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Green Synthesis and Characterization of Silver Nanoparticles from *Millingtonia hortensis* and Their Antidiabetic and Antioxidant Efficacy

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Authors' contributions

This work was carried out in collaboration among all authors. The concept was designed by authors SKT, NVLSRV and GRM. The idea was executed by authors PS, SS, SLS and SJ. It was supervised by author SKT. The manuscript was written by author SKT. It was corrected and proof read by authors NVLSRV and GRM. All authors read and approved the final manuscript.

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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

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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.

1. 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 applications [1-5].

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 nanoparticles [6].

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 model [7-11].

2. MATERIALS AND METHODS

2.1 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.

2.2 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 it [12].

2.3 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 ethanol [13].

2.4 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 Nano Particles of *Millingtonia hortensis*.

2.5 *In vitro* Antidiabetic Activity

2.5.1 Alpha amylase inhibition assay

Procedure:

"Alpha-amylase activity was carried out by starch-iodine method. 10 µL of α-amylase solution (0.025 mg/mL) was mixed with 390 µL 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 µL 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].

2.5.2 Alpha glucosidase inhibition assay

Procedure:

Plant extract is added to 50µl of 0.1 M phosphate buffer containing 0.2µ/ml of alpha glucosidase solution for5µl 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 50µl of 0.1 M phosphate buffer containing 0.2µ/ml of alpha glucosidase solution for all different concentration. Incubate both test, standard and nanoparticle at 37 ^{o} C for 20 min. After pre incubation 50µl 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 160µl of 0.2M Na2Co3 in each test tube. Measure the absorbance at 405 nm, pink colour was observed and calculates percentage inhibition and IC_{50} [16,17].

2.6 *In vitro* Antioxidant Activity

2.6.1 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^{o}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, reducing power (%) =Abs test – Abs blank /Abs blank * 100

Where 'Abs test' is absorbance of test solution: 'Abs blank' is absorbance of blank [18,19].

2.6.2 Nitric oxide scavenging assay

Procedure:

"Extract, nanoparticles prepared in ethanol, was added to different test-tubes in varying concentrations (10, 20, 40,60,80 µg/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^{o}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 IC_{50} were calculated" [20,21].

3. RESULTS AND DISCUSSION

3.1 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.

3.1.1 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.

3.1.2 *In vitro* antidiabetic assay

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

Table 1. Preliminary phytochemical analysis

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

Note: ++ indicates present; - indicates absent.

3.2 α- Amylase Inhibitory Assay

3.2.1 α- amylase inhibitory assay acarbose, EEMH and NPMH

α-amylase inhibitory assay, IC_{50} 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 Fig. 1.

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

S.No	Compounds	Concentration (ug/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.	Ethanolic extract of *Millingtonia hortensis* (EEMH)	10	10.3	44.6
		20	20.6	
		40	34.4	
		60	44.8	
		80	79.3	
3.	Nanoparticle of *Millingtonia hortensis* (NPMH)	10	10.3	41.49
		20	34.4	
		40	44.8	
		60	48.2	
		80	75.8	

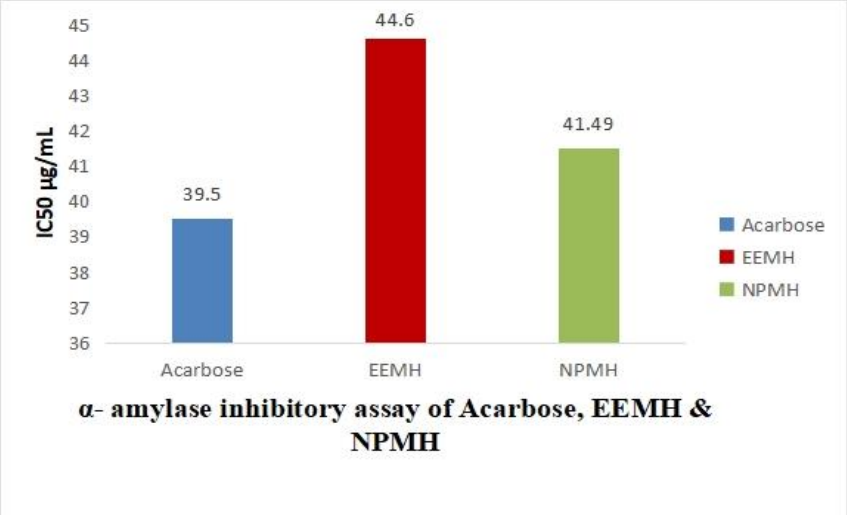

Fig. 1. α-amylase inhibitory assay of Acarbose, EEMH & NPMH

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.	Ethanolic extract of *Millingtonia hortensis* (EEMH)	10	14.2	39.3
		20	33.3	
		40	38.09	
		60	66.6	
		80	90.4	
3.	Nanoparticle of *Millingtonia hortensis* (NPMH)	10	28.5	35.04
		20	33.3	
		40	42.8	
		60	61.9	
		80	90.4	

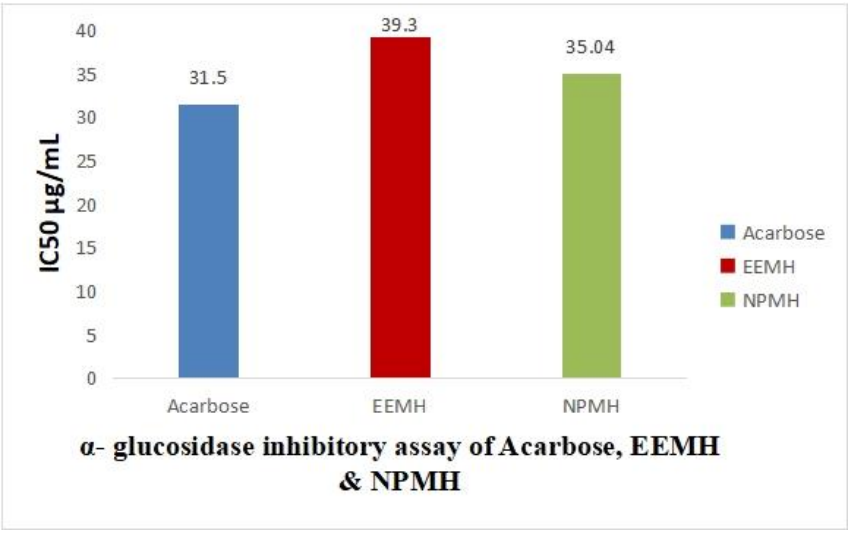

Fig. 2. α- glucosidase inhibitory assay of Acarbose, EEMH & NPMH

3.3 α- Glucosidase Inhibitory Assay

3.3.1 α- glucosidase inhibitory assay acarbose, EEMH AND NPMH

α-glucosidase inhibitory assay, IC_{50} values of standard drug, EEMH and NPMH was found to be 31.5, 39.3 and 35.04 µg/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 Fig. 2.

Frap assay of ascorbic acid, EEMH and NPMH

3.4 *In vitro* 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.

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

S.No	Compounds	Concentration (ug/mL)	% inhibition	IC_{50} values
1.	Ascorbic acid	10	25	24.03
		20	41	
		40	58	
		60	75	
		80	100	
2.	Ethanolic extract of *Millingtonia hortensis* (EEMH)	10	16.6	48.07
		20	33.3	
		40	41.6	
		60	66.6	
		80	91.6	
3.	Nanoparticle of *Millingtonia hortensis* (NPMH)	10	16.6	30.3
		20	33.3	
		40	75	
		60	91.6	
		80	100	

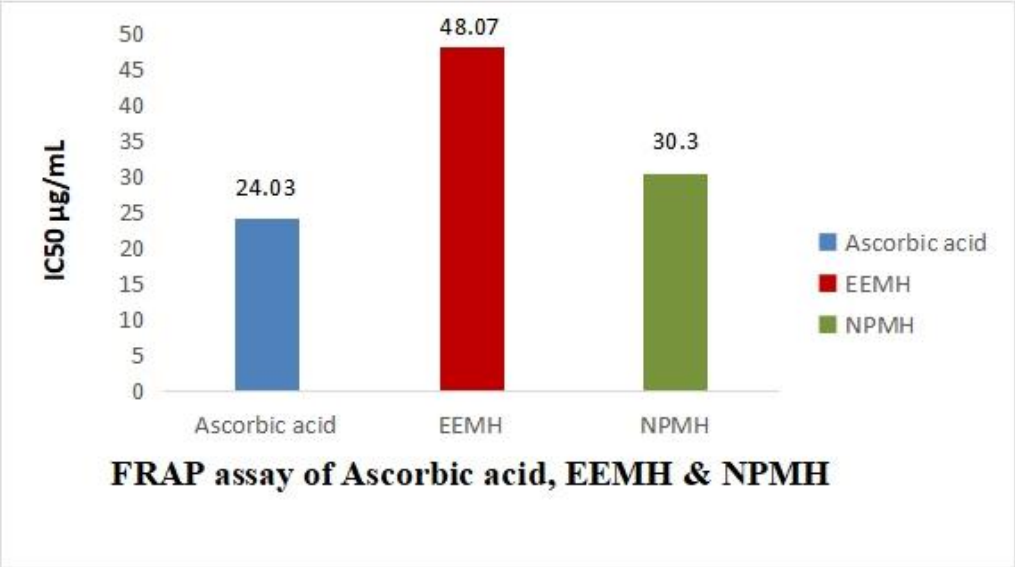

Fig. 3. FRAP assay of Ascorbic acid, EEMH & NPMH

FRAP assay, IC_{50} values of standard drug, EEMH and NPMH was found to be 24.03, 48.07 and 30.3 µg/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 Fig. 3.

3.5 Nitric Oxide Scavenging Assay

3.5.1 Nitric oxide scavenging assay ascorbic acid, EEMH and NPMH

Nitric oxide radical scavenging assay, IC_{50} values of standard drug, EEMH and NPMH was found to be 23.7, 31.7 and 27.1µg/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 Fig. 4.

3.5.2 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-H4stretching (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 bio reduction of Ag^{+} to Ag nanoparticle.

3.6 Particle Size and Zetapotential

NPMH:

Particle size and zetapotential 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.

Table 5. 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 *Millingtonia hortensis* (EEMH)	10	26.3	31.7
		20	31.5	
		40	52.6	
		60	68.4	
		80	89.4	
3.	Nanoparticle of *Millingtonia hortensis* (NPMH)	10	26.3	27.1
		20	36.8	
		40	57.8	
		60	68.4	
		80	94.7	

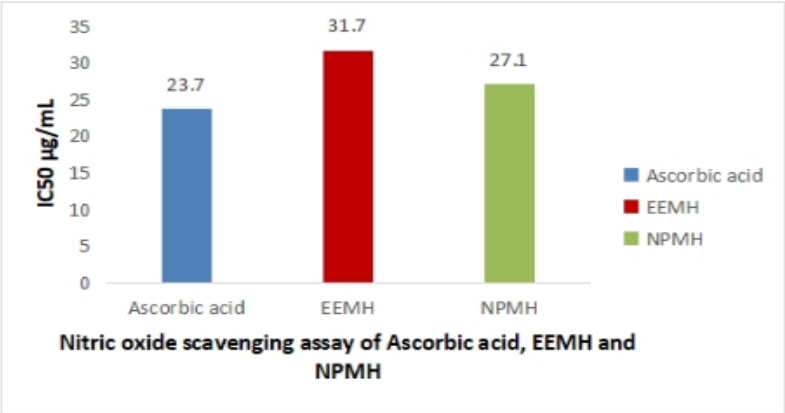

Fig. 4. No scavenging assay of Ascorbic acid, EEMH & NPMH

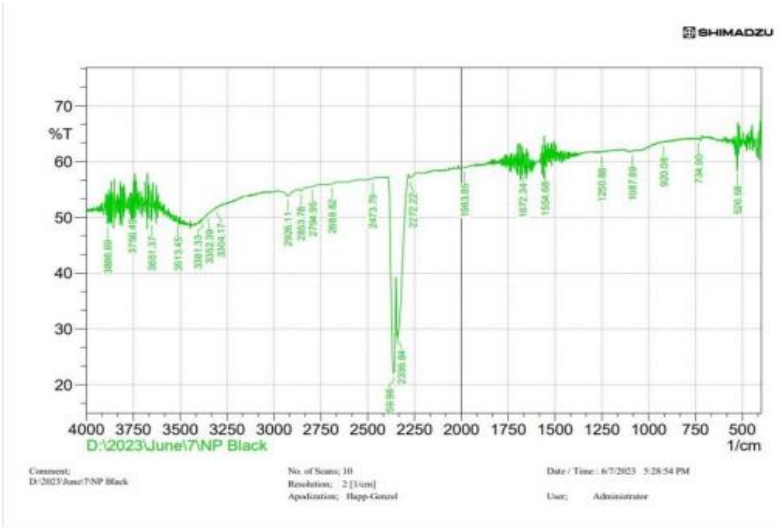

Fig. 5. FT-IR Spectrum of synthesized silver nanoparticles using leaves extract of *Millingtonia hortensis*

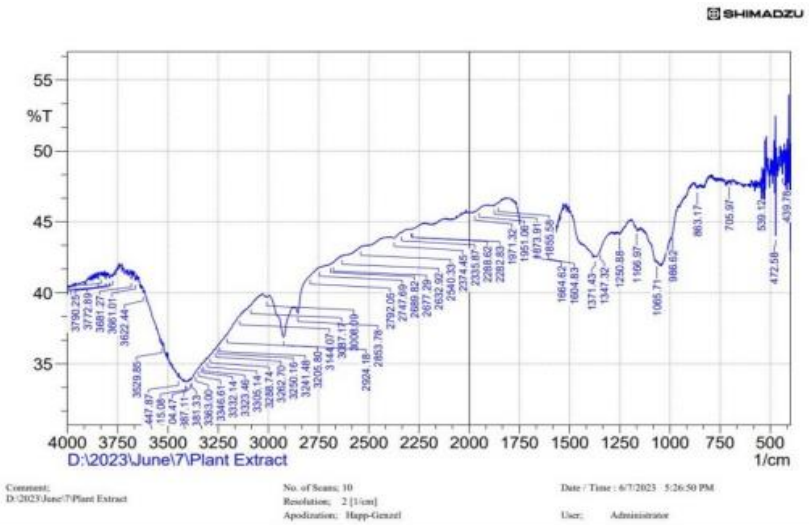

Fig. 6. FT-IR Spectrum of *Millingtonia hortensis* leaves extract

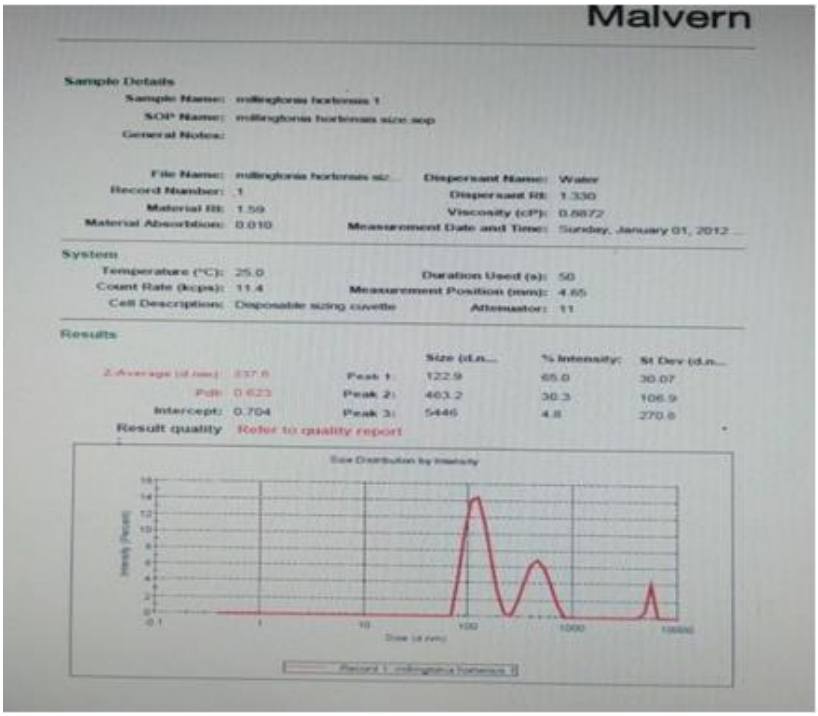

Fig. 7. Particle size of NPMH

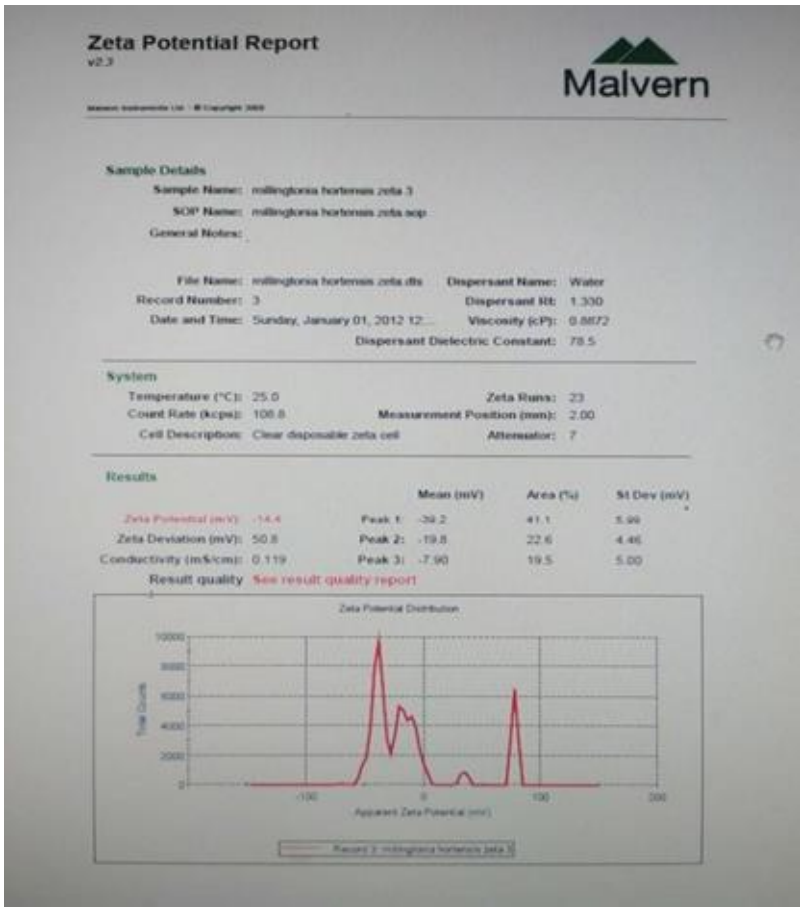

Fig. 8. Zeta potential of NPMH

4. 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.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Balan K, Qing W, Wang Y, Liu X, Palvannan T, Wang Y, Ma F, Zhang Y. Antidiabetic activity of silver nanoparticles from green synthesis using Lonicera japonica leaf extract. Rsc Advances. 2016;6(46):40162-8.
2. Rajaram K, Aiswarya DC, Sureshkumar P. Green synthesis of silver nanoparticle using Tephrosia tinctoria and its antidiabetic activity. Materials Letters. 2015;138:251-4.
3. Bal KE, Bal Y, Cote G, Chagnes A. Morphology and antimicrobial properties of Luffa cylindrica fibers/chitosan biomaterial as micro-reservoirs for silver delivery. Materials Letters. 2012;79:238-41.
4. Velavan S, Arivoli P, Mahadevan K. Biological reduction of silver nanoparticles using Cassia auriculata flower extract and evaluation of their in vitro antioxidant activities. Nanosci Nanotechnol Int J. 2012;2(4):30-5.
5. Gajendran B, Chinnasamy A, Durai P, Raman J, Ramar M. Biosynthesis and characterization of silver nanoparticles from *Datura inoxia* and its apoptotic effect on human breast cancer cell line MCF7. Materials Letters. 2014;122:98-102.
6. Jini D, Sharmila S. Green synthesis of silver nanoparticles from Allium cepa and its in vitro antidiabetic activity. Materials Today: Proceedings. 2020;22:432-8.
7. Sharma M, Puri S &Sharma PD. Antifungal activity of *Millingtonia hortensis*. Indian J. Pharm. Sci. 2007;69(4):599-601.
8. Jetty A &Iyengar DS. Antimicrobial activity of *Millingtonia hortensis* leaf extract. Pharma. Biotechnol. 2000;38(2):157-160.
9. Kaushik R, Sani P. Larvicidal activity of leaf extract of *Millingtonia hortensis* (Family: Bignoniaceae) against *Anopheles stephensi*, *Culex quinque fasciatus* and *Aedes aegypti*. J. Vector. Borne. Dis. 2008;45:66–69.
10. Babitha S, Banji D, Banji OJF. Antioxidant and hepatoprotective effects of flower extract of *Millingtonia hortensis* Linn. on carbon tetrachloride induced hepatotoxicity. J. Pharm. Bioallied Sci. 2012;4(4):307-312.
11. Janaki A, Kaleena PK, Elumalai D, Hemalatha P, Babu M, Velu K, Sudha R. Phytochemical screening antioxidant and antibacterial activities of Millingtonia Hortensis. Int. J. Curr. Pharm. 2017; 9(5):162-167.
12. Ghomari O, Sounni F, Massaoudi Y, Ghanam J, Kaitouni LB, Merzouki M, Benlemlih M. Phenolic profile (HPLC-UV) of olive leaves according to extraction procedure and assessment of antibacterial activity. Biotechnology Reports. 2019;23:e00347.
13. Kumar V, Singh S, Srivastava B, Bhadouria R, Singh R. Green synthesis of silver nanoparticles using leaf extract of Holoptelea integrifolia and preliminary investigation of its antioxidant, anti-inflammatory, antidiabetic and antibacterial activities. Journal of Environmental Chemical Engineering. 2019;7(3):103094.
14. Poovitha S, Parani. *In vitro* and *In vivo* α-amylase and α-glucosidase inhibiting

activities of the protein extracts from two varieties of bitter gourd (*Momordica charantia* L.). BMC complementary and alternative medicine. 2016;16(1):1-8.

15. Suvarchala Reddy NVL, Akhila M, Subrahmanyam CVS, Trimurtulu G, Raghavendra NM. Isolation, *In vitro* antidiabetic, antioxidant activity and molecular docking studies of pentacyclic triterpenoids from *Syzygium alternifolium* (wt.) Walp. Journal of Pharmacy and Biological Sciences. 2015;10(6):22-27.
16. Suvarchala Reddy NVL, Mary JL, Raghavendra NM, Subrahmanyam CVS. Antidiabetic and Antioxidant activity of *Rhynchosia beddomei* Baker. American Journal of Phytomedicine and Clinical Therapeutics. 2014;2(11):1323-1332.
17. Nagaraja S, Ahmed SS, D R B, et al. Green synthesis and characterization of silver nanoparticles of *Psidium guajava* leaf extract and evaluation for its antidiabetic activity. Molecules. 2022; 27(14):4336. Published 2022 Jul 6. DOI:10.3390/molecules27144336
18. Kannan RR, Arumugam R, Anantharaman P. *In vitro* antioxidant activities of ethanol extract from Enhalus acoroides (LF) Royle. Asian Pacific Journal of Tropical Medicine. 2010;3(11): 898-901.
19. Kandiah M, Chandrasekaran KN. Green synthesis of silver nanoparticles using Catharanthus roseus flower extracts and the determination of their antioxidant, antimicrobial, and photocatalytic activity. Journal of Nanotechnology. 2021;2021: 1-8.
20. Awah FM, Verla AW. Antioxidant activity, nitric oxide scavenging activity and phenolic contents of *Ocimum gratissimum* leaf extract. Journal of Medicinal Plants Research. 2010;4(24):2479-87.
21. Rameshwari KS, Abirami M, Christobel R. Partial Characterization of green synthesized silver nanoparticles of *Cardiospermum halicacabum* and *Butea monosperma* aqueous extract combination (CHBMCSNP). Research Journal of Pharmacy and Technology. 2020;13(12): 6218-23.

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