RESEARCH PAPER

Evaluation of Biological Activities of Gold Nanoparticles Derived from Vitis vinifera Peel Extract.

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ABSTRACT
The study aims to synthesize Vitis vinifera peel gold nanoparticles (VvAuNPs). an eco-friendly green synthesis and with precise biological activities. Its assessment focuses on the antimicrobial, antioxidant and its potential to show ant diabetic property of AuNPs. In this study it is revealed that we use to evaluate the biological and green synthesis of gold nana particle the (AuNPs) derived from Vitis vinifera peel extract. The phytometabolites present in the peel extract acts as a natural reducing and stabilizing agent during synthesis. VvAuNPs structural prediction were conformed with spectral studies. Antibacterial assay revealed significant (zone of inhibitions) against Staphylococcus aureus, Bacillus subtilis and Escherichia coli, pseudomonas aeruginosa bacterial strains, indicating strong bactericidal efficiency. The polyphenolic activity is determined through DPPH and FRAP radical scavenging assays, exhibited excellent free radical neutralization capacity. Antidiabetic activity assessed via α-amylase and α-glucosidase inhibition demonstrated effective enzyme suppression, suggesting glucose-regulating potential. Moreover, anti-inflammatory studies based on protein denaturation and membrane stabilization assays confirmed substantial inhibition of inflammatory mediators.
Keywords: Vitis vinifera peel; gold nanoparticles; green synthesis; antibacterial; antioxidant; antidiabetic; Activity.
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INTRODUCTION

The escalating prevalence of chronic diseases, including diabetes, and inflammatory disorders, has intensified the search for novel therapeutic agents. Nanomedicine, particularly gold nanoparticles (AuNPs), has emerged as a promising avenue due to their unique physicochemical properties and biocompatibility. Among various synthesis methods, green synthesis using *Vitis vinifera* extracts offers an eco-friendly and cost-effective approach. Vitis vinifera, commonly known as the grapevine, is a rich source of Antioxidant components, anti-inflammatory, and anticancer properties. Utilizing grape peel extract for the synthesis of AuNPs not only valorises agricultural waste but also harnesses the therapeutic potentials of these phytochemicals.

The demand of the biosynthesis of gold nano particle is very high through the process of green synthesis so as to mitigate adverse effects the devasting effects in therapy and other medical applications (Al-Ansari, M.M., 2021., et al.,).

Medicinal plants have long played an important part in therapeutic practices and are widely recognised as best source of bioactive substances capable of preventing and treating a variety of ailments. Twenty first century have, proved pharmacological usefulness of neutraceutical plants has increased the importance of herbal medicine, establishing it as a promising and expanding field in modern healthcare. The increasing scientific interest in therapeutic plants originates from their numerous natural biomolecules, which demonstrate a broad spectrum of biological functions (Khan MSA, Ahmad I.., 2010… et al.,).

The *Vitises* is among the most important medicinal plants. The Vitis vinifera is a perennial fruit known for its high nutritional value (Fernandes L, Ramalhosa., 2017… et al.,). *Vitis vinifera*, a well-established plant of the Vitaceae family and Vitis genus, comes in a variety of seeded or seedless types and is typically available in red, black, and white (Patel S.,and Sharma D. 2021… et al.,) and (Da Silva AA, Kamatou HP., 2021… et al.,).

Extensive study has revealed that the plant's root, stem, leaves, seeds, fruit, pomace, and skin contain a diverse range of phytochemical elements that contribute to significant anticancer, antibacterial, and antioxidant characteristics (Ibeas S, Melo TS, 2021… et al.,). Because of these significant chemicals, *Vitises* are also regarded an important natural source for cosmetic compositions (Khan MSA, Ahmad I..,

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2010… et al.,). On comparing the harmful synthetic chemical medicines, the use of the plant and microbes have a wide range of applications in in the field of imaging, drug administration and photo thermal treatment fields. As a result, plant-mediated biosynthesis through the bio reduction process has improved anti-diabetic, anti-oxidant, and antimicrobial properties. Bio-converted NPs are made from metals such as Au, Ag, Cu, Pt, Zn, and Fe, which are processed against organic agents derived from plant materials (I Al-Dahmash, N.D., 2021… et al.,). Gold nanoparticles (AuNPs) have a long history of use in bioimaging, medication delivery, and other applications. Gold nanoparticles' unique physiochemical and photothermal capabilities control the conjugation and delivery of targeted medications to particular tissues or cells. Au-NPs exhibit various distinguishing characteristics, including the resonance of the plasma and also for optical criteria.

In the field of the Ayurveda, it is found that the Draksha vitises are the natural substances having many neutraceutical properties. The use of the vitises as a natural substance having various therapeutic uses is proven, as it peel contains many bioactive compounds such as oligomeric proanthocyanin complex (OPC), the presence of the antioxidant is considered to be important for cureing certain diseases. It is scientifically evident that the Vitis peel in the powered form may increase the blood flow in the legs, reduces stress of the eyes and also helps in decreasing the level of cholesterol and boosts the immune system as well. It is also effective against the muscular degeneration due to aging.

The biosynthesis of metal Nano particles are both compatible to body and also ecofriendly.. Au-NPs used lately been studied in therapeutic use for better understand their unique features. The designed nanoparticles can now be tagged with biological, physiological, or biomedical carriers and employed in nano-based systems to offer effective and accurate medication in cancer imaging zones cancer cells(Al-Radadi, N.S., 2022… et al.,). Proanthocyanidins have also been shown in studies to have anti-inflammatory properties. These bioactive polyphenols serve to control inflammation by inhibiting the release of arachidonic acid. Furthermore, phenolic constituents—such as resveratrol—show antibacterial efficacy by producing oxidative stress, which destroys bacterial membranes while maintaining the structural integrity of host cells (Sharayei P, Solgizadeh S, 2019… et al.,) and (Karthikeyan G, Parthipan S., 2020… et al.,).

Vitis has important drug forming capacity against the cancer because due to presence of high antioxidant components (Martin ME, Gonzalez S., 2020… et al.,).

The several forms of gold nanoparticles, including spheres, nano cubes, nanorods, nano stars, and nanocages, are the subject of current research. Furthermore, monoclonal antibodies, polymers, and probes are used to functionalise gold nanoparticles for human medicines (Al-Ansari, M.M., 2021… et al.,).

The neutraceutical property such as antixodant, antidiabetic and anti microbial has been investigated using various characterizations. This research collectively supports the therapeutic potential of black Vitis vinifera extract as a multifunctional agent, displaying antioxidant, antibacterial and anti-cancer effects. Black Vitis vinifera is therefore a viable option for nanotechnological uses, such as the creation of nano emulsions and the creation of pharmaceuticals. The synthesized AuNPs from Vitis vinifera peel extract have attracted research interest due to their broad biological activities. Studies demonstrate potent antimicrobial properties attributed to the bioactive phytochemicals on the AuNP surface, which can disrupt bacterial membranes and inhibit microbial growth. Additionally, these AuNPs exhibit strong antioxidant activity by scavenging free radicals through electron donation from the phenolic groups associated with the nanoparticle surface. This antioxidant potential supports their further investigation for therapeutic applications.

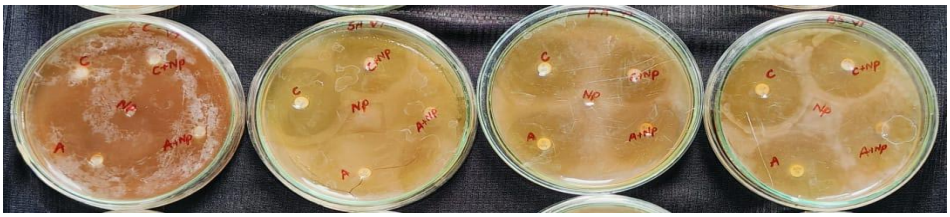
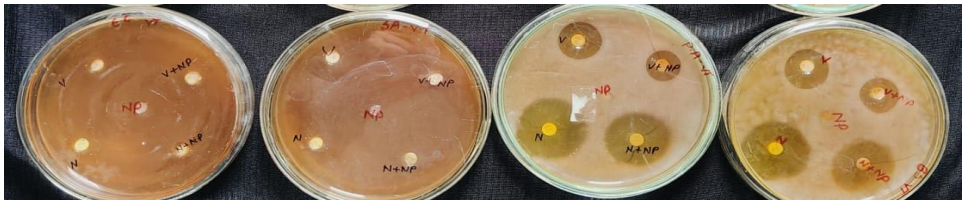
MATERIALS AND METHODS:

Preparation of Peel Extract

Peels were washed, air-dried, powdered, and boiled in distilled water (10 g/100 mL) for 30 minutes. The extracted portion is filtered and stored at 5-5˚C

Synthesis of AuNPs

For synthesis we use to add10 mL of peel to 90 mL of 1 mM $HAuCl_4$ stock solution, stirred at 60°C until ruby red colour appeared. The centrifugation of nanoparticles was carried out at (12,000 rpm, 15 min), washed, dried, and stored

STANDARD DRUG	ZONE OF INHIBITIONS			
	Escherichia coli	***Staphylococcus aureus***	***pseudomonas aeruginosa***	***Bacillus subtilis***
Tetracycline-30mcg Erythromycin-15mcg				
	Fig:3-- *(a)*	*(b)*	*(c)*	*(d)*
Ciprofloxacin-5mcg Amoxycillin-30mcg				
	Fig:3- *(a)*	*(b)*	*(c)*	*(d)*
Vancomycin-30mcg Nitrofurantoin-300mcg				
	Fig:4- *(a)*	*(b)*	*(c)*	*(d)*
Azithromycin Ampicillin				
	Fig:5- *(a)*	*(b)*	*(c)*	*(d)*

Antioxidant Activity Evaluation:

DPPH Assay for Vitis vinifera Peel–Derived Gold Nanoparticles:

The antioxidant activity of gold nanoparticles (AuNPs) synthesized using aqueous Vitis vinifera peel extract was evaluated by the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical-scavenging assay, following the general procedure described by Gulçin and Alwasel (2023) and Singh et al. (2022), with modifications for nanoparticle systems. A fresh 0.004% (w/v) DPPH solution was prepared in ethanol and protected from light (Gulçin & Alwasel, 2023).

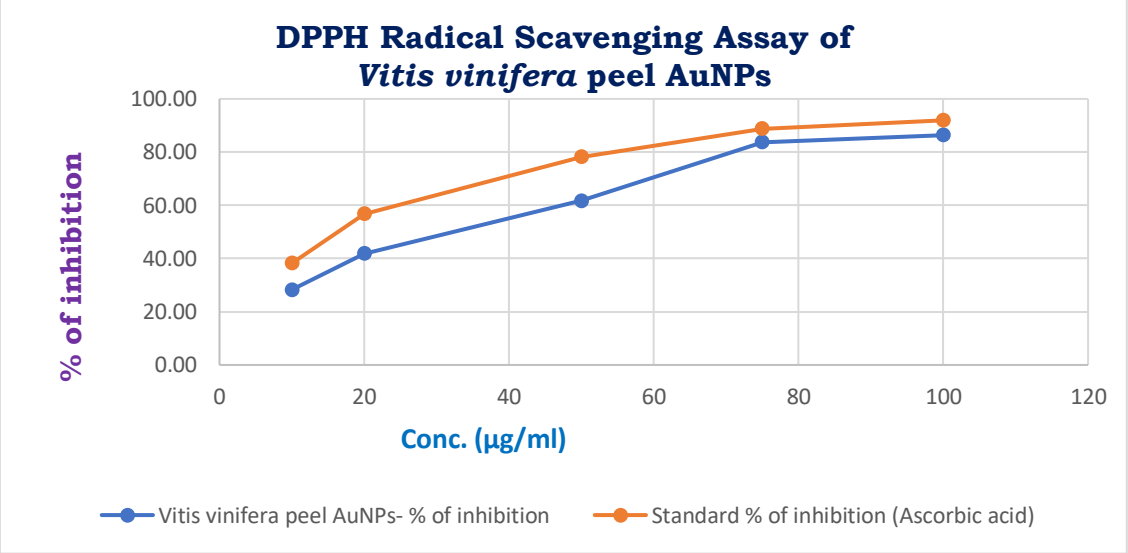
AuNP stock dispersions (prepared as described in the synthesis protocol) and the parent Vitis vinifera peel aqueous extract were diluted in the same solvent system to obtain working concentrations of 10, 20, 50, 75 and 100 $\mu g \cdot mL^{-1}$, expressed as gold or dry-mass equivalent **[Table 5 and 6]** and Graphical presentation (Singh et al., 2022) [**Graph-1**]. Ascorbic acid was used as a positive control (da Silva et al., 2025).

DPPH Radical Scavenging Assay;

Table-5: Presents the percentage of DPPH free-Radical Scavenging Activity exhibited by *Vitis Vinifera* derived gold nanoparticles in comparison with the standard percentage of inhibition by using various concentration

Conc. (µg/ml)	Vitis vinifera peel AuNPs % of inhibition	IC_{50}	Standard % of inhibition (Ascorbic acid)	IC_{50}
10	28.29±0.10	35.27± 0.15	38.21±0.06	14.86± 0.40
20	41.92±0.35		56.59±0.25	
50	61.76±0.17		78.24±0.55	
75	83.74±0.21		88.81±0.06	
100	86.36±0.11		91.96±0.60	

*Each value is represented as mean ± SD (n=3).



Graph 1: Vitis vinifera peel AUNps percentage of Inhibitions and standard Inhibition

Table-6: Represents a graph % values by using different concentrations

For Graph

Conc. (µg/ml)	Vitis vinifera peel AuNPs- % of inhibition	Standard % of inhibition (Ascorbic acid)
10	28.29	38.21
20	41.92	56.79
50	61.76	78.24
75	83.74	88.81
100	86.36	91.96

For each test, 1.0 mL of sample (AuNP or extract) was mixed with 2.0 mL of DPPH solution and incubated in the dark at room temperature for 30 minutes. All measurements were performed in triplicate to ensure reproducibility. Because biologically synthesized AuNPs typically exhibit a surface plasmon resonance (SPR) band in the 510–560 nm range, which may cause baseline interference, a nanoparticle blank (A NB) consisting of 1.0 mL sample + 2.0 mL solvent (no DPPH) was included for each concentration and subtracted from the test reading.

A DPPH control (A control) (2.0 mL DPPH + 1.0 mL solvent) and a solvent blank were also prepared.

Absorbance was recorded at 517 nm using a UV–Vi's spectrophotometer (or scanned 300–800 nm to confirm SPR behaviour and absence of spectral overlap). The percentage of

radical-scavenging activity was calculated using the following formula (Yamauchi et al., 2024):

DPPH scavenging activity (%) = (A_ "control" −(A_ test−A_NB))/A_ "control" ×100

where

A control = Absorbance of DPPH solution without sample (represents 100% free

radicals),

A test = Absorbance of DPPH solution with AuNP or extract,

A NB = Absorbance of nanoparticle blank (without DPPH).

Dose–response curves were plotted between percent inhibition and concentration, and

IC_{50} values were calculated as the concentration required to scavenge 50% of DPPH

radicals, expressed as mean ± SD (n = 3) (da Silva et al., 2025).

All solvent systems, incubation times, and instrumental parameters were maintained constant across samples to ensure comparability and to minimize artefacts caused by nanoparticle optics or residual phytochemicals

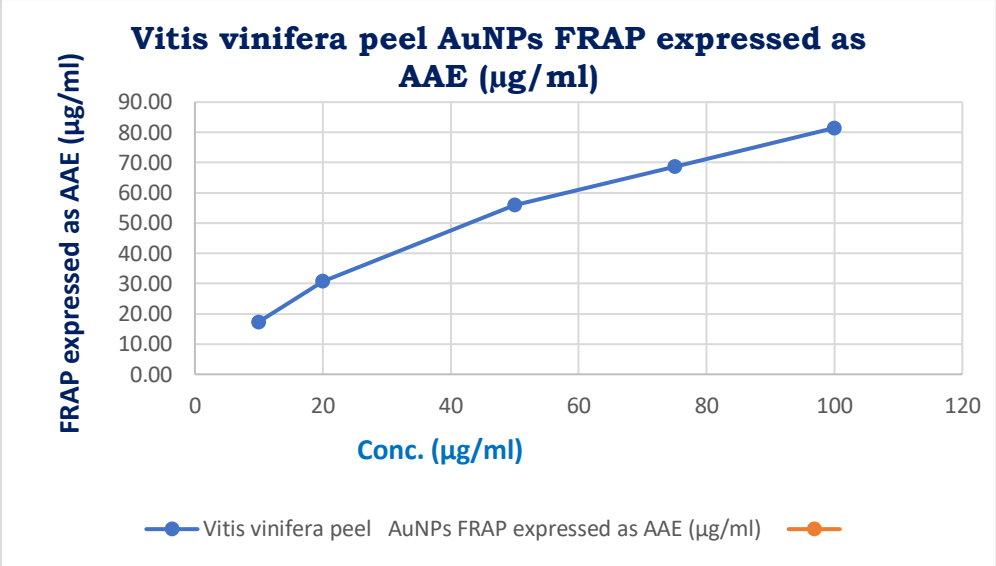
FRAP Assay for *Vitis vinifera* Peel–Derived Gold Nanoparticle

Table 7. *Vitis vinifera* peel AuNPs FRAP expressed as AAE (µg/ml)

	***Vitis vinifera peel* AuNPs FRAP expressed as AAE (µg/ml)**	**IC_{50}**		
Conc. (µg/ml)				
10	17.35±0.41	49.79± 0.21	17.35	0.41
20	30.81±0.50		30.81	0.50
50	55.92±0.36		55.92	0.36
75	68.67±0.14		68.67	0.14
100	81.46±0.95		81.46	0.95

The ferric reducing antioxidant power (FRAP) of gold nanoparticles (AuNPs) synthesized using aqueous *Vitis vinifera* peel extract was evaluated according to the method originally described by Benzie and Strain (1996) and modified by Banerjee et al. (2008), with additional precautions to correct for nanoparticle optical interference (Kumari et al., 2020). Briefly, 1.0 mL of each test sample—*Vitis vinifera* peel–derived AuNP dispersion or the parent aqueous peel extract **Table 3.** (10, 20, 50, 75, and 100 µg·mL^{-1})—was mixed with 1.0 mL of 0.2 M sodium phosphate buffer (pH 6.6) and 1.0 mL of 1% (w/v) potassium ferricyanide in separate test tubes. The mixtures were incubated at 50 °C for 20 minutes in a temperature-controlled water bath to promote the reduction of ferricyanide to ferrocyanide (Banerjee et al., 2008). After incubation, 1.0 mL of 10% (w/v) trichloroacetic acid (TCA) was added to each tube to stop the reaction. The tubes were centrifuged at 3,000 rpm for 10 minutes at room temperature to obtain clear supernatants. An aliquot of 1.0 mL of each supernatant was transferred into a fresh tube containing 1.0 mL of deionized water and 200 µL of 0.1% (w/v) ferric chloride ($FeCl_3$). After 5 minutes of incubation at room temperature, the absorbance of the resulting Prussian blue complex was recorded at 700 nm using a UV–Vis spectrophotometer. A nanoparticle blank (A NB) (1.0 mL of AuNP dispersion treated identically but without potassium ferricyanide) was included for each concentration to correct for baseline interference caused by AuNPs, whose surface plasmon resonance (SPR) typically lies between 510–560 nm and may cause scattering in the visible range (Kumari et al., 2020; Singh et al., 2022). Ascorbic acid (10–100 µg·mL^{-1}) was used as a positive control and calibration standard to express results as µg ascorbic acid equivalents (AAE) per mL of sample (Benzie & Strain, 1996). The ferric reducing antioxidant power was expressed as the increase in absorbance at 700 nm after blank subtraction, calculated as follows:

ΔA_700= (A_700 ("sample") −A_ "blank") −A_NB



Graph 1: Vitis vinifera peel AuNPs FRAP expressed as AAE (µg/ml)

Table 8: shows a graph value of *Vitis vinifera peel AuNPs FRAP expressed as AAE (µg/ml)*by using different concentrations (µg/ml).

Conc. (µg/ml)	Vitis vinifera peel AuNPs FRAP expressed as AAE (µg/ml)
10	17.35
20	30.81
50	55.92
75	68.67
100	81.46

All measurements were performed in triplicate (n = 3) and expressed as mean ± SD. Solvent systems, incubation times, and instrument parameters were maintained identical across all samples to minimize artefacts from nanoparticle scattering or residual phytochemicals.

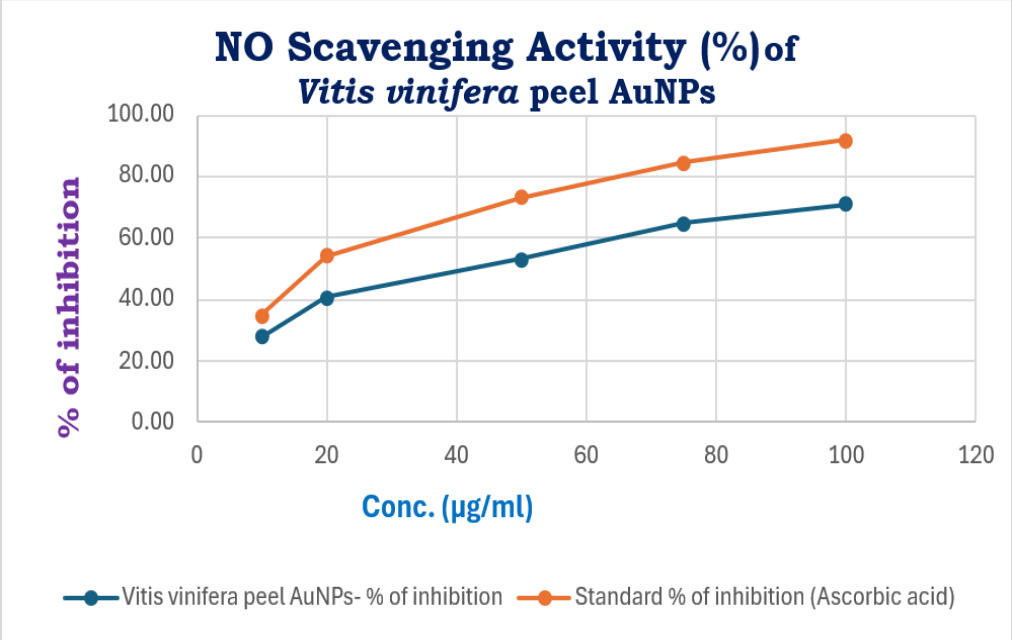
Nitric Oxide (NO) Scavenging Assay for *Vitis vinifera* Peel–Derived Gold Nanoparticles

The nitric oxide (NO) radical-scavenging activity of gold nanoparticles (AuNPs) synthesized using aqueous *Vitis vinifera* peel extract was determined using the sodium nitroprusside (SNP) method described by Garrat (1964) and Marcocci et al. (1994), with modifications to account for nanoparticle interference. In this assay, 10 mM sodium nitroprusside (SNP) in phosphate-buffered saline (PBS, pH 7.4) was used as the source of nitric oxide. Various concentrations of *Vitis vinifera* peel–derived AuNP dispersions and the parent extract (10, 20, 50, 75, and 100 µg·mL^{-1}) **Table 9.** were prepared in deionized water (Green et al., 1982).

Table- 9: NO Scavenging Activity (%) by using various concentrations.

Conc. (µg/ml)	**Vitis vinifera peel AuNPs - NO Scavenging Activity (%)**	**IC_{50}**	**Standard % of inhibition (Ascorbic acid)**	**IC_{50}**
10	27.86±0.10	47.81± 0.46	35.00±0.50	21.19± 0.91
20	40.61±0.25		54.05±0.61	
50	53.04±0.30		73.31±0.50	
75	64.96±0.67		84.37±0.69	
100	70.84±0.35		91.94±0.75	

*Each value is represented as mean ± SD (n=3).



Graph 2: Vitis vinifera peel percentage inhibition of Vv.Aunps and Absorbic acid

Table-10: Percentage of DPPH radical Scavenging activity of V.vinifera peel-Mediated Gold nanoparticles and the standard drug (ascorbic acid) at different concentrations

Conc. (µg/ml)	Vitis vinifera peel AuNPs- % of inhibition	Standard % of inhibition (Ascorbic acid)
10	27.86	35.00
20	40.61	54.05
50	53.04	73.31
75	64.95	84.37
100	70.84	91.64

For each reaction, 1.0 mL of sample was mixed with 2.0 mL of SNP solution and incubated under light at room temperature for 150 minutes to facilitate nitric oxide generation. After incubation, 0.5 mL of the reaction mixture was combined with 1.0 mL of freshly prepared Griess reagent (1% sulphanilamide in 5% phosphoric acid and 0.1% naphthyl ethylenediamine dihydrochloride), mixed well, and allowed to stand for 5 minutes for colour development (Bryan & Grisham, 2007). The absorbance of the resulting pink chromophore was measured at 540 nm against the reagent blank. A nanoparticle blank (A NB) (1.0 mL AuNP dispersion + 2.0 mL PBS, no SNP) was included for each concentration to correct for background absorbance or direct reaction of nanoparticles with the Griess reagent (Kumari et al., 2020). Ascorbic acid (10–100 $\mu g \cdot mL^{-1}$) served as a positive control (Singh et al., 2022). Nitric oxide scavenging activity was calculated using the following formula (Marcocci et al., 1994):

"NO Scavenging Activity (%)"= (A_ "control" −(A_ "test" −A_NB))/A_"control" ×100

where A control = Absorbance of SNP + Griess reagent (without sample, representing 100% NO generation), A test = Absorbance of reaction mixture containing AuNPs or extract, A NB = Absorbance of nanoparticle blank (no SNP).

The percentage inhibition was plotted against concentration to obtain IC_{50} values (the concentration required to scavenge 50% of NO radicals). All experiments were performed in triplicate and expressed as mean ± SD (n = 3).

Evaluation of Anti-Diabetic Activity:
Assay of α-Amylase Inhibition Activity

The α-amylase inhibitory activity of *Vitis vinifera* peel–derived AuNPs was evaluated using the starch–DNSA method. A starch solution (1% w/v) was prepared by dissolving 1 g of soluble starch in 100 mL of 20 mM phosphate buffer (pH 6.9) containing 6.7 mM sodium chloride. Porcine pancreatic α-amylase (27.5 mg) was dissolved in 100 mL of the same buffer to prepare the enzyme solution. To 100 µL of AuNP suspension at different concentrations (2, 4, 8, 10, and 15 µg/mL), 200 µL of α-amylase solution was added and incubated at 37 °C for 20 minutes. Subsequently, 100 µL of 1% starch solution was added and the mixture was further incubated at 37 °C for 10 minutes. The reaction was terminated by adding 200 µL of DNSA reagent (prepared by dissolving 1 g of 3,5-dinitrosalicylic acid, 30 g sodium potassium tartrate, and 20 mL of 2 N sodium hydroxide and making the volume up to 100 mL with distilled water), followed by heating in a boiling water bath for 5 minutes. After cooling, the reaction mixture was diluted with 2.2 mL of distilled water and absorbance was measured at 540 nm using a UV–Visible spectrophotometer. Blank samples were prepared by replacing the enzyme solution with distilled water, while the control contained all reagents except the AuNPs, representing 100% enzyme activity. Acarbose was used as a positive control. All experiments were performed in triplicate.

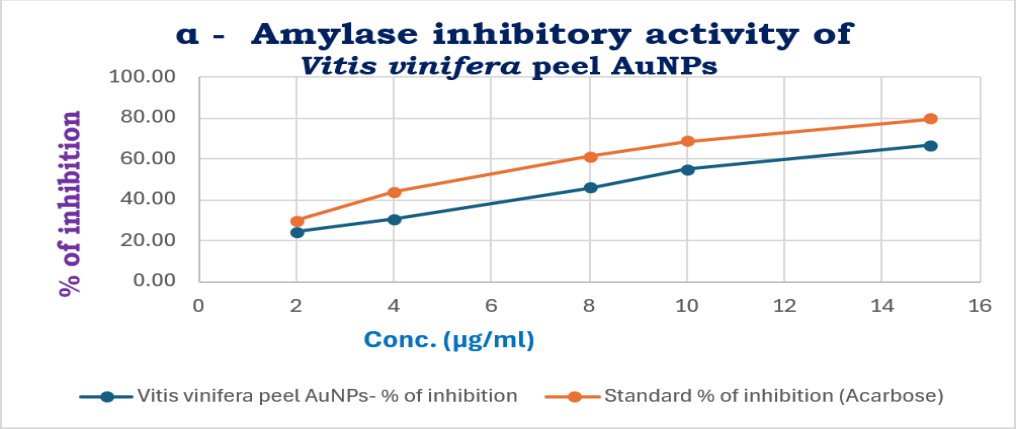
Table-11: α-Amylase Inhibition (%) OF Vitis Vinifera peel based AU-nanoparticles and acarbose at varying concentrations.

Conc. (µg/ml)	Vitis vinifera peel AuNPs % of inhibition	IC_{50}	Standard % of inhibition (Acarbose)	IC_{50}
2	24.30±0.45	9.44± 0.01	29.94±0.63	6.05± 0.08
4	30.57±0.18		43.98±0.30	
8	46.00±0.30		60.99±0.38	
10	54.91±0.31		68.56±0.25	
15	66.59±0.30		79.53±0.31	

*Each value is represented as mean ± SD (n=3).

CALCULATION

$$\% \text{ α-amylase inhibition} = \frac{(\text{Abs control} - \text{Abs sample})}{\text{Abs control}} \times 100$$



Graph 3: Vitis vinifera peel percentage of AuNps and Acarbose

Table- 12: Concentration-dependent α- glucosidase inhibitory activity (%) of V.v AuNps in comparison with acarbose.

Conc. (µg/ml)	Vitis vinifera peel AuNPs- % of inhibition	Standard % of inhibition (Acarbose)
2	24.30	29.94
4	30.57	43.98

8	46.00	60.99
10	54.91	68.56
15	66.59	79.53

Assay of α-Glucosidase Inhibition Activity

The α-glucosidase inhibitory activity of *Vitis vinifera* peel–derived AuNPs was assessed following a modified method described by Kim et al. (2011). α-Glucosidase enzyme (1 mg) was dissolved in 100 mL of phosphate buffer (pH 6.8). To 100 µL of AuNP suspension at various concentrations (2, 4, 8, 10, and 15 µg/mL), 200 µL of α-glucosidase solution was added and incubated at 37 °C for 20 minutes. The reaction was initiated by adding 100 µL of 3 mM p-nitrophenyl-α-D-glucopyranoside (p-NPG) and incubated for 10 minutes at 37 °C. The reaction was terminated by adding 2 mL of 0.1 M sodium carbonate solution. The release of p-nitrophenol was measured spectrophotometrically at 405 nm using a UV–Visible spectrophotometer (Shimadzu UV-1800). Acarbose was used as a standard inhibitor. The IC_{50} value was defined as the concentration of AuNPs required to inhibit 50% of enzyme activity under assay conditions. All experiments were carried out in triplicate.

Table-13: α-Glucosidase Inhibition assay showing OD values and percentage inhibition of biosynthesized nanoparticles at different concentrations.

		Sample OD values							
Control	**Sample1**	**Sample2**	**Sample3**	**NB**	**% of Inhibition**			**Mean**	**STD**
2	0.912	0.642	0.638	0.635	29.60526	30.04386	30.37281	30.01	0.39
4	0.912	0.528	0.525	0.522	42.10526	42.43421	42.76316	42.43	0.33
8	0.912	0.396	0.394	0.391	56.57895	56.79825	57.12719	56.83	0.28
10	0.912	0.302	0.298	0.296	66.88596	67.32456	67.54386	67.25	0.33
15	0.912	0.192	0.19	0.194	78.94737	79.16667	78.72807	78.95	0.22

Calculation

$$\% \text{ α-glucosidase inhibition} = \frac{(\text{Abs control} - \text{Abs sample})}{\text{Abs control}} \times 100$$

Glucose Uptake by Yeast Cells Method

The glucose uptake potential of *Vitis vinifera* peel–derived AuNPs was evaluated using a yeast cell model as described by Gupta et al. (2013). Commercial baker's yeast was washed repeatedly with distilled water and centrifuged at 3000 g for 5 minutes until a clear supernatant was obtained. A 10% (v/v) yeast suspension was prepared. AuNP samples at concentrations ranging from 10 to 50 µg/mL were added to 1 mL of glucose solutions (5, 10, and 20 mM) **Table-1,2,3** and incubated at 37 °C for 10 minutes. The experiment was initiated by adding 100 µL of yeast suspension, followed by vortexing and incubation at 37 °C for 60 minutes. After incubation, the reaction mixtures were centrifuged at 3000 rpm for 5 minutes, and the glucose content in the supernatant was determined spectrophotometrically. Metformin was used as a reference drug. All experiments were performed in triplicate.

Table-14: Assay of α-Glucosidase Inhibitory Activity of the test sample at different concentrations (10-50µg/mL) showing OD values, Percentage inhibition, Mean and standard deviation at 5Mm.

		Sample OD values							
Control	**Sample1**	**Sample2**	**Sample3**	**NB**	**% of Inhibition**			**Mean**	**STD**
10	0.615	0.558	0.555	0.553	9.268293	9.756098	10.0813	9.70	0.41
20	0.615	0.518	0.514	0.513	15.77236	16.42276	16.58537	16.26	0.43
30	0.615	0.482	0.481	0.485	21.62602	21.78862	21.13821	21.52	0.34
40	0.615	0.449	0.451	0.446	26.99187	26.66667	27.47967	27.05	0.41
50	0.615	0.42	0.419	0.421	31.70732	31.86992	31.54472	31.71	0.16

Table-15: Assay of α-Glucosidase Inhibitory Activity of the test sample at different concentrations (10-50µg/mL) showing OD values, Percentage inhibition, Mean and standard deviation at 10Mm.

		Sample OD values							
Control	**Sample1**	**Sample2**	**Sample3**	**NB**	**% of Inhibition**			**Mean**	**STD**
10	0.675	0.608	0.604	0.603	9.925926	10.51852	10.66667	10.37	0.39
20	0.675	0.554	0.551	0.556	17.92593	18.37037	17.62963	17.98	0.37
30	0.675	0.502	0.506	0.501	25.62963	25.03704	25.77778	25.48	0.39
40	0.675	0.463	0.46	0.458	31.40741	31.85185	32.14815	31.80	0.37
50	0.675	0.433	0.427	0.426	35.85185	36.74074	36.88889	36.49	0.56

Table-16: Assay of α-Glucosidase Inhibitory Activity of the test sample at different concentrations (10-50µg/mL) showing OD values, Percentage inhibition, Mean and standard deviation at 20Mm.

		Sample OD values							
Control	**Sample1**	**Sample2**	**Sample3**	**NB**	**% of Inhibition**			**Mean**	**STD**
10	0.759	0.701	0.699	0.704	7.641634	7.905138	7.246377	7.60	0.33
20	0.759	0.641	0.644	0.646	15.54677	15.15152	14.88801	15.20	0.33
30	0.759	0.587	0.585	0.584	22.6614	22.9249	23.05665	22.88	0.20
40	0.759	0.547	0.543	0.548	27.93149	28.4585	27.79974	28.06	0.35
50	0.759	0.508	0.506	0.503	33.06983	33.33333	33.72859	33.38	0.33

Calculation

$$\% \text{ increase in glucose uptake} = \frac{(\text{Abs control} - \text{Abs sample})}{\text{Abs control}} \times 100$$

where *Abs control* represents the absorbance of the control reaction containing all reagents except the test sample, and *Abs sample* represents the absorbance in the presence of AuNPs

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