

GREEN SYNTHESIS AND CHARACTERIZATION OF SILVER NANOPARTICLES FROM *MILLINGTONIA HORTENSIS* AND THEIR ANTIDIABETIC AND ANTIOXIDANT EFFICACY

ABSTRACT

The current study focuses on the green production of silver nanoparticles utilising an ethanolic extract of *Millingtonia hortensis* leaves. This medicinal plant was high in the phenol and flavonoids classes of chemicals. They converted silver nitrate into silver nanoparticles (AgNPs), which were then characterised by FT-IR, particle size analysis, and Zeta potential. The FT-IR analysis confirmed the existence of several functional groups around AgNPs. The particle size examination revealed that the synthesised silver nanoparticles were spherical in shape and nano in size. The zeta potential of AgNPs was determined to be -14 mV; this negative value validates particle repulsion and so increases the formulation's stability. The antioxidant and anti-diabetic properties of AgNPs were investigated, and the results revealed significant free radical scavenging ability, inhibition of carbohydrate digesting enzymes (α -Amylase and α -glucosidase). The green synthesized silver nanoparticles were found to be a promising phyto-medicine for the treatment of diabetes.

Keywords: Antidiabetic activity; Antioxidant activity; Green synthesis; phytochemical studies; silver nanoparticle.

INTRODUCTION

A significant area of research now is nanomedicine. Scientists are primarily focused on developing safe, efficient, and most importantly, less expensive and hazardous medications to treat diseases like diabetes, cancer, epilepsy, etc. Due to the site-specific actions of these nanoparticles, only a safe and recommended dosage of medication molecules must be supplied, aiding in the reduction of unintended toxicity. These nanoparticles boost the drug's effectiveness because of their focused activity. Their little size gives them an advantage since it allows them to dodge immunological responses and get through membranes that are often impervious. Since chemical synthesis requires the reduction of metals using numerous toxic agents, green synthesis of nanoparticles is favoured. Plants naturally have both primary and secondary metabolites to perform green synthesis. Green methods of synthesising silver nanoparticles are widely employed in industry as well as for a variety of uses, including anti-diabetic, anti-microbial, antioxidant, and anti-cancer applications¹⁻⁵.

Diabetes is a chronic illness that is brought on by a mix of inherited and environmental factors that result in abnormally high blood sugar levels. Both wealthy and underdeveloped nations struggle with this serious health issue. Although there are several treatments for diabetes, they do not completely cure the condition and have a host of negative side effects. The world is looking for new anti-diabetic drugs from plant sources with fewer side effects, since many plants and vegetables have been evaluated and proven for their antidiabetic actions in animal models. One of the most prominent treatment methods to lower

high blood sugar levels by preventing the intake of glucose is the identification of inhibitors for carbohydrate-hydrolyzing enzymes like α -amylase and α -glucosidase. Numerous studies have been conducted to find substances that can inhibit the carbohydrate-hydrolyzing enzymes. As a result of advances in nanotechnology, α -glucosidase and α -amylase enzyme inhibitors for the treatment of diabetes were successfully made from natural materials as silver nanoparticles⁶.

A huge ornamental tree from Southern Asia, *Millingtonia hortensis* Linn, is grown in many parts of India and is a member of the Bignoniaceae family. Antifungal, antibacterial, Larvicidal, hepatoprotective, and antioxidant properties of *Millingtonia hortensis* are employed. In traditional medicine, the leaves of *M. hortensis* are used as an antipyretic, sinusitis, cholagogue, and tonic. These plants have yielded a number of isolated substances, including phenols, terpenoids, saponins, flavonoids, and glycosides. The current study focuses on the synthesis, characterisation, and antidiabetic action of AgNPs made from an ethanolic leaf extract of *Millingtonia hortensis* by using FTIR, UV-vis spectroscopy, particle size analysis, and zeta potential, For the current investigation, antidiabetic targets such α -amylase, α -glucosidase, and free radical inhibition were used as a model⁷⁻¹¹.

MATERIALS AND METHODS

COLLECTION OF PLANT

The leaves of *Millingtonia hortensis* was collected from Hyderabad district, telangana in the month of October and was authenticated by botanist Harikrishna from Osmania university. The leaves were dried under shade at room temperature for about 20 days and coarsely powdered in a mixer grinder. The powdered material was stored or taken up for extraction process.

PREPARATION OF PLANT EXTRACT

A beaker containing 20 grammes of *Millingtonia hortensis* dry leaf powder was filled with 200 ml of ethanol, sealed with aluminium foil, and left to stand for 24 hours. During that time, it was periodically shaken to help the ethanol-soluble compounds dissolve, and the mixture was then filtered. The filtrate product will now be evaporated at room temperature for 4 to 5 days. Scrape out the resulting extract when the filter has completely evaporated and measure it¹².

GREEN SYNTHESIS OF AgNPs

The leaves of *Millingtonia hortensis* were used to make a plant extract. To remove dust and other contaminants prior to extract processing, leaves were first washed with tap water and then with distilled water. Using a grinder, shade-dried leaves were ground into powder. 200 ml of ethanol and 8 g of powder were mixed together, then the mixture was heated at 60 °C for 15 minutes. cooled and Whatman filter paper No. 1 was used to filter the supernatant. The extract was made into a clear light green solution that was kept between 4 and 10 °C. 0.0225 gramme of silver nitrate was dissolved in 75 ml of distilled water to create a 2 mM solution. Then, 100 ml of leaf extract was combined with 50 ml of silver nitrate solution. The creation of silver nanoparticles was indicated by a change in colour from green to a colloidal yellowish green black colour. After 30 minutes, 100 mL of the reaction mixture was centrifuged at 5000 rpm for 5 minutes. The sediment's solid components were collected, cleaned with distilled water, then dried at room temperature after being rinsed with ethanol¹³.

CHARACTERIZATION OF AgNPs

Silver nanoparticle of *Millingtonia hortensis* leaves was prepared and was characterized to determine the biochemical properties. FTIR, Particle size and zeta potential used to characterize the NanoParticles of *Millingtonia hortensis*.

IN VITRO ANTIDIABETIC ACTIVITY

ALPHA AMYLASE INHIBITION ASSAY

PROCEDURE:

Alpha-amylase activity was carried out by starch-iodine method. 10 μ L of α -amylase solution (0.025 mg/mL) was mixed with 390 μ L of phosphate buffer (0.02 M containing 0.006 M NaCl, pH 7.0) containing different concentration of extracts. After incubation at 37 °C for 10 min, 100 μ L of starch solution (1%) was added, and the mixture was re-incubated for 1 h. Next, 0.1 mL of 1% iodine solution was added and after adding 5 mL distilled water, the absorbance was taken at 565 nm. Sample, substrate and α -amylase blank determinations were carried out under the same reaction conditions^{14,15}.

ALPHA GLUCOSIDASE INHIBITION ASSAY

PROCEDURE:

Plant extract is added to 50 μ l of 0.1 M phosphate buffer containing 0.2 μ /ml of alpha glucosidase solution for 5 μ l of all test concentration. To the standard tubes add all the reagents which are adding in the test except the plant extract. Nanoparticles are added to 50 μ l of 0.1 M phosphate buffer containing 0.2 μ /ml of alpha glucosidase solution for all different concentration. Incubate both test, standard and nanoparticle at 37 ° C for 20 min. After pre incubation 50 μ l of 5mm 4-Nitrophenyl-beta-D-glucopyranoside (P-NPG) will added to each test tube (extract, standard and nanoparticles). Incubate at 37°C for another 20 min. Then the reaction was stopped by adding 160 μ l of 0.2M Na₂CO₃ in each test tube. Measure the absorbance at 405 nm, pink colour was observed and calculates percentage inhibition and IC₅₀^{16,17}.

INVITRO ANTIOXIDANT ACTIVITY:

FERRIC REDUCING POWER ASSAY (FRAP)

PROCEDURE:

To 1 mL of test (EEMH, NPMH) and standard compounds, add 2.5 mL of potassium ferricyanide (1% w/v), 2.5 mL of phosphate buffer pH 6.6, and incubate at 50°C for 30 minutes. 2.5 mL of the aforementioned supernatant liquid was mixed with 2.5 mL of distilled water and 0.5 mL of FeCl₃ solution (0.1% w/v). The absorbance of ferric ferrous complex was measured at 700 nm using a UV-Visible spectrophotometer with phosphate buffer pH 6.6 as a control and the increase in absorbance was estimated. The present increase in reducing power was calculated using the following equation,

$$\text{reducing power (\%)} = \frac{\text{Abs test} - \text{Abs blank}}{\text{Abs blank}} * 100$$

Where 'Abs test' is absorbance of test solution: 'Abs blank' is absorbance of blank^{18,19}.

NITRIC OXIDE SCAVENGING ASSAY

PROCEDURE:

Extract, nanoparticles prepared in ethanol, was added to different test-tubes in varying concentrations (10, 20, 40,60,80 μ g/mL). Sodium nitroprusside (5mM) in phosphate buffer was added to each test tube to make volume up to 1.5ml. Solutions were incubated at 25°C for 30 minutes. Thereafter, 1.5ml of Griess reagent (1% sulphanilamide, 0.1% Naphthyl ethylenediamine dichloride and 3% phosphoric acid was added to each test tube. The absorbance was measured, immediately at 546nm and percentage of scavenging activity was measured with reference to ascorbic acid as standard and IC₅₀ were calculated^{20,21}.

RESULTS AND DISCUSSION

RESULTS

In vitro antioxidant and antidiabetic activity of green synthesized silver nanoparticle of *Millingtonia hortensis* was explored for its antidiabetic activities. All the results obtained in the studied were included below.

PRELIMINARY PHYTOCHEMICAL ANALYSIS

The preliminary phytochemical investigation of EEMH extract of *Millingtonia hortensis* leaves showed the presence of phenolic compounds, glycosides, flavonoids, terpenoids, tannins, steroids etc.

Table 1. Preliminary Phytochemical analysis

Phytochemical Constituents	Results
Flavonoids	++
Phenols	++
Terpenoids	++
Tannins	++
Glycosides	++

Note: ++ indicates present; - indicates absent.

INVITRO ANTIDIABETIC ASSAY

The EEMH and NPMH was subjected to invitro antidiabetic assay. In vitro antidiabetic assay was performed using α - amylase and α - glucosidase.

α - AMYLASE INHIBITORY ASSAY:

α - AMYLASE INHIBITORY ASSAY ACARBOSE, EEMH AND NPMH:

Table 2: α - amylase inhibitory assay of Acarbose, EEMH AND NPMH

S.No	Compounds	Concentration (μ g/mL)	% inhibition	IC ₅₀ values
1.	Acarbose	10	6.8	39.5
		20	27.5	
		40	37.9	
		60	55.17	
		80	93.1	
2.	Ethanollic extract of <i>Millingtonia hortensis</i> (EEMH)	10	10.3	44.6
		20	20.6	
		40	34.4	
		60	44.8	
		80	79.3	

3.	Nanoparticle of	10	10.3	41.49
	<i>Millingtonia hortensis</i>	20	34.4	
	(NPMH)	40	44.8	
		60	48.2	
		80	75.8	

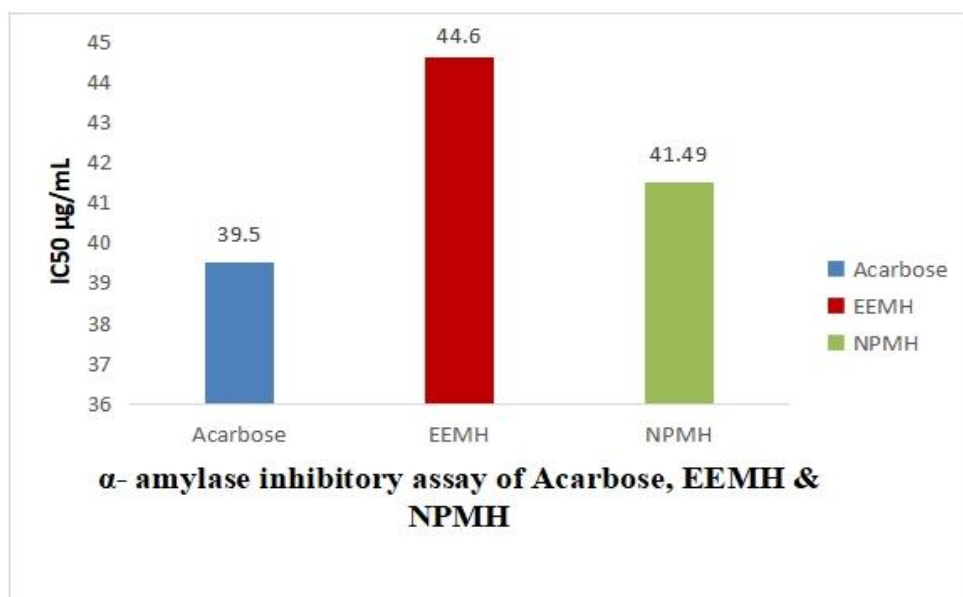


Fig 1: α-amylase inhibitory assay of Acarbose, EEMH & NPMH

α-amylase inhibitory assay, IC₅₀ values of standard drug, EEMH and NPMH was found to be 39.5, 44.6 and 41.49 µg/ml respectively. From the above result it is clear that NPMH showed good antidiabetic activity. The results were expressed in the table 2 and the profile was expressed in the figure 1.

α- GLUCOSIDASE INHIBITORY ASSAY:

α- GLUCOSIDASE INHIBITORY ASSAY ACARBOSE, EEMH AND NPMH:

Table 3: α- glucosidase inhibitory assay of Acarbose, EEMH AND NPMH

S.No	Compounds	Concentration (ug/mL)	% inhibition	IC ₅₀ values
1.	Acarbose	10	14.2	31.5
		20	33.3	
		40	47.6	
		60	52.3	
		80	85.7	
2.	Ethanollic extract of	10	14.2	39.3

	<i>Millingtonia hortensis</i>	20	33.3	
	(EEMH)	40	38.09	
		60	66.6	
		80	90.4	
	Nanoparticle of	10	28.5	35.04
3.	<i>Millingtonia hortensis</i>	20	33.3	
	(NPMH)	40	42.8	
		60	61.9	
		80	90.4	

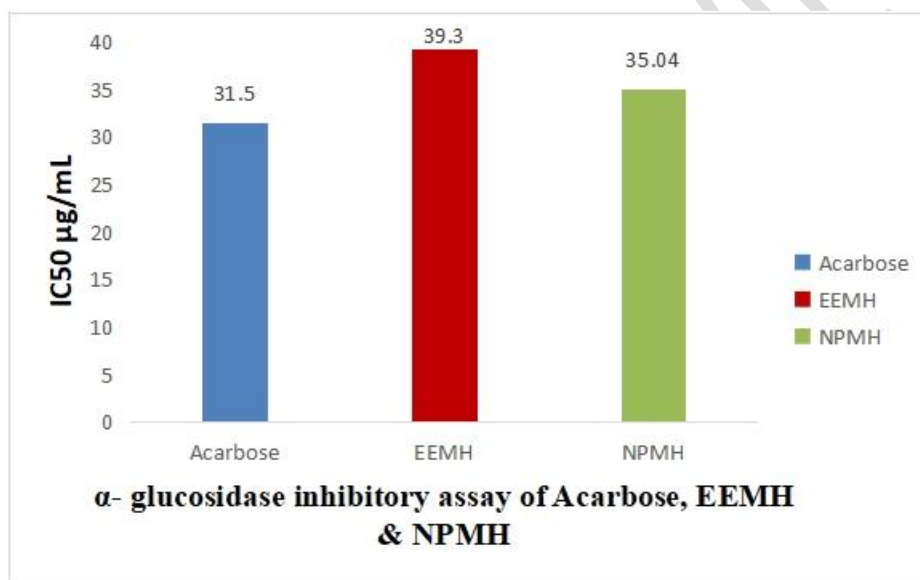


Fig 2: α -glucosidase inhibitory assay of Acarbose, EEMH & NPMH

α -glucosidase inhibitory assay, IC₅₀ values of standard drug, EEMH and NPMH was found to be 31.5, 39.3 and 35.04 μ g/ml respectively. From the above result it is clear that NPMH showed good antidiabetic activity. The results were expressed in the table 3 and the profile was expressed in the figure 2.

INVITRO ANTIOXIDANT ACTIVITY:

The EEMH and NPMH was subjected to invitro antioxidant assay. In vitro antioxidant assay was performed using FRAP and nitric oxide scavenging assay.

FRAP ASSAY OF ASCORBIC ACID, EEMH AND NPMH

Table 4: FRAP assay of Ascorbic acid, EEMH & NPMH

S.No	Compounds	Concentration ($\mu\text{g/mL}$)	% inhibition	IC_{50} values
1.	Ascorbic acid	10	25	24.03
		20	41	
		40	58	
		60	75	
		80	100	
2.	Ethanollic extract of <i>Millingtonia hortensis</i> (EEMH)	10	16.6	48.07
		20	33.3	
		40	41.6	
		60	66.6	
		80	91.6	
3.	Nanoparticle of <i>Millingtonia hortensis</i> (NPMH)	10	16.6	30.3
		20	33.3	
		40	75	
		60	91.6	
		80	100	

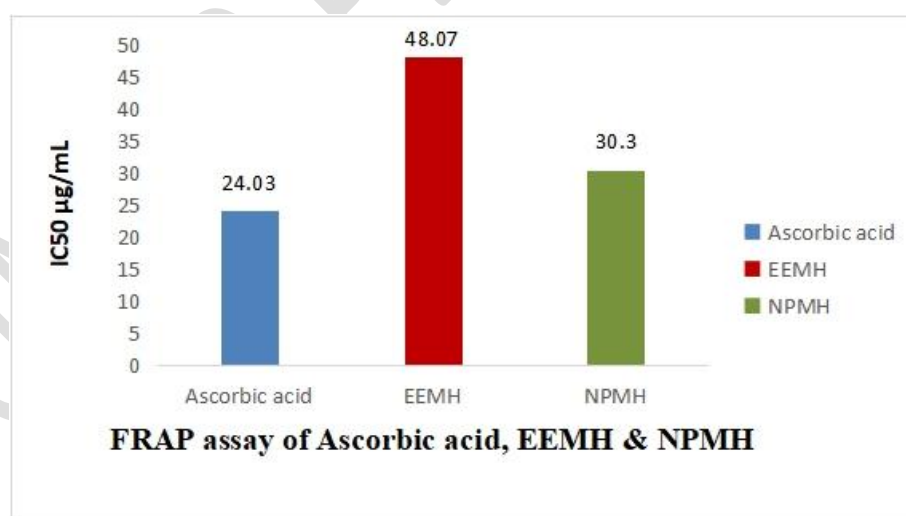


Fig 3: FRAP assay of Ascorbic acid, EEMH & NPMH

FRAP assay, IC_{50} values of standard drug, EEMH and NPMH was found to be 24.03, 48.07 and 30.3 $\mu\text{g/ml}$ respectively. From the above result it is clear that NPMH showed good antioxidant activity. The results were expressed in the table 4 and the profile was expressed in the figure 3.

NITRIC OXIDE SCAVENGING ASSAY

NITRIC OXIDE SCAVENGING ASSAY ASCORBIC ACID, EEMH AND NPMH

Table5: NO scavenging assay of Ascorbic acid, EEMH and NPMH

S.No	Compounds	Concentration (ug/mL)	% inhibition	IC ₅₀ values
1.	Ascorbic acid	10	31.5	23.7
		20	42.1	
		40	73.6	
		60	89.4	
		80	100	
2.	Ethanolic extract of <i>Millingtonia hortensis</i> (EEMH)	10	26.3	31.7
		20	31.5	
		40	52.6	
		60	68.4	
		80	89.4	
3.	Nanoparticle of <i>Millingtonia hortensis</i> (NPMH)	10	26.3	27.1
		20	36.8	
		40	57.8	
		60	68.4	
		80	94.7	

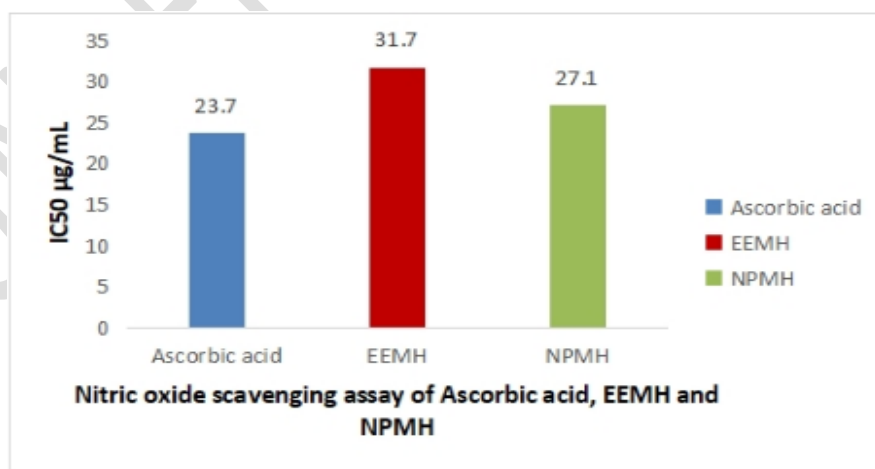


Fig 4: NO scavenging assay of Ascorbic acid, EEMH & NPMH

Nitric oxide radical scavenging assay, IC_{50} values of standard drug, EEMH and NPMH was found to be 23.7, 31.7 and 27.1 $\mu\text{g/ml}$ respectively. From the above result it is clear that NPMH showed good antioxidant activity. The results were expressed in the table 5 and the profile was expressed in the figure 4.

FT-IR SPECTROSCOPIC ANALYSIS

FT-IR measurements were performed to identify the potential biomolecules in the leaf of *Millingtonia hortensis* responsible for the formation of silver nanoparticles. The FT-IR spectrum of ethanolic leaf extract of *Millingtonia hortensis* before reaction, showed several absorption peaks at 3404, 2924, 2853, 2288, 1971, 1873, 1604, 1371, 1166, 1065, 863 cm^{-1} . The FT-IR spectrum of silver nanoparticles showed absorbance peak at 3381, 2926, 2853, 2359, 2336, 2272, 1983, 1250, 1087, 734 cm^{-1} . represent O-H stretching (alcohol, amides), C-H stretching (alkane, ether), O-H stretching (carboxylic acid), C-H bending (aromatic compounds), C=C=C (allene), C=C (conjugated alkene), O-H bending (phenol), C-O stretching (ester), C-N stretch (amine), C-Cl stretch (halo compounds) An analysis of the plant extract using FTIR measurements confirmed the presence of active biomolecules that acted as capping and reducing agents. functional groups present in sample might be responsible for bioreduction of Ag^+ to Ag nanoparticle.

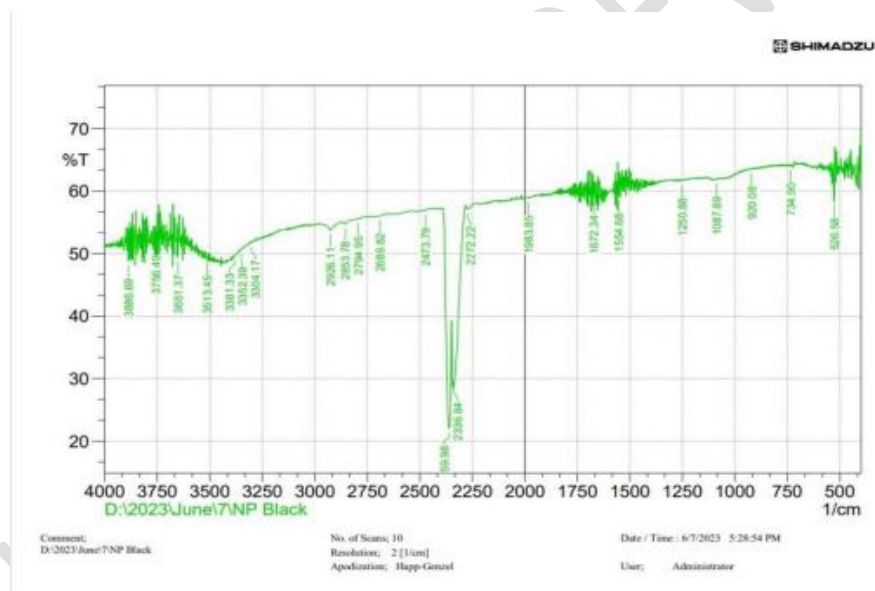


Fig 5: FT-IR Spectrum of synthesized silver nanoparticles using leaf extract of *Millingtonia hortensis*

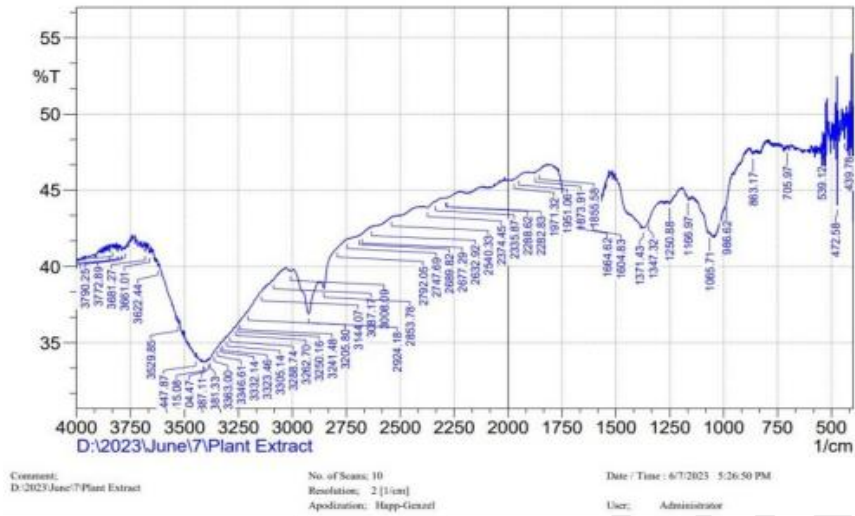


Fig 6:FT-IR Spectrum of *Millingtonia hortensis* leaves extract

PARTICLE SIZE AND ZETAPOTENTIAL

NPMH:

Particle size and zeta potential for nanoparticle of *Millingtonia hortensis* was found to be 237nm and -14.4 mV respectively. Zeta potential shows the charge on the particle surface which indicates the physical stability of the dispersed systems. A high zeta potential will confer stability of the preparation that will resist aggregation.

Sample Details

Sample Name: nifloglona bacterias 1
SOP Name: nifloglona bacterias size.asp
General Notes:
File Name: nifloglona bacterias alc...
Record Number: 1
Material ID: 1.59
Material Absc: 0.010
Dispersant Name: Water
Dispersant RH: 1.330
Viscosity (cP): 0.8872
Measurement Date and Time: Sunday, January 01, 2012

System

Temperature (°C): 25.0
Count Rate (kcps): 11.4
Cell Description: Disposable sizing cuvette
Duration Used (s): 50
Measurement Position (mm): 4.65
Attenuator: 11

Results

Z-Average (d.nm)	Peak 1	Peak 2	Peak 3	Size (d.nm)	% Intensity	St Dev (d.nm)
237.9	122.9	463.2	544.6	122.9	65.0	30.07
PDI: 0.823				463.2	30.3	106.9
Intercept: 0.704				544.6	4.8	270.6

Result quality Refer to quality report



Fig 7: ParticlesizeofNPMH

Zeta Potential Report v2.3



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Sample Details

Sample Name: malingorea horfensis zeta 3
SOP Name: malingorea horfensis zeta.sop
General Notes:

File Name: malingorea horfensis zeta.dts Dispersant Name: Water
Record Number: 3 Dispersant RH: 1.300
Date and Time: Sunday, January 01, 2012 12:00:00 Viscosity (cP): 0.8672
Dispersant Dielectric Constant: 78.5

System

Temperature (°C): 25.0 Zeta Runs: 23
Count Rate (kcps): 108.8 Measurement Position (mm): 2.00
Cell Description: Clear disposable zeta cell Attenuator: 7

Results

	Mean (mV)	Area (%)	St Dev (mV)
Zeta Potential (mV): -14.4	Peak 1: -38.2	41.1	5.80
Zeta Deviation (mV): 50.8	Peak 2: -19.8	22.6	4.46
Conductivity (mS/cm): 0.119	Peak 3: -7.90	19.5	5.00

Result quality [See result quality report](#)

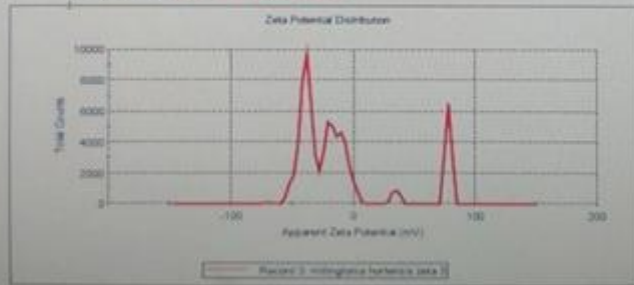


Fig 8: Zeta potential of NPMH

CONCLUSIONS

The current research focused on the green synthesis of silver nanoparticles utilising *Millingtonia hortensis* and the assessment of its anti-diabetic effect by blocking carbohydrate hydrolyzing enzymes such as α -amylase and α -glucosidase. Furthermore, it demonstrated high antioxidant activity, which will scavenge free radicals generated by hyperglycemia. The results of FT-IR, particle size, and zeta potential examination revealed that the synthesised particles were nano in size and spherical in form, and were attached by the functional groups of metabolites extracted from *Millingtonia hortensis*. According to the findings, green synthesised silver nanoparticles from *Millingtonia hortensis* show antioxidant and anti-diabetic properties. As a result, the synthesised nanoparticles will be an effective therapeutic agent for diabetes treatment by inhibiting carbohydrate hydrolyzing enzymes. More research is needed to clarify its specific mechanism of action in animal and human models before this medication may be recommended as a therapeutic drug for the treatment of diabetes.

UNDER PEER REVIEW

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