DNS) method[14]. Various concentrations (25 µg/ml To 200 µg/ml) of methanol extract (200 µL) were added to 1 ml of 0.02 M sodium phosphate buffer (pH 6.9, containing 6 mM sodium chloride) along with 1 ml of α-amylase solution. This mixture was incubated at 37°C for 10 minutes. Subsequently, 500 µL of a 1% starch solution (prepared in 0.02 M sodium phosphate buffer, pH 6.9) was added to all test tubes.
The reaction was terminated by adding 500 µL of DNS reagent. The test tubes were then placed in a boiling water bath for 5 minutes and cooled to room temperature. The reaction mixture was diluted with 10 ml of distilled water, and absorbance was measured at 540 nm. Control samples, prepared without any plant extracts, were used for comparison against the test samples containing various concentrations of the plant extract. Acarbose served as a positive control. The results were expressed as percentage inhibition, calculated using the formula:
Inhibition activity (%) = Abs (control) − Abs (extract) × 100/Abs (control)

Results and Discussion

Phytochemicals such as phenolic and flavonoid compounds present in *S. nigrum* are well-known for their antioxidant and antidiabetic activities. Due to these properties, there is interest in using extracts rich in phenolics and flavonoids for the treatment of diabetes and its complications.
The quantitative analysis of *S. nigrum* was conducted to determine its total phenol and flavonoid content. The total phenol content in the plant extract is expressed in terms of catechol equivalent. The equation for the standard curve (Figure-1) is $y= 0.00521x+0.05537$, $R^2 =0.99939$ and the concentration of total phenolic content is given below in Table-1. The flavonoid content in the plant extract is expressed in terms of quercetin equivalent. The equation for the standard curve (Figure-2) is $Y= 0.0057x+0.08682$, $R^2=0.99697$ and the concentration of flavonoid content is given below in Table-1.

Table:-1 TPC and TFC Quantitative photochemical analysis in methanolic extract different parts of *Solanum nigrum* L

Name of part extract	absorbance for Total Phenol content	µg/ml	absorbance for Total Flavonoids content	µg/ml
Solanum nigrum (SNF)	**0.385**	63.31	**0.222**	23.72
Solanum nigrum (SNL)	**0.590**	102.70	**0.817**	128.11
Solanum nigrum (SNS)	**0.305**	47.96	**0.164**	13.54
Solanum nigrum (SNR)	**0.379**	62.17	**0.230**	25.12

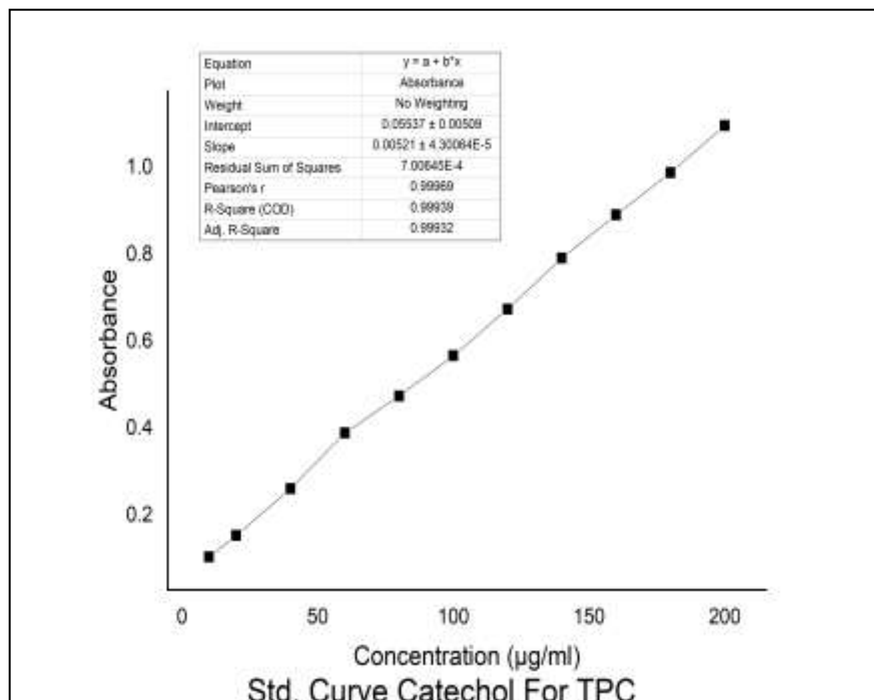

Fig: 1- Standard curve of Catechol for TPC

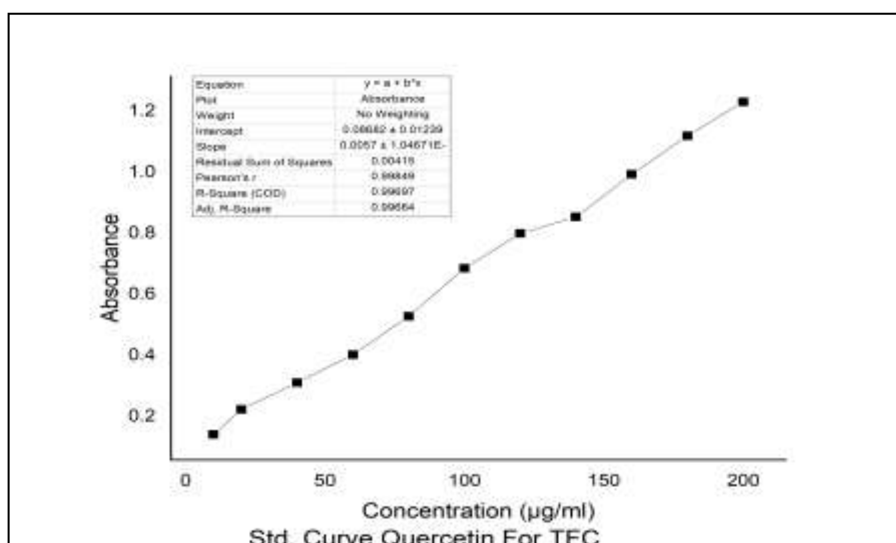

Fig: 2- Standard curve of Quercetin for TFC

DPPH and ABTS scavenging activity (% inhibition) are presented in Tables 2 and 3, respectively. Figure 3-4 depicts the standard curve of ascorbic acid with sample. This assay evaluates the capacity of the stable free radicals, 1,1'-diphenyl-2-picrylhydrazyl (DPPH) and ABTS, to decolorize in the presence of antioxidants. In our study, ascorbic acid served as the reference standard, 80% methanol was used as the blank and the DPPH and ABTS solutions without any sample acted as controls. Percentage inhibition was calculated, and a curve plotting % inhibition against concentrations was constructed. Linear regression analysis was utilized to

determine the inhibition concentration of the sample required to scavenge DPPH and ABTS radicals by 50% (IC_{50} value). The IC_{50} value of standard ascorbic acid was calculated to be 31.01µg/ml for DPPH and 33.69 µg/ml for ABTS. Table 2 and 3 list the IC_{50} values of all samples. Compared to the standard ascorbic acid, the selected plant parts exhibited significant DPPH and ABTS scavenging activity, based on IC_{50} values. The highest DPPH and ABTS radical scavenging activity was demonstrated by the fruit hydro methanolic extracts, with IC_{50} values of 38.77µg/ml and 46.93 µg/ml respectively.

Table: 2- DPPH percentage inhibition with IC_{50} value

Conc. µg/ml	% Inhibition				
	Std. Ascorbic Acid	SNF	SNL	SNS	SNR
10	28.24	31.87	42.45	47.39	20.28
25	29.35	46.48	45.98	49.51	31.27
50	31.17	52.03	51.62	49.81	43.97
100	33.79	68.46	56.26	51.73	47.90
200	41.65	73.70	73.19	54.55	54.45
400	53.54	77.93	92.24	57.17	67.04
IC_{50}	31.01	38.77	51.45	56.35	114.57

Note: - SNF = *Solanum nigrum* fruit, SNL= *Solanum nigrum* leaves, SNS= *Solanum nigrum* stem, SNR= *Solanum nigrum* root

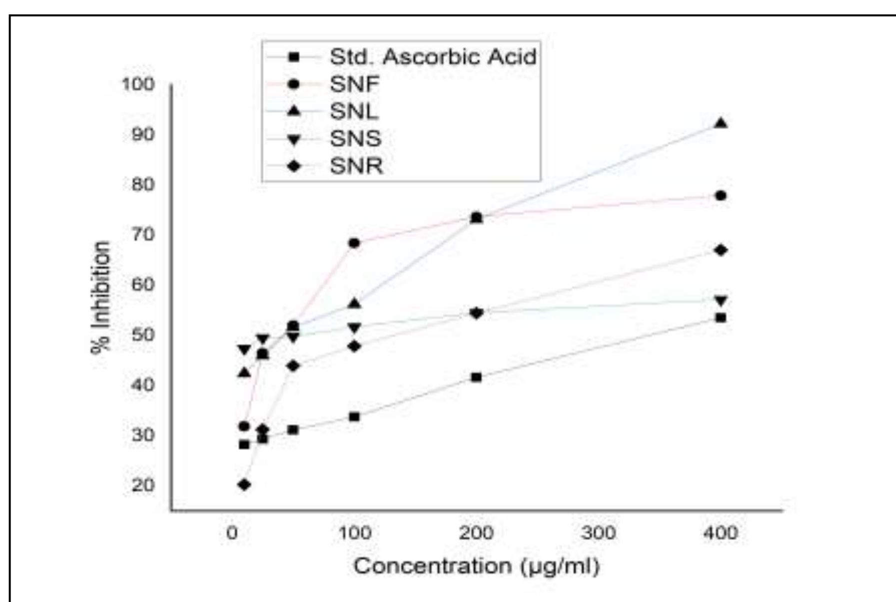

Fig: 3- Standard (Ascorbic acid) curve with sample curve for DPPH

Table: 3- ABTS percentage inhibition with IC_{50} value

Conc. µg/ml	% Inhibition				
	Std. Ascorbic Acid	SNF	SNL	SNS	SNR
25	43.87	45.36	43.85	40.10	25.81
50	54.58	51.40	49.79	48.73	38.52
100	61.08	57.50	61.14	59.51	51.57
200	67.04	66.71	69.72	70.19	75.79
IC_{50}	33.69	46.93	51.26	55.96	101.35

Note: - SNF = *Solanum nigrum* fruit, SNL= *Solanum nigrum* leaves, SNS= *Solanum nigrum* stem, SNR= *Solanum nigrum* root

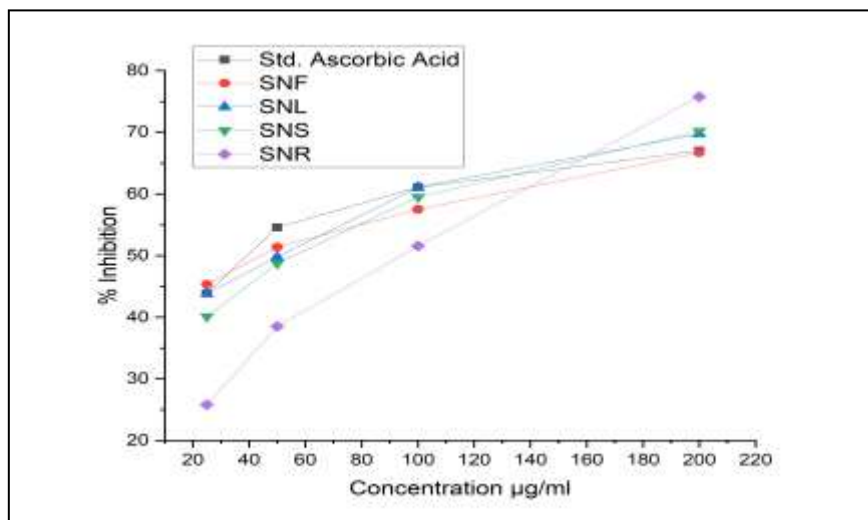

Fig: 4- Standard (Ascorbic acid) curve with sample curve for ABTS

Table: 4- α-amylase percentage inhibition with IC_{50} value

Conc. µg/ml	%Inhibition				
	Std. Acarbose	SNF	SNL	SNS	SNR
25	22.29	14.68	43.11	15.03	4.17
50	55.26	50.90	49.09	37.5	6.89
100	59.97	59.97	55.26	52.18	24.63
200	65.03	72.82	67.76	71.20	52.18
IC_{50}	32.92	49.47	62.83	84.42	178.75

Note: - SNF = *Solanum nigrum* fruit, SNL= *Solanum nigrum* leaves, SNS= *Solanum nigrum* stem, SNR= *Solanum nigrum* root

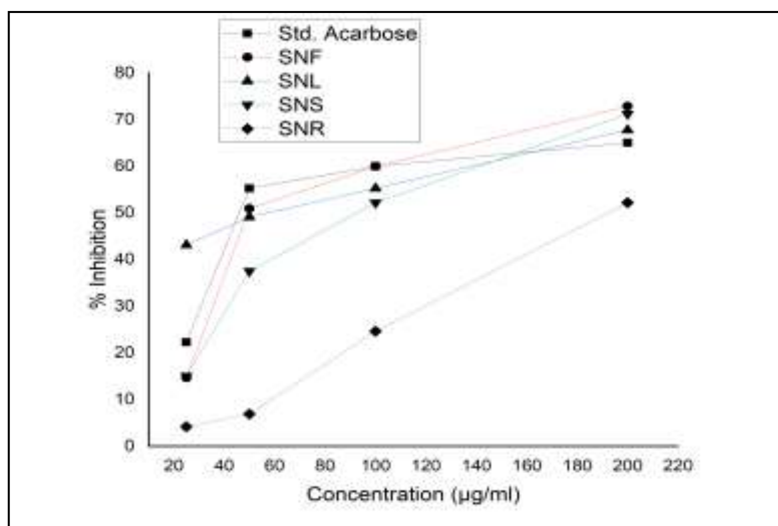

Fig: 5- Standard (Acarbose) curve with sample curve for **α-amylase**

Table: 5- α-glucosidase percentage inhibition with IC_{50} values

Conc. µg/ml	% Inhibition				
	Std. Acarbose	SNF	SNL	SNS	SNR
25	6.10	4.22	4.94	3.78	3.49
50	25.29	9.59	14.39	13.37	11.05
100	42.30	33.43	30.81	30.09	23.55
200	55.38	56.10	60.90	54.36	51.79
IC_{50}	122.65	154.36	163.93	180.10	194.17

Note: - SNF = *Solanum nigrum* fruit, SNL= *Solanum nigrum* leaves, SNS= *Solanum nigrum* stem, SNR= *Solanum nigrum* root

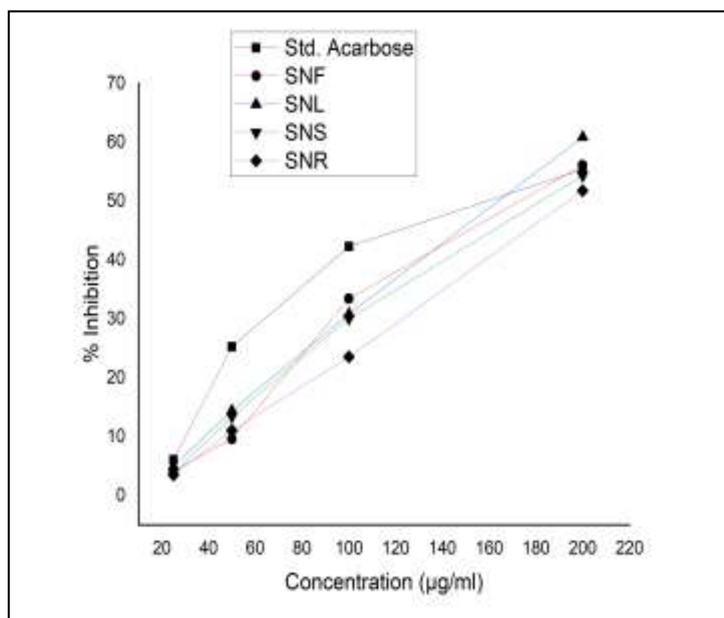

Fig: 6- Standard (Acarbose) curve with sample curve for **α-glucosidase**

This study also compared the α-amylase and α-glucosidase inhibitory activity of *S. nigrum* with the standard acarbose. Figure 5-6 graphically represents the % inhibition of α-amylase and α-glucosidase, while table 4-5 provides the IC_{50} values of the selected plant parts and acarbose used as a standard. The standard positive control Acarbose showed an IC_{50} value 32.92 µg/ml for α-amylase activity and 122.65 µg/ml of α-glucosidase activity. α-Glucosidase enzymes are crucial in many biological processes, including the breakdown of dietary carbohydrates. α-Glucosidase, located in the brush border surface membrane of intestinal cells, is a key enzyme in carbohydrate digestion.α-amylase is degradation to starch molecule then hydrolyzing them into small chain dextrin's by acting upon the hydrolyzing α-1,4 glycoside bonds of polysaccharides to produce glucose, which easily enters the bloodstream. The inhibition of α-amylase and α-glucosidase by *S. nigrum* fruit can be attributed to the presence of phenolic and flavonoid with potential antioxidant activity. The crude fruit powder of MeOH extract of *S. nigrum* plant exhibited strong antidiabetic α-amylase and α-glucosidase activity, with IC_{50} values of 49.47 µg/ml and 154.36 µg/ml, respectively.

Conclusion

The present study concludes that *S. nigrum* is an important beneficial herbal plant used for curing various diseases due to the variety of phytochemicals present in different parts of the plant. The overall result obtained from this study indicates that the fruit part of *S. nigrum* exhibits strong antioxidant (ABTS & DPPH) and antidiabetic (α-amylase and α-glucosidase inhibitory) activities. This suggests that the phytoconstituents found in fruit part of *S. nigrum* are responsible for its antidiabetic activity.

Acknowledgement

The authors are thankful to MPCST Bhopal for providing grant to research project sanction no. 2724/CST/R&D (SVTK)/2022.

Reference

1. Kaveeshwar SA, Cornwall J. The current state of diabetes mellitus in India. Australas Med J. 2014; 7(1):45-48. doi:10.4066/AMJ.2013.1979

2. Roglic G. WHO Global report on diabetes: A summary. Int J Noncommunicable Dis. 2016; 1(1):3. doi:10.4103/2468-8827.184853
3. Pareek H, Sharma S, Khajja BS, Jain K, Jain GC. Evaluation of hypoglycemic and anti-hyperglycemic potential of Tridax procumbens (Linn.). BMC Complement Altern Med. 2009; 9: 48. doi:10.1186/1472-6882-9-48
4. Aslan M, Orhan N, Orhan DD, Ergun F. Hypoglycemic activity and antioxidant potential of some medicinal plants traditionally used in Turkey for diabetes. *J Ethnopharmacol*. 2010;128(2):384-389. doi:10.1016/j.jep.2010.01.040
5. Mukherjee PK, Maiti K, Mukherjee K, Houghton PJ. Leads from Indian medicinal plants with hypoglycemic potentials. J Ethnopharmacol. 2006;106(1):1-28. doi:10.1016/j.jep.2006.03.021
6. Chen Y, Huang L, Qi X, Chen C. Insulin Receptor Trafficking: Consequences for Insulin Sensitivity and Diabetes. Int J Mol Sci. 2019;20(20):5007. doi:10.3390/ijms20205007
7. Tiwari AK, Rao JM. Diabetes mellitus and multiple therapeutic approaches of phytochemicals: Present status and future prospects. Curr Sci. 2002;83(1):30-38.
8. Saleem TM, Chetty C, Ramkanth S, Alagusundaram M, Gnanaprakash K, Rajan VT, Angalaparameswari S. Solanum nigrum Linn.-A review. Pharmacognosy reviews. 2009 Jul 1; 3(6):342.-45.
9. Maniyar YA, Umamageswari MS, Karthikeyan TM. Evaluation of antihyperglycaemic activity of aqueous extract of leaves of Solanum nigrum in Alloxan induced diabetic rats. International Journal of Pharmacy and Biological Sciences. 2012; 2(4):312-19.
10. Tiwari P, Kumar B, Kaur M, Kaur G, Kaur H. Phytochemical screening and extraction: a review. Internationale pharmaceutica sciencia. 2011 Mar;1(1):98-106.
11. Tripathi IP, Mishra MK, Mishra C, Tripathi R, Kamal A, Tripathi P, Shukla VP, Gangele R, Pandey KB. Assessment of antioxidant and total polyphenolic content of some plants of euphorbiaceae family. Indian J. Appl. Res. 2013; 3(10):1-4.
12. Chatattikun M, Choabchalard A. Phytochemical screening and free radical scavenging activities of orange baby carrot and carrot (Daucus carota Linn) root crude extracts. Journal of Chemical and Pharmaceutical Research. 2013; 5(4):97-102.
13. Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radic Biol Med. 1999;26(9-10):1231-1237. doi:10.1016/S0891-5849(98)00315-3
14. Asokan A, Thangavel M, Nisha P. Invitro antioxidant activity of Saraca indica methanolic bark extract. Int. J. Curr. Microbiol. App. Sci. 2015; 4(3):515-20.