One-pot phytosynthesis of nano-silver from Mentha longifolia L.: their characterization and evaluation of photodynamic potential

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Abstract
The present study deals with the eco-friendly one-pot synthesis and stabilization of silver nanoparticles (AgNPs) by using aqueous extract of Mentha longifolia branches. Spectrophotometric analysis of different ratios of reactants revealed that a 1 to 9 ratio of plant extract and silver salt solution respectively is the most suitable proportion for synthesis. Synthesis of AgNPs was confirmed initially by the observation of change in the color of the reaction mixture which was carried out at 60 °C by using 3 mM of silver salt and the pH of the reaction medium was maintained at 5.22. A characteristic surface plasmon resonance (SPR) band was observed at 495 nm of light wavelength. SEM images revealed that the nanoparticles are in ∼20–80 nm and are anisotropic and nearly spherical while EDX analysis showed the presence of elemental Ag with ∼90% signal intensity. Size distribution analysis of AgNPs was performed by dynamic light scattering technique and AgNPs were found in the range of ∼8–30 nm. ROS quantification revealed that the AgNPs have a quantum yield of 0.09 Φ which provides them the ability to proteolytically treat cancer and other microbial pathogenic cells. AgNPs did not report any photothermal activity to be used as photodynamic agents. These findings explain the redox potential of M. longifolia to bio-fabricate AgNPs and their abilities to generate ROS may help to curb dreadful diseases.

1. Introduction
Photodynamic therapy is an advanced, non-invasive treatment method and plays a vital role to cure malignant and neoplastic growth. Photothermal therapy involves the systematical or local administration of nanoparticles to the infected area followed by the irradiation of the tumor site with the light of the suitable wavelength usually near-infrared or visible light that help to excite nanoparticles and generate Reactive Oxygen Species (ROS) such as singlet oxygen (1O₂) that can be cytotoxic to cancerous and other malignant growth by disruption of the homeostasis and ionic balance of the cells [1, 2]. The success rate of photodynamic therapy depends on the compatibility of nanoparticles with the particular wavelength of the light that works in a synergic manner. Successful treatment of the ailment generally relies on the type and the biocompatibility of the nanoparticles with the human body. It is well known that the use of physical and chemical methods of metal reduction results in the generation of many hazardous substances. These processes are also cost-ineffective and result in the association of hazardous byproducts with the nanocore which makes them unfit for biomedical applications [3, 4].

The use of hazardous chemicals to manufacture nano-moieties curb their use to a very small sector and have limitations as therapeutics because of their unknown toxicological effects which results in a great loss of economy and environmental degradation [5, 6]. Green chemistry involves the use of natural ingredients or chemicals that are safer to use and results in the manufacturing of products that are less harmful to the
environment and humans [7, 8]. Manufacturing of nanostructures via a green approach not only limits the use of hazardous industrial chemicals but also results in one-step manufacturing of nanostructures [9]. The present study involves the use of a plant’s aqueous extract for the reduction and stabilization of metal nanoparticles and the determination of their hidden photodynamic potential.

Various studies [10–12] have been carried out to screen the abilities of plants to synthesize nanoparticles of different sizes and shapes and to find their role in a multitude of biological fields. The plant body contains several functional groups belong to the secondary metabolites. Functional groups provide the surface modifications and functionalities to phyto-fabricated nanoparticles and help them to selectively treat pathogens. Previous studies explain that the carboxylic, amine and hydroxyl groups of secondary metabolites play an important role in the reduction process [13].

Silver is an important naturally occurring noble metal that is flexible and shiny. Silver has many different physical, chemical, optical and biological properties e.g. electrical conductivity, surface enhanced Raman scattering, high thermal behavior, optical behavior, and nonlinear catalytic activity, that make it a suitable candidate for a lot of applications such as currency, cleaning agent, medical devices, healthcare medicines, food coat, food preservation, textile industry, food storage, cleansing agent, catalyst, water treatment, filtration and have used in various biomedical and pharmaceutical industries [14–16]. The present study was designed (detailed layout is given in scheme 1) for the ecofriendly biogenic synthesis of AgNPs by using the reducing and stabilizing abilities of aqueous extract of Mentha longifolia branches and determination of their photodynamic potential for future biological applications to treat cancer and other bacterial pathogens.

2. Materials and methods

2.1. Processing of plant material and preparation of plant aqueous extract

M. longifolia was collected from the banks of the Jinnah stream (33.751870 °N 73.135138 °E) on the premises of Quaid-i-Azam University Islamabad. Plant samples were washed, dried to remove the moisture, chopped into small pieces and converted into powder form by using kitchen grinding machine. Aqueous extract of the branches was prepared by taking a 1 to 10 ratio of plant material and distilled water respectively and heated on a hot plate at 45 °C–65 °C for 10–20 min. After the boiling, the aqueous extract was filtered under vacuum by using celite and used immediately as fresh for the reduction of silver salt into AgNPs [13].

2.2. Chemical reagents

The chemical reagents purchased for the synthesis of AgNPs were of analytic grade and were used as received without any further purification. AgNO₃ was purchased from J.T. baker chemical company Phillipsburg, USA.
AgNPs. Analysis of the spectrum manifested that the 1 to 9 ratio of plant extract and silver nitrate respectively as different mixing ratios of the plant extract and silver nitrate solution have different impacts on the synthesis of 3.1. Effects of mixing of different ratios of reactants on the synthesis of AgNPs

For the determination of the optimum mixing ratio of AgNO₃ and plant extract 2 mM solution of AgNO₃ was prepared. 100 μl of plant extract was mixed with 900 μl of AgNO₃ and the concentration of plant extract increased gradually from 100 μl to 900 μl and the concentration of silver salt was decreased gradually from 900 μl to 100 μl to record the effects of different mixing concentrations over the synthesis of silver nanoparticles. Mixtures were prepared in glass vials and incubated in an oven at 60 °C for 24 h. After the end of the incubation period aliquot was taken from each vial and diluted with distilled water to record the absorbance in the range of 200–900 nm wavelength of light by using UV-Visible spectrophotometer (Shimadzu 1601, Japan) [17].

2.3. Effects of mixing of different ratios of reactants on the synthesis of AgNPs

For the determination of the optimum mixing ratio of AgNO₃ and plant extract 2 mM solution of AgNO₃ was prepared. 100 μl of plant extract was mixed with 900 μl of AgNO₃ and the concentration of plant extract increased gradually from 100 μl to 900 μl and the concentration of silver salt was decreased gradually from 900 μl to 100 μl to record the effects of different mixing concentrations over the synthesis of silver nanoparticles. Mixtures were prepared in glass vials and incubated in an oven at 60 °C for 24 h. After the end of the incubation period aliquot was taken from each vial and diluted with distilled water to record the absorbance in the range of 200–900 nm wavelength of light by using UV-Visible spectrophotometer (Shimadzu 1601, Japan) [17].

2.4. Synthesis of AgNPs

Silver nitrate solution of a 3 mM concentration was mixed with freshly prepared plant extract in a ratio of 1 to 9 of plant extract and silver nitrate solution respectively. The reaction was carried out at a pH of 5.22 as described in the previous study [18]. The temperature was maintained at 60 °C and reflux was installed to prevent the loss of reaction solvent and the reaction mixture was stirred continuously. An aliquot was taken out from the flask and the absorbance was measured by using crystalline quartz cuvettes with the help of UV-Visible spectrophotometer (Shimadzu 1601, Japan) in the range of 200–900 nm of the light wavelength. Absorbance was measured after a specified interval of time to observe the reaction kinetically and to determine the synthesis of AgNPs with respect to the incubation period. After the completion, the reaction was quenched by removing the flask from the hot plate and diluted with a small amount of distilled water. The reaction mixture was centrifuged at 1000 × g (Sorvall RT 7 Plus) for 1 h thrice to separate the AgNPs from the reaction mixture. AgNPs were dried and stored for further use [9, 19].

2.5. Morphological and optical characterization of AgNPs

Characterization of synthesized AgNPs was performed by using different material characterization techniques to explore their morphological and optical characteristics. Characterization was done initially by visual observation, photographs were taken before and after reaction completion and were compared. UV-Visible Spectrophotometer (Shimadzu 1601, Japan) was used to measure the absorbance of the reaction mixture as a representative of reaction kinetics and the height of the peak was compared with each other with respect to time intervals of incubation period [20]. Scanning Electron Microscope (SEM) JEOL 7500F HRSEM was used to manifest the size and shape of silver nanoparticles, the reaction mixture was dropped on a copper grid and was observed on different voltages and powers of microscope, images were taken and analyzed [21]. Energy Dispersive x-ray Analysis (EDX) was performed to confirm the presence of Ag in the sample and the elemental composition of the sample was also determined, using detectors associated with JEOL 7500F HRSEM [22]. The hydrodynamic diameter of NPs was measured by using the Dynamic Light Scattering (DLS) machine of Malvern Instruments particle sizer, Zetasizer Nano S, Malvern Instruments, UK. The sample was prepared by suspending nanoparticles into a 1× PBS solution [23]. The sample was analyzed at 25 °C and the rest of the machine parameters were adjusted by the machine’s automatic setting.

2.6. ROS quantification of AgNPs

ROS generation by AgNPs was measured by preparing the DPBF solution (0.1 mM DPBF in ethanol). 10 μg ml⁻¹ of AgNPs was dissolved into 2 ml of DPBF solution. The reaction was carried out into sealed quartz cuvettes with IR filter (400–800 nm) for 30 s under sunlight. After each 30 s photobleaching of the DPBF was measured by using UV-3000 Spectrophotometer for 5 min Methylene blue was used as a control [4, 24].

2.7. Photothermal activity measurement of AgNPs

AgNPs were suspended into distilled water at a concentration of 1 mg ml⁻¹. A test tube containing the test sample was exposed to sunlight and after each minute temperature of the sample was recorded by dipping the temperature probe (Fisher Scientific). This procedure was continued for 15 min and a plot was drawn between time and temperature changed [24].

3. Results and discussion

3.1. Effects of mixing of different ratios of reactants on the synthesis of AgNPs

Different mixing ratios of the plant extract and silver nitrate solution have different impacts on the synthesis of AgNPs. Analysis of the spectrum manifested that the 1 to 9 ratio of plant extract and silver nitrate respectively as...
the most suitable proportion for the optimized synthesis of silver nanoparticles (figures 1 and 2). 2 to 8 ratio of
the plant extract and silver nitrate was having the lowest peak height while the peak height was increasing slightly
up to the concentration of 9 to 1 ratio of the plant extract and AgNO₃ but the characteristics peak was not
observed by any other proportion. These results show that the proper contribution of both reactants is
important to report synthesis. Change in the proportion of the silver salt and reducing agent can have marked
effects on the process or it can halt the process of synthesis. Higher the concentration of plant extract can
increase the number of secondary metabolites that act as reducing and stabilizing agents of silver salt in the
reaction medium and eventually affect the molecular dynamics, protein conformations, and reaction kinetics. A
very similar study was performed earlier that reported the effects of the different mixing ratios of reactants for
the synthesis of AgNPs by using *Artemisia absinthium* [17].

### 3.2. Phytosynthesis of AgNPs (spectrophotometric studies)

The reduction of 3 mM of AgNO₃ was carried out at 60 °C and the pH of the reaction mixture was maintained at
5.22 resulted in the synthesis of AgNPs by mixing plant aqueous extract and silver salt in 1 to 9 ratio respectively
(figure 3). UV-visible spectrums showed an SPR band at 495 nm in between 350 to 550 nm of the wavelength of
light characteristic of AgNPs synthesis (figure 3). Springer band is a response to the interaction of free electrons of
silver in oscillation with the electromagnetic waves of the light [25]. Synthesis of AgNPs was confirmed by the change in the color of the reaction mixture from golden to tea brown or dark brown which is characteristic for the synthesis of AgNPs. Change in color of the reaction mixture (figure 1) appears as a response to the interaction of the AgNPs with a light wavelength [7]. The reaction mixture was observed at a defined time interval to determine the effects of incubation period on the synthesis of AgNPs. The highest SPR band was recorded in between 2 h to 2 h 15 min (120–135 min), which shows that incubation period is also very important for sustainable synthesis of AgNPs and continuation of the process of synthesis beyond optimum threshold results in the reduction of synthesis, yield and can also affect the physical and chemical attributes of nanoparticles (figure 3). It was also observed that increasing the incubation period increased the absorbance at a higher wavelength (485 nm to 495 nm). An increase in absorbance at a higher wavelength is called the Redshift. According to Mie’s theory, small particles absorb light at a smaller wavelength while large particles absorb light at a longer wavelength [26]. It confirms that the increasing incubation period affects the morphological attributes of AgNP. A narrow and symmetrical peak showed that the nanoparticles are monodisperse and spherical [13]. Our results are in favor of some previously published reports [7, 27, 28].

3.3. Morphological and optical characterization of AgNPs
The physical characterization of AgNPs was performed mainly by using SEM. Micrographs showed that NPs are anisotropic and spherical (figures 4(A), (B)) and have a size range of ~10–100 nm. Our results are in favor of a scientific study that used a green method for the reduction of silver salt [29]. The elemental composition of AgNPs was obtained by using EDX analysis (figure 4(C)). The characteristic peak of the Ag was observed at 3 KeV and the intensity of the Ag signal was ~90.37%. It was also observed that the colloidal AgNPs have 13.73% of elemental O2 which is responsible for the generation of the free radicals and Reactive Oxygen Species (ROS). EDX analysis also reported the presence of S, Si, C, Cu, and Mg in a small amount. These elements originally belong to plant secondary metabolites that help to cap silver nanocore and also provide functionalization to nanostructures. Cu was reported because of the involvement of laser signals with copper grids. Our results support previous findings [30].

3.4. Particle size analysis of AgNPs
The hydrodynamic diameter of AgNPs was measured by using a dynamic light scattering technique. It collectively represents the size of a metallic silver core and biochemical attire of AgNPs that is designed by functional groups originally belong to plant secondary metabolites. Size distribution (by intensity) of AgNPs in Brownian motion was measured as ~10–1000 nm which represents a number bigger than SEM because of the
hydrodynamic diameter and presence of some agglomerated or clumped AgNPs in the reaction mixture (figure 5(A)). It was further confirmed that most of the AgNPs are in the range of ∼8–30 nm (figure 5(B)). It shows that the majority of nanoparticles are small in size and are suitable for biomedical applications. Polydispersity index of AgNPs was recorded at 0.461 which shows that these nanoparticles are moderately polydisperse which express their suitability for drug delivery applications. These results explain that the plant secondary metabolites have strong abilities to reduce and stabilize Ag by various redox chemical reactions into nano-silver that have various unique physical, chemical, and optical properties because of their small size. Our results are in favor of previous studies [10, 12].

3.5. Photo-thermal activity of AgNPs
Photo-thermal activity measurement reported no change in temperature and the temperature of the AgNPs remained constant even after 15 min of exposure to sunlight which expressed that they cannot be used as photodynamic agents to treat cancer cells (figure 6). The previous researcher reported [4, 24, 31] the photothermal abilities of O2-doped nanoparticles e.g. Zn oxide, Fe oxide, and Cu oxide nanoparticles. The absence of the photothermal abilities of Mentha-fabricated AgNPs shows that they cannot be used in photothermal therapy of cancer and other pathogenic bacterial cells.

3.6. ROS quantification of AgNPs
The generation of free radicals plays an important role to increase the cytotoxic abilities of anticancer drugs. A previous report [4] suggested a direct relationship of the ROS generation with the cytotoxicity of the drugs in most of the O2-doped nanoparticles. Our results expressed the generation of ROS by AgNPs phyto-fabricated from M. longifolia branches aqueous extract and quantum yield was reported at 0.09 Φ (figure 7). The quantum yield has a direct relationship with the EDX analysis which showed the presence of elemental oxygen at 13.73%. Our results explain that the generation of ROS by M longifolia synthesized nanoparticles may have the potential to treat cancer and perilous microbial disease.
Figure 5. Size distribution analysis of silver nanoparticles by using DLS machine (a) size distribution of the silver nanoparticles by intensity was measured between \( \sim 10 \)–\( 1000 \) nm (b) distribution by the number representing the average size of most of the nanoparticles in the colloidal mixture and was reported between \( \sim 8 \)–\( 30 \) nm.

Figure 6. Photodynamic potential measurement of silver nanoparticles. A linear line is representing constant temperature in response to time.
4. Conclusion

The present study was designed to synthesize silver nanoparticles by using ecofriendly one-pot economical approach to reduce and stabilize silver salt into nano-silver. It was observed that the ratio of reactants such as plant extract and AgNO₃ greatly affect the process of synthesis. Different incubation periods have different effects on the yield and synthesis process and increasing the incubation period up to optimum threshold can alter the process of reduction, yield and morphological and biochemical attire of nanoparticles. Quantum yield was measured at 0.09 which shows the ability of phytosynthesized nano-silver to kill cancer and microbial pathogens by the generation of free radical oxygen species while they were confirmed non-photodynamic. This study supports the use of phytosynthesized silver nanoparticles to proteolytically kill bacterial pathogens and malignant cancerous cells.

Conflict of interest

The authors declare no conflict of interest.

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