Synergistic response of physicochemical reaction parameters on biogenesis of silver nanoparticles and their action against colon cancer and leishmanial cells

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**ABSTRACT**

Physicochemical parameters include pH, temperature, the concentration of the AgNO\(_3\), ratio of reactants, agitation and incubation period that act synergistically and provide a steering force to modulate the biogenesis of nanoparticles by influencing the molecular dynamics, reaction kinetics, protein conformations, and catalysis. The current study involved the bio-fabrication of silver nanoparticles (SNPs) by using the reducing abilities of \textit{Mentha longifolia} (L.) L. leaves aqueous extract. Spectrophotometric analysis of various biochemical reactions showed that 3 mM of AgNO\(_3\) at 120°C in an acidic pH when mixed in 1–9 ratio of plant extract and AgNO\(_3\) respectively, are the optimised conditions for SNPs synthesis. Different analytical techniques confirmed that the nanoparticles are anisotropic and nearly spherical and have a size range of 10–100 nm. The ~10 \(\mu\)g/ml of SNPs killed ~66% of \textit{Leishmania} population and IC\(_{50}\) was measured at 8.73 \(\mu\)g/ml. SRB assay and Annexin V apoptosis assay results showed that the plant aqueous extract and SNPs are not active against HCT116 colon cancer cells and no IC\(_{50}\) (80% survival) was reported. ROS generation was quantified at 0.08 \(\Phi\), revealed that the SNPs from \textit{M. longifolia} can generate free radicals and no photothermal activity was recorded which makes them non-photodynamic.

**GRAPHICAL ABSTRACT**

**INTRODUCTION**

Different physicochemical factors play an important role to control the reaction kinetics, molecular dynamics, protein conformations, and catalysis and have a marked influence on the shape, size and biochemical corona of nanostructures. The physicochemical parameters provide a full range of toolboxes including the temperature, pH, the concentration of the metal salt, concentration of the reducing agent, agitation or without stirring and the reaction incubation period that acts synergistically to bio-fabricate nanoparticles [1]. The temperature plays a promising role to control the reaction kinetics and molecular dynamics by increasing or decreasing the activation energy that affects agitation of the reactants at an atomic level and contributes towards their collision, while the pH is a factor towards the concentration of the H\(^+\) ions.
The concentration of the H\(^+\) ions depends on the pH level, the lower the pH higher the concentration of H\(^+\) ions that result in increasing the rate of collision of reactants and change the conformation of reactants by altering the electro-negative state [2]. The concentration of the substrate or metal salt along with the concentration of the reducing agent or plant extract has a significant role in redox chemical reactions, act in a synergic manner to carve the final product by changing the ionic ratio of metal to the reducing agent [3]. These physicochemical parameters also contribute greatly to design the functional corona of nanoparticles by altering protein conformations and controlling molecular dynamics [4]. The bio-fabrication requires a rigorous optimisation of reaction dynamics for the better synthesis of nanoparticles and has an advantage over other chemical and physical methods that involves energy-intensive and hazardous chemical reactions. Some research studies [4–10] were performed before that involves the evaluation of the effects of individual reaction physicochemical conditions on the biogenesis of nanoparticles but the physicochemical parameters act synergistically and the synthesis and yield of nanoparticles alter under the influence of other reaction condition.

Biogenesis of silver nanoparticles by steering the natural reducing abilities of the plant secondary metabolites through the physicochemical parameter-driven approach provides a one-step process to tailor biocompatible nanoparticles for human applications to treat resistant diseases such as Leishmania and cancer [11,12]. Conventional cancer treatment involves the surgical removal of the malignant parts, administration of the chemotherapeutic drugs to shrink the tumours and the radioactive killing of the growth of the abnormal cells [13]; these methods involve many side effects including the resistance of the patient's body to receive treatment, development of the resistance against chemotherapeutic drugs and perilous effects of the drugs on the normal or healthy cells of the body [14,15]. Leishmaniasis is another common disease which usually transmits by phlebotomine sand-fly and has intracellular disease spreading. It can cause mild to chronic illness and can cause cutaneous ulcers to mutilating mucocutaneous disease which can further result in systematic illness. The microorganisms develop resistance against conventional drugs which severely affects the patients’ health and results in decreasing disease success rate [16,17].

Green nanotechnology provides a new avenue to use nanoparticles of various metals especially silver because of its historic significance, diverse physicochemical properties [18] and its involvement in pharmaceutical preparations due to its strong cytotoxic action to curb cancer, multiple drug resistance and other perilous pathogenic organisms such as Leishmania [19,20]. The plant secondary metabolites provide an easy approach to reduce, chelate and stabilise the nanoparticles. Some scientific studies reported the presence of sesquiterpenes and monoterpenes as the active compounds of the *M. longifolia* that belong to the class of terpenoids and phenols and play their functional role in various biomedical applications and the synthesis of nanomaterials [21].

The present study was envisioned to study the synergistic response of various physicochemical reaction parameters on the optimisation of the biogenesis of silver nanoparticles from *Mentha longifolia* leaves aqueous extract. The structural characterisation was performed to study the size, shape and optical characteristics of nanoparticles followed by the evaluation of nanoparticles for the treatment of Leishmania and HCT116 colon cancer cells. The pictorial layout of work is given in Graphical abstract.

### Experimental methods

#### Collection of plant and extraction of leaves aqueous extract

*Mentha longifolia* leaves were collected from the healthy plants growing on the Jinnah stream bank (33.751870° N 73.135138° E) located at Quaid-i-Azam University Islamabad. Plants were growing in patches on the side of the stream. The plant samples were identified by plant taxonomists and the flora of Pakistan was considered to confirm the taxonomic identification. The voucher specimen was allotted accession number (UAAR-js-0398) and deposited in the herbarium of the botany department of PMAS Arid Agriculture University. The plant aqueous extract was prepared by mixing the powdered plant material and distilled water in a 1–10 ratio respectively and heated at 45–65 °C for 10–20 min and the colour was changed to greenish. The plant aqueous extract was filtered by using Celite and filter paper and used as fresh [22].

#### Biogenesis of silver nanoparticles

The freshly prepared plant aqueous extract was mixed with a specific molar concentration of the silver salt in a 1–9 ratio respectively. The physicochemical reaction parameters such as the temperature, pH were maintained and reactions were stirred continuously. After a predetermined period, an aliquot was taken in the quartz cuvettes to measure the absorbance between 200 and 900 nm of the light wavelength by using a UV-Visible spectrophotometer (Shimadzu 1601, Japan). After the confirmation of the synthesis, SNPs were separated from the reaction mixture by centrifuging the colloidal solution at 1000 × g (Sorvall RT 7 Plus) and washed thrice with the distilled water [17].

#### Optimisation of physicochemical parameters

The reactions were arranged according to the multifactorial experimental design. The physicochemical reaction parameters that include the concentration of AgNO\(_3\), pH, temperature, incubation period and plant extract ratio in the reaction mixture were optimised. The reactions were arranged to study their synergistic response. The spectrophotometric results were compared to evaluate the synergistic effects of physicochemical reaction parameters and for the determination of suitable conditions for the synthesis of SNPs [1].
**Determination of suitable reactants ratio**

Different combinations of reactants such as the plant extract and AgNO₃ (2 mM) were prepared. 1–9 ratio of plant extract and AgNO₃ was mixed and incubated at 60 °C for 24 h in a lab oven. Series of experiments were arranged and the ratio of the plant extract was increased (1 to 9) while the proportion of AgNO₃ was decreased (9 to 1) gradually. The absorbance of the colloidal reaction mixtures was measured in the range of 200–900 nm of light wavelength to determine the suitable reactants ratio [23].

**Structural and optical evaluation of SNPs**

The structural morphologies of the phyto-synthesised SNPs were manifested by using different material characterisation instruments. The Scanning Electron Microscopy (SEM) was performed on JEOL 7500 F HRSEM. A drop coating method was used to mount the sample on the copper grid [24]. The elemental composition of the colloidal SNPs was evaluated through the Energy Dispersive X-Ray Analysis (EDX) (JEOL 7500F HRSEM) [25]. The Atomic Force Microscopy (AFM) images were obtained on an Agilent 5500 AFM. The nanoparticle size analysis of SNPs was performed by using the Dynamic Light Scattering (DLS) machine (Zetasizer Nano S, Malvern Instruments, UK). The sample was prepared in a 1× PBS solution at 25 °C [27].

**Cell culture maintainance of L. tropica**

The promastigote form of Leishmania was used during the study. The cultures of the L. tropica (KWH23) cells were maintained on the M199 medium (Gibco, Invitrogen, USA). Streptomycin (100 μg/ml, Sigma, USA), penicillin (100 μg/ml, Sigma, USA) and heat-inactivated foetal bovine serum (10%, PAA, Austria) was used to supplement the medium [28].

**Anti-leishmania activity measurement**

The cells of L. tropica were maintained for 7 days at 24 °C on the M199 medium. A stock solution of the SNPs was prepared in the distilled water and the concentration was maintained at 1 mg/ml. A 96-well microtiter plate was used to suspend L. tropica cells. The serial dilution of SNPs were prepared as 500 μg/ml, 250 μg/ml, 100 μg/ml, 50 μg/ml, 20 μg/ml, 1 μg/ml, 0.1 μg/ml, and 0.001 μg/ml. The Neubauer chamber (Thermo Fisher) was used to measure the viability of the cells and trypan blue dye was used to observe the motility of cells. The GraphPad Prism® software was used to measure the IC₅₀ (Half-maximum inhibitory concentration) of SNPs [28, 29].

**SRB assay (IC₅₀ evaluation of SNPs)**

The Sulforhodamine B assay was used to measure the IC₅₀ of the SNPs against the HCT116 colon cancer cells. 100 μl of the DMEM complete medium was used to seed 2000 colon cancer cells in 96 well plates. The serial dilution was used to prepare different concentrations of SNPs. The following day the cancer cells were treated with various concentrations of nanoparticles. 41 μl of 10% TCA was added after 3 days to fix the cells directly to the wells. The plate was then incubated overnight at 4 °C. When the incubation period finished, plates were washed 4 times with the tap water and dried at 37 °C for 15 min. 100 μl of 0.06% SRB was added to the wells for 30 min at room temperature to stain the cells. The cells were washed 4 times by using 1% acetic acid in tap water and again dried for 15 min at room temperature. The dye was washed by using 100 μl of 10 mM Tris and the plate was incubated at room temperature on a shaker at 300 rpm for 5 min. In the end, the absorbance was obtained at 490 nm. The IC₅₀ was calculated using GraphPad Prism® software [1].

**Measurement of the apoptotic abilities of the plant aqueous extract (Annexin V apoptosis assay)**

After the incubation period ended the harvested cells were washed in 1× PBS ice-cold solution. The colon cancer cells were resuspended in 1× PBS ice-cold solution and centrifuged at 300× g for 5 to 10 min at 4 °C to collect the cell’s pallet and were resuspended in 1× annexin binding buffer (ice cold). The cell’s density was adjusted in 1× annexin binding buffer at 1× 10⁵ cells per ml. 100 μl of the cell suspension (resuspended in 1× Annexin binding buffer) was taken into a new Eppendorf tube and 5 μl of FITC or Alexa fluor 488 labelled Annexin V was added to each 100 μl cell suspension. 1 μl of propidium iodide (100 μg/ml) was also added to each 100 μl cell suspension. The cell suspension was incubated for 15 min in dark at the room temperature. After the incubation period, 400 μl of ice-cold annexin binding buffer was added and mixed gently. The samples were incubated on ice and data was collected on a flow cytometer [1].

**ROS quantification of SNPs**

1,3-Diphenylisobenzofuran (DPBF) solution was prepared (0.1 mM DPBF in ethanol) to determine the quantum yield of the SNPs. 2 ml of DPBF solution was used to dissolve 10 μg/ml of SNPs. The quartz cuvettes were used and an IR filter (400–800 nm) was installed to carry the reaction under sunlight for 30 s. After 30 s photobleaching abilities of DBPF were measured for 5 min by using a UV-3000 Spectrophotometer. Methylene blue was used as a positive control experiment [30].

**Photothermal activity of SNPs**

A colloidal solution of 1 mg/ml of SNPs was prepared to determine the photothermal ability. The test tube of the sample was exposed to the sunlight for one minute and the temperature was measured by using a temperature probe (Thermo scientific, USA). The Temperature was measured after each minute and the readings were taken for the next 15 min [17].
Data analysis

The student's t-test was performed to evaluate the significance of anti-leishmanial activity and \( p < .05 \) was considered a significant difference.

Results and discussion

Synergistic response of different physicochemical reaction parameters on the biogenesis of SNPs (spectrophotometric analysis)

Role of different concentrations of AgNO₃ and temperature

Different physicochemical parameters act synergistically and have a major impact on the size, shape, morphology and other biochemical attributes of the SNPs. The relationship of these parameters to tailor nanoparticles was studied extensively by using a multifactorial experimental design arrangement.

A different set of temperatures were maintained along with various molar concentrations of the silver salt. The experiments were initiated by maintaining the reaction temperature at 30°C with 1 mM of AgNO₃ (Figure 1(A)). The analysis of the spectrum expressed a very low and broad peak. The next reaction mixture was monitored at 30°C with 3 mM and 5 mM of the AgNO₃, separately (Figure 1(B,C)). There was a gradual increase in the height of the peak after every hour but that was a very slight increase. It can be explained that the phytometabolites have their specific conformation that contributes towards their action and the enzymes that play a role in the process of synthesis do not show activity at the low temperature.

The temperature plays a pivotal role to control the reaction kinetics, molecular dynamics and protein conformations in the process of the synthesis of SNPs [31]. In the next set of reactions, the temperature of the reaction mixture was increased to 60°C (Figure 1). A very broad Surface Plasmon Resonance (SPR) band was observed at low absorbance (0.32 a.u.) when 1 mM of the AgNO₃ was used (Figure 1(D)). The broad peak suggests the synthesis of anisotropic and large size nanoparticles. In the next experiment, 3 mM of the AgNO₃ solution at 60°C (Figure 1(E)) was used which showed better synthesis while there was a marked decline in the synthesis of SNPs when 5 mM of AgNO₃ was used (Figure 1(F)). It was observed that an increase in the incubation period resulted in an increase in synthesis and it was declined after reaching an optimum threshold. Beer Lambert's law states that the height of the SPR band is proportional to the concentration of the reacting species in the system [32]. A narrow and symmetrical SPR band was observed when 3 mM of the AgNO₃ solution was used at 60°C. It represents the synthesis of monodisperse and spherical nanoparticles [33]. It was also observed that the increase in the incubation period resulted in an increase in the absorbance at a higher wavelength which is called the blueshift. Mie's scattering theory describes the interaction of light with nanoparticles. According to this theory, small nanoparticles absorb light at a lower wavelength while larger molecules absorb light at a higher wavelength [1].

To understand the effects of increasing temperature on the synthesis of SNPs a higher temperature of 120°C was also used (Figure 2). There was a slight increase in the height of the peak when 1 mM of AgNO₃ was used that showed the synthesis of the SNPs but the yield of the product was very low expressed by the low absorbance unit (a.u. 0.37) which can be because of the low concentration of AgNO₃ in the reaction mixture (Figure 2(A)). 3 mM of the AgNO₃ at 120°C (Figure 2(B)) was found most suitable conditions for the synthesis of SNPs. The higher temperature did not show good synthesis at a low molar concentration of silver salt and synthesis increased synergistically at 3 mM and declined at 5 mM (Figure 2(C)). It can be evaluated that increasing temperature results in increasing the reaction kinetics and provide useful conformational changes to the enzymes and biomolecules so the reaction was executed more quickly. The other reason is the high kinetic energy, that helps molecules in the reaction mixture to move with strong kinetic force and aid reactants to collide and convert into the product. Our results are in support of previously published scientific reports which studied the individual effects of temperature [34], incubation period [4,10] and concentration of metallic salt [8] on the synthesis of nanoparticles.

Role of different pH ranges and concentrations of AgNO₃

The biogenesis of nanoparticles can be steered by using different physicochemical reaction conditions. The pH along with the temperature and the molar concentration of silver salt plays a vital role to affect the morphology and the biological covering of the metallic nanoparticles [16]. The pH scale is divided into three basic parts acidic, neutral and basic so the synthesis of nanoparticles was tested against all three types of pH conditions. The pH was studied in conjunction with the molar concentration of the silver salt. The reaction was initiated with the maintenance of the reaction temperature at 60°C and the pH were adjusted at 5, 7 and 9 (Figure 2(D,E,F)). 1 mM of the silver nitrate solution was used to get reduced by the plant extract. No synthesis of SNPs was recorded when AgNO₃ was used in 1 mM which expressed that the concentration of the silver ions was very low in the reaction mixture to enter the process of reduction.

Followed by increasing the concentration of AgNO₃ to 3 mM to understand the effects of varying pH conditions on the synthesis of SNPs (Figure 3). The highest synthesis of SNPs was recorded in acidic (pH 5) conditions (Figure 3(A)) and there was no marked synthesis on neutral and basic pH (Figure 3(B,C)). The next phase of the study includes the increase of AgNO₃ concentration up to 5 mM and there was synthesis similar to 3 mM at acidic pH (Figure 3(D)). The asymmetrical peaks were observed to represent the meagre synthesis of SNPs at basic and neutral pH (Figure 3(E,F)). The above experiments showed 3 mM of AgNO₃ and maintenance of the acidic pH as the optimum conditions for the synthesis of SNPs by using M. longifolia leaves aqueous extract. The pH of the reaction mixture provides a steering force to...
increase or decrease the concentration of H\(^+\) ions which directly alters the electronegative state of reactants. At lower pH the concentration of the H\(^+\) ions increases and vice versa. Increasing the concentration of H\(^+\) ions in the reaction mixture subsequently increases the collision or interaction of the reactants and directly influences the reaction kinetics. The pH does not directly influence the structural conformation of enzymes and proteins. However, the change in the concentration of H\(^+\) ions in the reaction mixture can shape and alter the morphological properties of the substrate by changing

Figure 1. Spectrophotometric analysis of SNPs biogenesis under different physicochemical conditions (a) Temperature 30 °C, AgNO\(_3\) 1 mM (b) Temperature 30 °C, AgNO\(_3\) 3 mM (c) Temperature 30 °C, AgNO\(_3\) 5 mM (d) Temperature 60 °C, AgNO\(_3\) 1 mM (e) Temperature 60 °C, AgNO\(_3\) 3 mM (f) Temperature 60 °C, AgNO\(_3\) 5 mM.
Role of different reactants ratios (AgNO₃ and plant extract)

The different ratios of the reactants have different effects on the biogenesis of the SNPs (Figure 4(A)). Analysis of the UV-spectrum manifested a 1 to 9 ratio of the plant extract and AgNO₃ respectively as the most suitable reactant proportion for the optimised biosynthesis of SNPs. It can be explained that different ratios of the reactants provide different concentrations of Ag⁺ ions and plant secondary metabolites in the reaction mixture and carve the final nano-product. Higher the concentration of plant extract results in increasing the number of reducing agents that may alter their active conformations and affect the process of synthesis of nanoparticles. A study was performed earlier that manifested the effects of different reactant proportions on the synthesis of SNPs by using Artemisia absinthium L. aqueous extract [23].

Modulated Synthesis of SNPs by using M. longifolia aqueous extract

The spectrums obtained under different physicochemical reaction conditions were observed critically. The location, height, absorbance unit (Au) and the shape of the SPR band were observed. The location, height and symmetry of the peak depends on the size and shape of the nanoparticles. It was manifested that the 3 mM of the AgNO₃ with plant extracts in the ratio of 1 to 9 (Plant: AgNO₃) at 120°C in acidic pH results in the optimised synthesis of SNPs after the incubation period of one hour (Figure 4(B)). The mechanism of the biosynthesis of the SNPs involves the reduction, chelation and stabilisation with the potential functional groups originate from the plant secondary metabolites. The reduction takes place by the redox chemical reactions that occur between the plant secondary metabolites and the silver salt. The redox reactions result in the formation of ions and molecules. During the phase of chelation, ions and molecules coordinate and results in the bonding of ions and molecules to the metal ions. The functional groups originate from the plant secondary metabolites cover the metallic core and help the biogenic nanoparticles to remain stable for a longer period [35,36]. However, the exact mechanism of the synthesis of nanoparticles by phyto-reduction still needs to be investigated.

Structural and optical evaluation of SNPs

The structural characterisation of SNPs synthesised by steering physicochemical reaction parameters was examined by using microscopic techniques. Nanoparticles were observed spherical (Figure 5(A,B)) and existed in the size range of ~20–100 nm by using the SEM. The AFM images reported nanoparticles of medium sizes and most of the nanoparticles were found to be in a range of ~10–70 nm because large-sized nanoparticles have escaped in the air during the process of sample preparation (Figure 6(A,B)). The EDX analysis confirmed the elemental composition of SNPs (Figure 7). The characteristic peak of the Ag has been observed at 3 KeV and the intensity of the Ag signal was ~52.9% (Figure 7, Inset Bar-chart). The EDX analysis also reported the elemental O (5%) which shows that these nanoparticles have the potential to generate Reactive Oxygen Species (ROS) while the other elements were reported in trace amounts. The presence of the elemental oxygen confirms that these nanoparticles are oxidative [17]. The elemental carbon and oxygen usually appear in the sample because of the presence of the plant secondary metabolites that function in the process of reduction and stabilisation [17].

Particle size analysis of SNPs

The particle size analysis of colloidal SNPs in the Brownian motion was performed by using a dynamic light scattering technique. Figure 8 shows the hydrodynamic diameter of SNPs which collectively represent the size of the metallic core and the biological corona and was measured between ~17 and 500 nm (Figure 8(A)). It reported the nanoparticles of a larger size range because of the presence of some big clusters or clumps of SNPs in a colloidal sample. The average size of most of the nanoparticles was reported between 12 and 42 nm (Figure 8(B)) and the average size of a single nanoparticle was measured at 15.55 nm. The polydispersity index (PDI) of nanoparticles was recorded at 0.263 which represents that these nanoparticles are moderately dispersed and are suitable for biomedical applications [32]. The dispersity of the nanoparticles plays a very important role in their biomedical potential. Uniformly dispersed nanoparticles have better potential to be used in biomedical applications in contrast to polydisperse and moderately disperse nanoparticles [32].

Dose-responsive potential of SNPs against Leishmania tropica

Leishmaniasis is an intracellular disease that is transmitted by the bites of Phlebotomine sand-fly. According to the data collected by the WHO, Leishmaniasis is responsible to cause 26,000 to 65,000 deaths every year and the number of confirmed cases exists between 700,000 to 1 million [29,37]. IC₅₀ measurement showed that a very small amount of SNPs (8.73 μg/ml) are required to affect the cell’s growth. A dose-responsive activity of SNPs was measured against Leishmania and an indirect relationship between SNPs dose and % survival of pathogens was confirmed experimentally (Figure 9). 500 μg/ml was observed as the most lethal concentration to kill 100% Leishmanial population while the viability of pathogens increased sequentially with decreasing the drug dose (p < .05). Almost 50% of the Leishmanial population was found dead by using 10 μg/ml of SNPs which is showing that these nanoparticles are highly biocompatible as a very small dosage is required to affect the growth of pathogen and results are statistically significant (p < .05). The cytotoxicity of SNPs is usually because of the presence of a positive charge on silver ion that interacts with the negatively charged
plasma cell membrane and disrupts the ionic balance and finally the membrane structure [16,17]. Another reason is the small size of nanoparticles that have the advantage to cross the plasma cell membrane barrier of the pathogenic organisms and enter the cytoplasm where it disrupts the normal cells’ biochemical processes. The small size SNPs also have abilities to interact with the nucleic acids such as DNA and RNA because of the presence of a negatively

Figure 2. Spectrophotometric analysis of SNPs biogenesis under different physicochemical conditions (a) Temperature 120 °C, AgNO₃ 1 mM; (b) Temperature 120 °C, AgNO₃ 3 mM; (c) Temperature 120 °C, AgNO₃ 5 mM; (d) Temperature 60 °C, AgNO₃ 1 mM, pH 5; (e) Temperature 60 °C, AgNO₃ 1 mM, pH 7; (f) Temperature 60 °C, AgNO₃ 1 mM, pH 9.
charged phosphate backbone. This interaction results in the destabilisation of the DNA and RNA structure which affects cells’ further proliferation and finally leads to cell death [1,16,17]. Our results are in favour of some published scientific reports that studied the dose-dependent response of SNPs against Leishmania and found that the ROS can be responsible for the anti-leishmanial potential of nanoparticles [38–40].

Figure 3. Spectrophotometric analysis of SNPs biogenesis under different physicochemical conditions (a) Temperature 60 °C, AgNO₃ 3 mM, pH 5; (b) Temperature 60 °C, AgNO₃ 3 mM, pH 7; (c) Temperature 60 °C, AgNO₃ 3 mM, pH 9; (d) Temperature 60 °C, AgNO₃ 5 mM, pH 5; (e) Temperature 60 °C, AgNO₃ 5 mM, pH 7; (f) Temperature 60 °C, AgNO₃ 5 mM, pH 9.
Figure 4. (a) UV-Visible spectrum of the different concentrations of reactants; (b) UV-Visible spectrum representing the biogenesis of SNPs under optimised physico-chemical reaction conditions.

Figure 5. Scanning electron microscopic images of SNPs. Red circles are representing that SNPs are less than 100 nm and are spherical.

Figure 6. Atomic force microscopic images of SNPs. Red circles are highlighting SNPs.
Cytotoxicity of the plant aqueous extract and SNPs against colon cancer cells

Colorectal or colon cancer is the third most common cancer and is a leading cause of death. Colon cancer has severe pathophysiological actions on the human body and specialised biochemical mechanisms to resist the chemotherapeutic drugs which are the main reason for the poor treatment and less success rate to control the proliferating cancer cells [41]. The Sulforhodamine B assay (Figure 10) results showed that the SNPs bio-fabricated from *M. longifolia* did not express any cell death and no IC$_{50}$ was recorded. The

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**Figure 7.** Elemental composition (EDX) of SNPs. The inset shows the percentage composition of each element.

**Figure 8.** Particles size analysis of SNPs (a) size distribution by intensity is representing that most of the nanoparticles have a hydrodynamic diameter in between 17 and 500 nm, while the polydispersity (PDI) was recorded at 0.263 (b) size distribution by number is representing that most of the nanoparticles have a size range of 12–42 nm while the average size of single NP was observed 15.55 nm.

**Cytotoxicity of the plant aqueous extract and SNPs against colon cancer cells**

Colorectal or colon cancer is the third most common cancer and is a leading cause of death. Colon cancer has severe pathophysiological actions on the human body and...
Annexin V apoptosis assay (Figure 11) results showed that the plant aqueous extracts are also not effective to suspend the cells’ growth or induce cells’ death against HCT116 human colon cancer cells. It was observed experimentally that only 8.70% of the cells expressed apoptosis which shows that the plant aqueous extracts are not cytotoxic against colon cancer cells. It can be explained based on the above findings that the aqueous extract of M. longifolia is not embellished with the phytochemicals to exhibit cytotoxicity against cancer cells. Our results are in contrast to the findings of Prabhu et al. [42] who found SNPs phyto-synthesised by using Vitex negundo L., very efficacious against HCT15 colon cancer cells by inducing apoptosis, suppressing cells growth and arresting the G0/G1 phase of the cells cycle.

**ROS quantification of SNPs**

Free radicals contribute to the cytotoxic potential of pharmaceutical drugs. ROS are mainly responsible to scavenge the ionic potential of the cells. It eventually disturbs the osmotic balance which results in the disruption of the plasma cell membrane and the death of the cell. Some previous scientific studies reported a relationship between the ROS generation and the cytotoxic potential of the drugs in most of the metallic nano-oxides [29] and our results also expressed quantum yield (0.08 Φ) and these results are in direct favour of the EDX analysis (Figure 7). So it can be stated that the ROS is responsible for the anti-leishmanial potential of the SNPs along with their physical and chemical attributes while SNPs are not suitable for the treatment of HCT116 colon cancer cells that maybe because of the resistance of the colon cancer cells against SNPs bio-fabricated from M. longifolia.

**Photothermal activity evaluation of SNPs**

The photothermal activity evaluation manifested no change in the temperature of the SNPs and the temperature remained constant even after 15 min of exposure to the sunlight (Figure 12). In previous reports, the researcher found the photothermal activity of O2-doped nano-oxides which are in support of our reported findings [30,43,44]. The doping involves the introduction of impurities into a semiconductor crystal to enhance their electrical, optical and biological activities [28]. Change in the temperature embellishes the nanoparticles with special features that allow them to vibrate in response to specific light wavelengths to treat invasive diseases such as Leishmania and cancer. It was experimentally confirmed that SNPs bio-fabricated from M. longifolia cannot be used as a photodynamic agent because of the absence of photothermal potential.

**Conclusion**

Physicochemical parameters act as switches to control the reaction kinetics, molecular mechanics, protein dynamics and catalysis to synthesise sustainable nanoproducts. Herein we
have evaluated that the temperature, pH, concentration of the AgNO₃, agitation, incubation period, plant extract and the silver salt concentration affect the synthesis, yield and possibly the biochemical attributes of the nanoparticles. The morphological characterisation manifested that the nanoparticles are in the range of 10–100 nm synthesised by using *M. longifolia* leaves aqueous extract. The oxidative silver nanoparticles were found perilous (10 μg/ml of SNPs killed 66% of cells) against Leishmania. The SRB assay results revealed that the silver nanoparticles are not effective against HCT116 colon cancer cells which was further confirmed by Annexin V apoptosis assay. The ROS generation from the silver nanoparticles was quantified (0.08 Φ) while no photothermal activity was recorded which make them non-photodynamic. Findings from this study may help to understand the physicochemical switches for the modulated synthesis of biocompatible nanoparticles and the development of formulations against Leishmania responsible to cause Leishmaniasis in humans. The future perspectives of this study involve the use of phytofabricated silver nanoparticles for biomedical applications.

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**Author contributions**

ZRM and BJ devised the study. BJ performed the experiments, wrote and revised the manuscript. AN and AS assisted in biological assays. ZRM and NIR supervised the work. All authors read and endorsed the manuscript for the final submission.

**Disclosure statement**

The authors declare no conflict of interest.

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