CHARACTERIZATION OF GREEN SYNTHESIZED SILVER NANOPARTICLES USING MORINGA OLEIFERA LEAF EXTRACT FOR ANTIHYPERTENSIVE ACTIVITY

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Abstract. According to WHO more than 80% of the world's population relies on traditional medicine for their primary health need. Hypertension is a common problem facing many peoples today. Although billions of dollars are spent annually for the treatment and detection of cardiovascular disease, there is great interest, in the use of herbs for the treatment of hypertension and cardiovascular disease. Herbs and plants contain many phytochemicals that have been effective in the treatment of hypertension. In the present study, initially leaves of moringa oleifera was extracted with ethanol using Soxhlet apparatus then it has been formulated as a silver nanoparticle using silver nitrate by green synthesis method. The synthesized nanoparticle was evaluated using UV-Visible spectroscopy, Transmission Electron Microscopy and Zeta sizer. The results show the incorporation of the silver ions in the extract and also the reduction of particle size in nano range. The invitro antihypertensive activity was carried out using Angiotensin converting enzyme inhibitor assay for the standard drug and the formulated silver nanoparticle and found that the percentage inhibition of silver nanoparticle complies with the percentage inhibition of standard drug. It is seen that the silver nanoparticles have good hypertensive inhibition. Hence silver nanoparticles of moringa oleifera leaf extract can be used as a potential alternative to traditional synthetic drugs by using this non-toxic safe way of green synthesis.
Keywords: *moringa oleifera, silver nanoparticles, green synthesis, antihypertensive activity*

Introduction

Nanoparticles are solid or particulate dispersions with sizes between 10 and 1000 nm. The medication is dissolved, trapped, enclosed, or joined to a nanoparticle matrix. Nanoparticles, nanospheres, or nanocapsules can all be produced, depending on the preparation process. The main aims of developing nanoparticles as a delivery system are to manage particle size, surface characteristics, and release of pharmacologically active substances in order to accomplish the site-specific action of the drug at the therapeutically optimal rate and dose regimen (Machindra et al., 2023). Metal nanoparticles have been used in a wide-ranging application in various fields. Specifically, as shapes, sizes, and compositions of metallic nanomaterials are significantly linked to their physical, chemical, and optical properties, technologies based on nanoscale materials have been exploited in a variety of fields from chemistry to medicine. Recently, silver nanoparticles (AgNPs) have been investigated extensively due to their superior physical, chemical, and biological characteristics, and their superiority stems mainly from the size, shape, composition, crystallinity, and structure of AgNPs compared to their bulk forms. Efforts have been made to explore their attractive properties and utilize them in practical applications, such as anti-bacterial and anti-cancer therapeutics, diagnostics and optoelectronics, water disinfection, and other clinical/pharmaceutical applications. The synthesis methods of metal NPs are mainly divided into top-down and bottom-up approaches. The top-down approach

disincorporated bulk materials to generate the required nanostructures, while the bottom-up method assembles single atoms and molecules into larger nanostructures to generate nano-sized materials. Nowadays the synthetic approaches are categorized into physical, chemical, and biological green syntheses. The physical and chemical synthesis tend to be more labour-intensive and hazardous, compared to the biological synthesis of AgNPs which exhibits attractive properties, such as high yield, solubility, and stability.

To overcome the shortcomings of chemical methods, biological methods have emerged as viable options. Recently, biologically-mediated synthesis of nanoparticles have been shown to be simple, cost effective, dependable, and environmentally friendly approaches and much attention has been given to the high yield production of AgNPs of defined size using various biological systems including bacteria, fungi, plant extracts, and small biomolecules like vitamins and amino acids as an alternative method to chemical methods-not only for AgNPs, but also for the synthesis of several other nanoparticles, such as gold and graphene. Bio-sorption of metals by Gram-negative and Gram-positive bacteria provided an indication for the synthesis of nanoparticles before the flourishing of this biological method; however, the synthesized nanomaterials were as aggregates not nanoparticles. Several studies reported the synthesis of AgNPs using green, cost effective, and biocompatible methods without the use of toxic chemicals in biological methods. In this green chemistry approach, several bacteria, including Pseudomonas stutzeri AG259, Lactobacillus strains, Bacillus licheniformis; Escherichia coli (E. coli), Brevibacterium casei, fungi including Fusarium oxysporum, Ganoderma neo-japonicum Imazeki, plant extracts such as Allophylus cobbe, Artemisia princeps, and Typha angustifolia were utilized. In addition to these, several biomolecules, such as biopolymers, starch, fibrinolytic enzymes, and amino acids were used. The biological synthesis of nanoparticles depends on three factors, including (a) the solvent; (b) the reducing agent; and (c) the non-toxic material. The major advantage of biological methods is the availability of amino acids, proteins, or secondary metabolites present in the synthesis process, the elimination of the extra step required for the prevention of particle aggregation, and the use of biological molecules for the synthesis of AgNPs is eco-friendly and pollution-free.

Hypertension

High blood pressure is when the force of blood pushing against your artery walls is consistently too high. This damages your arteries over time and can lead to serious complications like heart attack and stroke. “Hypertension” is another word for this common condition. Healthcare providers call high blood pressure a “silent killer” because you usually don’t have any symptoms. So, you may not be aware that anything is wrong, but the damage is still occurring within your body. Blood pressure (BP) is the measurement of the pressure or force of blood pushing against blood vessel walls. Your BP reading has two numbers: (1) The top number is the systolic blood pressure, which measures the pressure on your artery walls when your heart beats or contracts; and (2) The bottom number is the diastolic blood pressure. This measures the pressure on your artery walls between beats when your heart is relaxing. Healthcare providers measure blood pressure in millimetres of mercury (mmHg). Two types of high blood pressure: (1) Primary hypertension: causes of this more common type of high blood pressure (about 90% of all adult cases in the U.S.) include aging and lifestyle factors like not getting enough exercise; and (2) Secondary hypertension: causes of this type of high blood pressure include different medical conditions or a medication you’re taking.

Primary and secondary high blood pressure (hypertension) can co-exist. For example, a new secondary cause can make blood pressure that's already high get even higher.

Materials and Methods

Preparation of ethanolic extract of leaf of Moringa oleifera

Fresh leaves of Moringa oleifera were collected and washed gently with running tap water twice and washed again with distilled water to remove sand and dried at room temperature for 14 days and crushed into coarse powder. 250 gm of Moringa oleifera leaves were extracted with 500ml of ethanol for 24 hours using Soxhlet apparatus and the extract was filtered and dried (Almatroudi, 2020; Ahmed et al., 2016).

Preformulation in silico studies

Docking

Molecular docking studies are used to determine the interaction of two molecules and to find the best orientation of ligands which would form a complex with overall minimum energy. The small molecule, known as ligand usually fits within the protein's cavity which is predicted by the search algorithm. These protein cavities become active when they come in contact with any external compounds and are thus called as active sites. Docking studies were performed using Schrodinger Maestro v12.6. Compounds with good ADME properties have been docked with the enzyme Angiotensin Converting Enzyme. Based upon the docking score and binding interactions top ranked compounds were selected for synthesis using conventional methods. In UV-visible spectral analysis of moringa oleifera leaf extract, 1ml of moringa oleifera leaf extract was taken in a 10 ml standard flask and diluted with ethanol. Then UV visible spectra were taken in the range of 200-400 nm using ethanol as blank (Khalid et al., 2018). While in FTIR spectroscopy of moringa oleifera leaf extract, 50 mg each of dried moringa oleifera leaf extract were mixed with 100 mg of spectral grade KBr and pressed into disc under hydraulic pressure. Then FTIR spectra were recorded in the 4000-400cm^{-1} range (Khalid et al., 2018).

Green synthesis of silver nanopacticles

Preparation of stock solution, silver nitrate aqueous solution and synthesis of silver nanoparticles

1 mg of extract was weighed and diluted to 10 ml with ethanol. 0.017g of silver nitrate was dissolved in 100 ml of distilled water to prepare 1mM solution of silver nitrate and stored in an amber colored bottle until further use. An aliquot (1ml, 3ml, 5ml) of ethanolic plant extract sample was separately added to 10ml of 1mM aqueous $AgNO^3$. To drive nanoparticle formation the reaction mixtures were kept in a magnetic stirrer with constant stirring at 120 rpm. Color Change of the reaction mixtures were monitored to determine silver nanoparticle formation which is indicated by a colloidal brown color.

Characterization of synthesized moringa oleufera silver nanoparticle

Characterization of Moringa oleifera silver nanoparticles is important in order to evaluate the functional aspects of the synthesized particles. Characterization is performed using a variety of analytical techniques, including UV-vis spectroscopy, Fourier transform infrared spectroscopy (FTIR), Transmission Electron Microscopy (TEM), particle size measurement, stability from zeta potential and drug entrapment efficacy for elemental analysis.

The visual examination, UV-visible spectroscopy and Fourier Transform Infrared (FTIR) spectroscopy

The primary confirmation of the synthesized Moringa oleifera silver nanoparticles is done by visual basis. The color change of reaction mixture (silver nitrate solution and leaf extract) with respect to time is observed (Acuram and Chichioco Hernandez, 2019; Mathur et al., 2018). UV-visible spectroscopy analysis characterizes the formulation and completion of Moringa oleifera silver nanoparticles. UV-visible spectroscopy is a very useful and reliable technique for the primary characterization of synthesized nanoparticles which is also used to monitor the synthesis and stability of silver nanoparticles. The reduction of pure silver ions was observed by measuring the UV-Visible spectrum of the reaction medium from the wavelength 400-800 nm by using distilled water as blank (Acuram and Chichioco Hernandez, 2019; Khalid et al., 2018). FTIR is a suitable, valuable, non-invasive, cost effective, and simple technique to identify he role of biological molecules in the reduction of silver nitrate to silver, Dried samples of silver nanoparticles of about 100mg were mixed with 100mg of spectral grade KBr and pressed into discs under hydraulic pressure. The FTIR spectrum of Moringa oleifera silver nanoparticle was recorded in the range of 4.000 cm^{-1}-400 cm^{-1}(Bhagwat and Vaidhya, 2013).

Determination of entrapment efficiency, particle size, and zeta potential

The entrapment efficiency of nanoparticles was determined by adding 10 ml of phosphate buffer of pH 7.4 and sonicated in a bath sonicator and filtered. 1 ml of filtrate is made up to 10 ml with phosphate buffer and was assayed using UV visible spectrophotometer at 375 nm using pH 7.4 phosphate buffer as blank. The amount of drug entrapped was determined by the formula (Eq. (1). The average mean diameter and size distribution of silver nanoparticles was determined by Dynamic Light Scattering method using Malvern zetasizer at 25°C. The dried silver nanoparticles were dispersed in water to obtain proper light scattering intensity of silver nanoparticles (Vitthal et al., 2013). Zeta potential is a measure of surface charge. The surface charge (electrophoretic mobility) of nanoparticles can be determined by using Zeta sizer (Malvern Instrument) having zeta cells, polycarbonate cells with gold plated electrodes and using water as medium for sample Preparation. It is essential for the characterization of stability of the silver nanoparticles (Vitthal et al., 2013).

$$\text{Drug entrapment (\%)} = \frac{\text{Amount of drug-Amount of free drug}}{\text{Amount of total drug}} \times 100 \quad \text{Eq. (1)}$$

In-vitro drug release studies

The in-vitro release study of Moringa oleifera silver nanoparticles was performed by dialysis bag method. The samples were placed into dialysis bags which are dialysed in 60 ml of phosphate buffer solution with pH 7.4. The drug release was assessed to start as soon as the dialysis into the reservoir compartment. The reservoir was kept under constant stirring. The sample was collected at regular intervals of time and replaced with an equal amount of buffer. The collected sample is filtered and diluted, further analysed using UV-visible spectrophotometer.

Extraction of angiotensin converting enzyme from sheep lung extract

Sheep lung was collected from the Corporation slaughterhouse and immediately transferred into a borate buffer (pH 8.3) and transported. It is stored in the refrigerator. One gram of sheep lung tissue was sliced and homogenised in 10ml of the ice cold 100 Mm borate buffer (pH 8.3) containing 50 mM KCl using a homogeniser at 4 °C. The homogenate was centrifuged at 8000g at 4 °C for half an hour, and the supernatant was collected. Remove, if any impurities are present and stored at 4 °C. This supernatant was used as the source of ACE.

Angiotensin converting enzyme inhibitory activity

ACE activity was measured using Hippuryl-L-Histidyl-L-Leucine as substrate. The reaction mixture contained 0.2 ml of 5mM HHL prepared in 200mM Borate buffer (pH 8.3), containing 1000 mM KCl was mixed with 10 µl of different concentration of drug sample (10-320 µg/ml), and captopril (10-320 µg/ml). The reaction was initiated by adding lung extract (50 µl) and distilled water in a volume of 1ml. The reaction mixtures were incubated at 37 °C for 30min. The reaction was stopped by adding 2 ml of HEPES buffer, after which 1 ml of 136 mM cyanuric chloride in 1,4-dioxan was added to the reaction mixture and shaken vigorously for 15 sec. The absorbance of the yellow colour developed was measured at 405 nm. Captopril was used as the standard drug for comparison with the assay system. ACE activity of the lung extract incubated with captopril was read against the standard curve (Eq. (2). All determinations were carried out in triplicate and the values are expressed as mean ± SEM.

$$\text{Percentage inhibition (\%)} = \frac{A-B}{A} \times 100 \qquad \text{Eq. (2)}$$

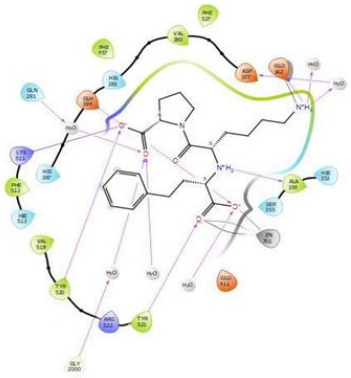
Results and Discussion

In silico studies

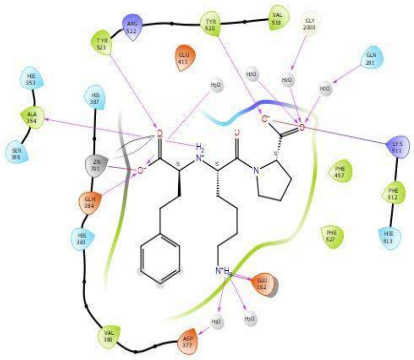
Captopril has been considered as a standard drug, which showed active binding i.e., interaction against ACE receptors by inhibiting the conversion of angiotensin I to angiotensin II. Therefore, the active binding energy of captopril was found to be -21.862 which is compared with the various test compounds of Moringa oleifera contain quercitin-O-glocoside (-41.2289), Benzyl glucosinolate (-36.6231), Benzyl isothiocyanate (-15.5942), Hexanamine (-12.4686), Tocopherol (-31.7296), Erythritol (-14.2635), Nonane (-13.7517), Heptanal (-13.4782), pentacosane (-24.5611) showed excellent active binding energy against ACE receptors as same as standard drug of captopril (*Table 1* and *Figure 1*).

Table 1. *Active binding energy of various compounds against ace.*

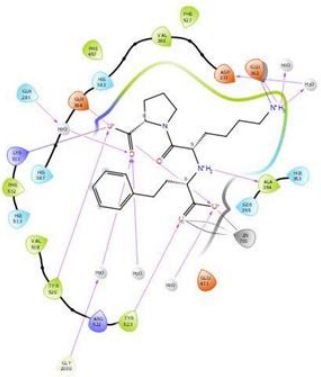
Compound name	Grid score	Grid energy
Quercitin-O-glucoside	-5.2185	-41.2289
Benzyl glucosinolate	-3.9801	-36.6231
Benzyl isothiocyanate	-3.3214	-15.5942
Hexanamine	-2.9454	-12.5942
Tocopherol	-2.8932	-31.7296
Erythritol	-1.6579	-14.2635
Nonane	-0.9089	-13.7517
Heptanal	-0.8783	-13.4782
Pentacosane	-0.8783	-24.5611
Captopril	-2.6931	-21.8622



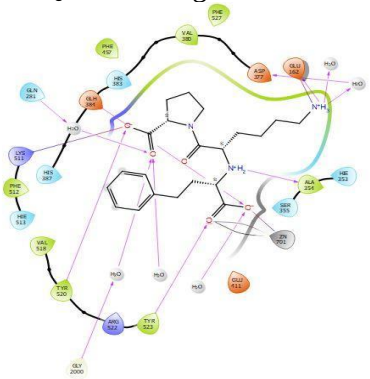
Quercitin-O-glucoside



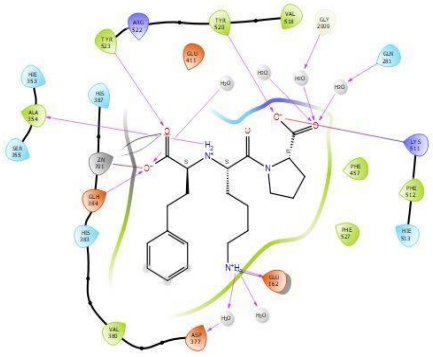
Quercitin-O-glucoside



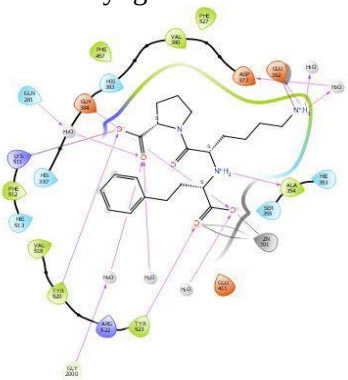
Benzyl isothiocyanate



Tocopherol
Benzyl glucosinolate



Erythritol



Hexanamine

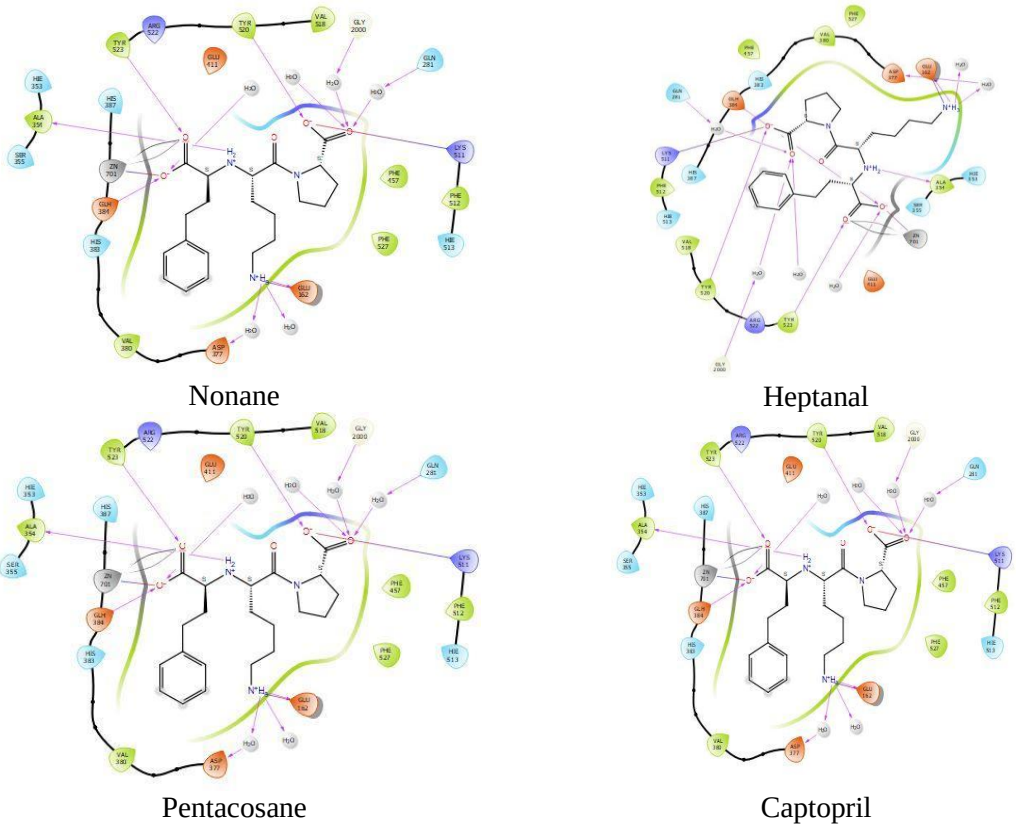


Figure 1. *Docking images of Binding effect of ligands with targets.*

UV-visible spectral analysis of moringa oleifern leaf extract

The maximum absorption of moringa oleifera leaf extract was found to be 375 nm, hence it is selected as the λ_{max} for further studies (*Figure 2*).

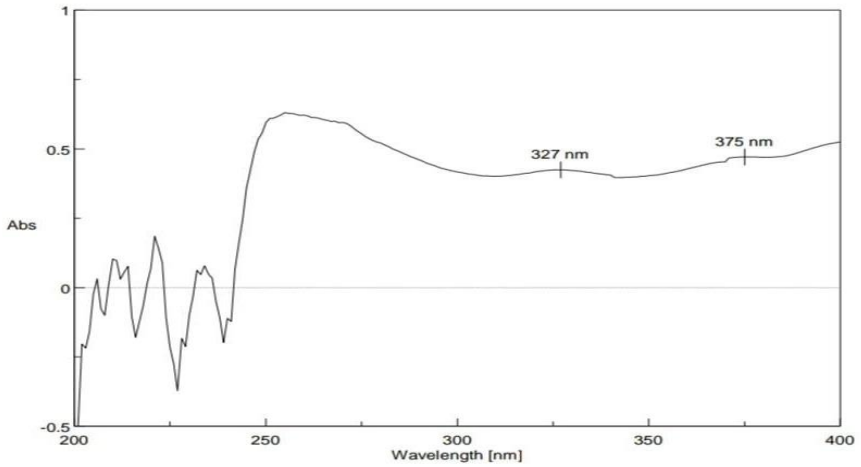


Figure 2. *UV-visible spectrum of moringa oleifera leaf extract.*

Green synthesis of silver nanoparticles

Color change from light green to brown indicated the formation of silver nanoparticles (*Figure 3*).

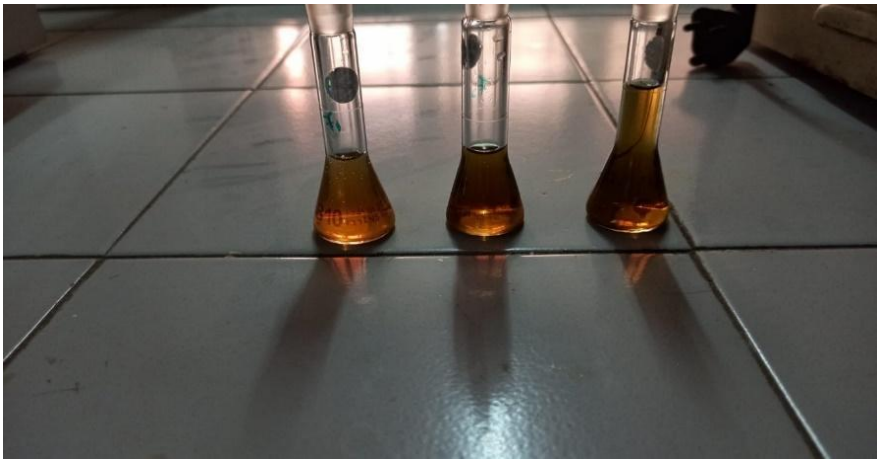


Figure 3. *Formulation of green synthesized silver nanoparticle of moringa oleifera leaf extract.*

Characterization of green synthesized moringa oleifera silver nanoparticles

Visual examination

From the formulation, addition of moringa oleifera leaf extract into aqueous silver nitrate leads to the color change from light green to brown color. This formation indicates that silver ions in reaction medium have been converted to elemental silver having the size of nanometric range.

UV visible spectral analysis of moringa oleifera silver nanoparticles

UV-visible spectral analysis characterizes the formation and completion of silver nanoparticles by using UV-visible spectrophotometer. The reduction of silver ions in solution was monitored by periodic sampling of aliquots at the time interval of 30mins were taken by distilled water using water as blank from the wavelength of 200-800 nm (*Figure 4*). The reduction of silver ions to silver nanoparticles was indicated by color change from yellow to brown color and it was reflected in spectral data obtained by using UV-Visible spectrophotometer. The color is characteristic of the surface plasmon resonance (SPR) of silver nanoparticles. It shows an absorption peak around 422 for F1, 420 nm for F2 and 420 nm for F3 formulation, which is specific for moringa oleifera silver nanoparticles.

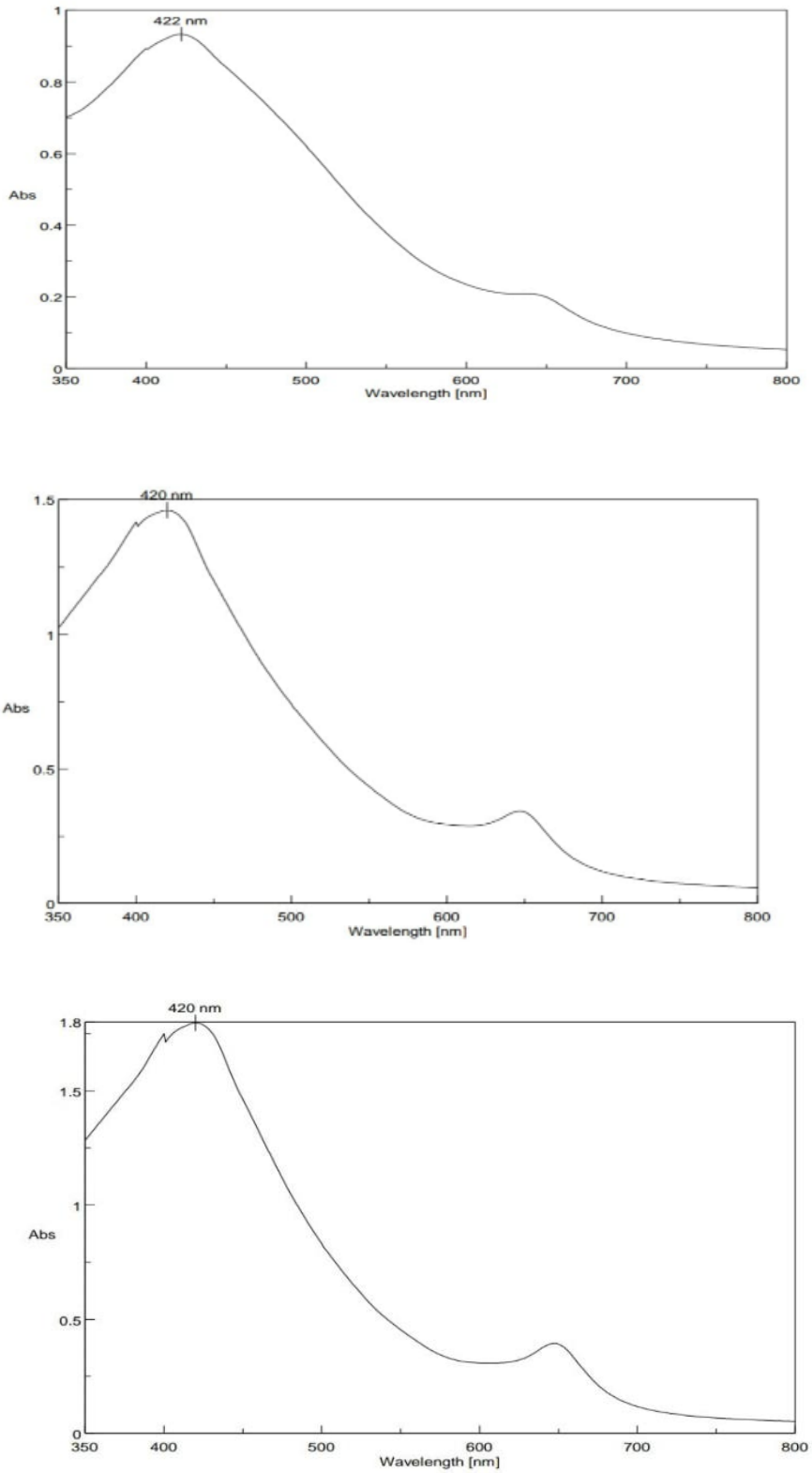


Figure 4. *UV-visible spectrum of of moringa oleifera silver nanoparticles.*

Drug entrapment efficiency

Drug entrapment efficiency is a measure of drug loading capacity of the system. Drug entrapment can be determined by using the phosphate buffer in a dialysis

membrane (*Table 2*). From the *Table 2*, the entrapment of drug or drug content of Moringa oleifera silver nanoparticles were found to be 81.09% for F1, 84.38% for F2 and 90.7% for F3. Based on entrapment results, the formulation F3 was chosen for further evaluation studies as it possessed high entrapment capacity as compared to other formulations.

***Table 2**. Drug entrapment efficiency of different formulations of Moringa oleifera silver nanoparticles.*

S.No.	Formulation code	% drug entrapment
1	F1	81.09%
2	F2	84.38%
3	F3	90.7%

Particle size measurement

The mean particle size (z-average), polydispersity index (PI) of moringa oleifera silver nanoparticles were determined by using Dynamic Light Scattering technique using a zeta size analyzer (Malvern Instruments). The study revealed average particle size (z-average) was found to be 324 nm. Particle size analysis showed the presence of nanoparticles with polydispersity indices PDI value 0.725 with intercept 0.528 (*Figure 5*).

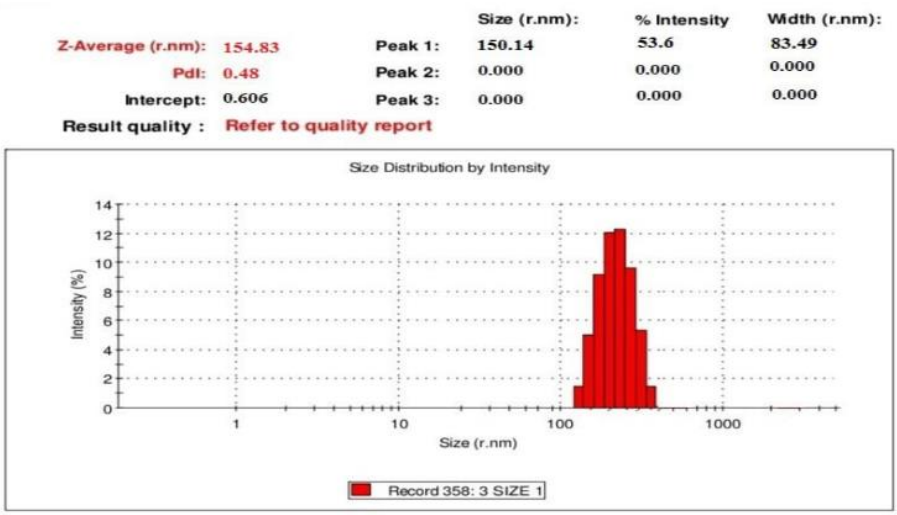


***Figure 5.** Particle size measurement of moringa oleifera silver nanoparticle.*

Zeta potential

Zeta potential was determined using the Malvern zeta-sizer instrument. The surface charge of the particles and stability of the solution was characterized by zeta potential. For moringa oleifera silver nanoparticles, zeta potential was found to be -4.28 Mv with peak area 100 intensity. These values indicate that moringa oleifera silver nanoparticles are stable (*Figure 6*).

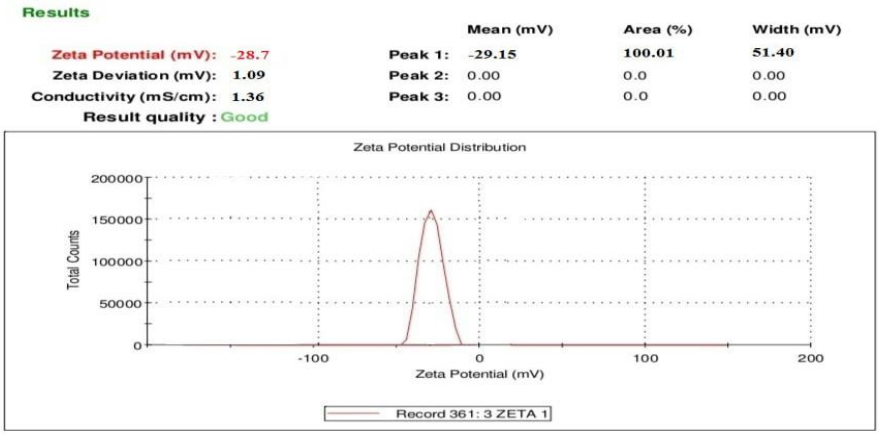


Figure 6. *Determination of zeta potential of moringa oleifera silver nanoparticles.*

In vitro drug release

The in vitro release of drug from the silver nanoparticle was measured using a phosphate buffer of pH 7.4 using dialysis bag diffusion method. Amount of drugs released at different time intervals including 1h, 2h, 4h, 8h, 12h, 14h, 16h, 18h, 20h, 24h were observed (*Table 3*). From the *Figure 7*, formulation F3 shows higher drug release when compared to other formulations F1 and F2

Table 3*. In-vitro drug release data of different formulations of Moringa oleifera silver nanoparticles.*

S.No	Time	Cumulative % drug release		
		F1	F2	F3
1	0	0	0	0
2	1	12.76	9.08	12.87
3	2	22.08	21.36	25.09
4	4	34.08	36.9	41.45
5	8	48.18	46.21	51.76
6	12	54.48	52.96	56.99
7	14	57.84	60.6	63.01
8	16	61.02	63.65	67.74
9	18	67.56	71.76	74.99
10	20	73.44	75.49	82.7
11	24	77.88	80.16	86.32

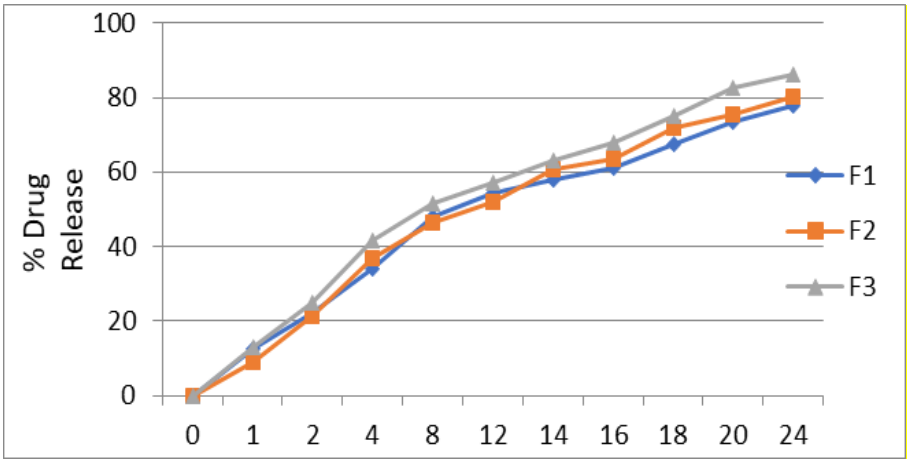


Figure 7. *In-vitro drug release of different formulations of moringa oleifera silver nanoparticles.*

In vitro antihypertensive activity

Angiotensin converting enzyme activity of standard drug captopril and the Moringa oleifera silver nanoparticles were determined using Hippuryl-L-Histidyl-L-Leucine as substrate. Different concentrations of drug sample and captopril are taken and mixed with borate buffer and lung extract and incubated for 30mins. Reaction mixture was stopped by adding HEPES Buffer. The absorbance of the yellow color developed was measured at 405nm. Captopril is used as a standard and the ACE activity of drug samples was compared with standard. From the *Table 4* and *Table 5*, it was confirmed that the concentration of drug increases with increase in % inhibition. IC50 of captopril and drug samples were compared, as it shows satisfactory % inhibition.

Table 4*. ACE Inhibitory data of control captopril.*

Concentration	Absorbance			% inhibition			Average
(µg/ml)	T1	T2	T3	T1	T2	T3	
10	1.5916	1.5816	1.5726	17.39	17.86	18.37	17.87± 0.2829
20	1.3829	1.3729	1.3916	28.22	28.74	27.77	28.24± 0.2802
40	1.1932	1,1829	1.1729	38.07	38.01	39.12	38.40 ± 0.3604
80	0.9746	0.9646	0.9529	49.41	49.41	50.54	49.96± 0.3265
160	0.7912	0.7626	0.7329	58.93	58.93	61.96	60.43± 0.8747
320	0.5819	0.5716	0.5629	69.79	6.79	70.86	70.3± 0.2861
IC_{50}	-	-	-	8.6	8.6	8.2	8.6± 0.1333

Table 5*. ACE Inhibitory data of moringa oleifera silver nanoparticles.*

Concentration	Absorbance			% inhibition			Average
(µg/ml)	T1	T2	T3	T1	T2	T3	
10	1.4129	1.4226	1.4629	26.66	26.16	24.09	25.63± 0.7932
20	1.2422	1.2329	1.2261	35.52	36.01	36.36	35.963± 0.2436
40	1.0226	1.0126	1.0236	46.92	47.44	46.41	46.92 ± 0.2973
80	0.8316	0.8229	0.8128	56.84	57.31	57.81	57.32± 0.2801
160	0.6219	0.5926	0.5816	67.72	69.24	69.81	68.92± 0.6237
320	0.5119	0.4926	0.4816	73.43	74.43	75.01	74.29± 0.4614
IC_{50}	-	-	-	5.2	5.1	5.2	5.2 ± 0.0333

Conclusion

The present study presents a non-toxic as well as eco-friendly procedure for synthesizing silver nanoparticles. This technique gives us a simple and efficient way for synthesis of nanoparticles with good antibacterial properties. Inhibition of moringa

oleifera silver nanoparticles against hypertension were observed, hence it can be used as a potential alternative to synthetic drugs as a non-toxic safe way of green synthesis.

Acknowledgement

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Conflict of interest

The authors confirm that there is no conflict of interest involve with any parties in this research study.

REFERENCES

[1] Acuram, L.K., Chichioco Hernandez, C.L. (2019): Anti-hypertensive effect of Moringa oleifera Lam. – Cogent Biology 5(1): 10p.
[2] Ahmed, S., Ahmad, M., Swami, B.L., Ikram, S. (2016): A review on plants extract mediated synthesis of silver nanoparticles for antimicrobial applications: a green expertise. – Journal of Advanced Research 7(1): 17-28.
[3] Almatroudi, A. (2020): Silver nanoparticles: Synthesis, characterisation and biomedical applications. – Open Life Sciences 15(1): 819-839.
[4] Bhagwat, R.R., Vaidhya, I.S. (2013): Novel drug delivery systems: an overview. – International Journal of Pharmaceutical Sciences and Research 4(3): 970-982.
[5] Khalid, S., Shahzad, A., Basharat, N., Abubakar, M., Anwar, P. (2018): Phytochemical screening and analysis of selected medicinal plants in Gujrat. – Journal of Phytochemistry and Biochemistry 2(1): 1-3.
[6] Machindra, M.G., Sanjay, P.P., Babasaheb, K.S., Nandkumar, P.S. (2023): Review on targeted drug delivery system and its carries as drug targeted to a specific organ. – International Journal of Multidisciplinary Research 5(2): 11p.
[7] Mathur, P., Jha, S., Ramteke, S., Jain, N.K. (2018): Pharmaceutical aspects of silver nanoparticles. – Artificial Cells, Nanomedicine, and Biotechnology 46(sup1): 115-126.
[8] Vitthal, K.U., Pillai, M.M., Kininge, P. (2013): Study of solid lipid nanoparticles as a carrier for bacoside. – International Journal of Pharmacy and Biological Sciences 3: 414-426.