Natural and Applied Sciences International Journal (NASIJ)
eISSN: 2788-4619 (online)
https://www.ideapublishers.org/index.php/nasij
Vol. 4, No. 1 (January-June), 2023, pp. 80-97
https://doi.org/10.47264/idea.nasij/4.1.6 Research Article

Using *Moringa oleifera* stem extract for green synthesis, characterization, and anti-inflammatory activity of silver oxide nanoparticles

Sheraz Muhammad*[1] | Asif Ali[2] | Jehangir Shah[1] | Muhammad Hamza[3] | Muhammad Kashif[1] | Behram Khan Ajat Khel[1] | Arshad Iqbal[1]

1. Department of Chemistry, Abdul Wali Khan University, Mardan, Pakistan.
2. School of Chemistry and Chemical Engineering, Beijing Institute of Technology, Beijing, China.
3. Department of Chemical Engineering, Ming Chi University of Technology, Taiwan, Republic of China.

* Corresponding Author Email: msherazahmad7770@gmail.com

Received: 06-Mar-2023 | Revised: 21-Jun-2023 | Accepted: 22-Jun-2023 | Published: 30-Jun-2023

Abstract

Recently, nanoparticles from 1 to 100 nm gained attention. Due to their eco-friendliness and medicinal potential, natural ingredients are used more to synthesise AgONPs. This work synthesises AgONPs from *Moringa oleifera* stem extract and tests their anti-inflammatory activity. Prepared AgONPs were characterised through UV-Visible, FTIR, XRD, SEM and EDX. According to the results, *Moringa oleifera* stem extract reduced silver ions to AgO with an average crystalline size of 23 nanometres. In addition, the study compared the anti-inflammatory properties of green AgONPs with those of standard pharmacological drugs. At a dose of 80 mL^{-1}, synthesised nanoparticles showed strong anti-inflammatory effects compared to the drug diclofenac sodium, which had an 85% inhibition rate. The AgONPs only had a 78% inhibition rate. Based on the results, AgONPs are compatible with diclofenac sodium. These findings show that AgONPs synthesised from *Moringa oleifera* stem extract may cure inflammation related diseases.

Keywords: AgONPs, *Moringa oleifera*, green synthesis, silver oxide nanoparticles, characterization techniques, anti-inflammatory activity, pharmacological drugs.

How to Cite: Muhammad, S., Ali. A., Shah, J., Hamza, M. Kashif, M., Khel, B. H. A., & Iqbal, A. (2023). Using *Moringa oleifera* stem extract for green synthesis, characterization, and anti-inflammatory activity of silver oxide nanoparticles. *Natural and Applied Sciences International Journal (NASIJ)*, *4*(1), 80-97. https://doi.org/10.47264/idea.nasij/4.1.6

1. Introduction

Material science's most significant field is nanotechnology. Because their surface area is much greater than their volume, nanoparticles have unique physical and chemical characteristics that cannot be found in bulk forms. Biological, chemical and even physical approaches have been developed to prepare NPs (Sharifi-Rad *et al.*, 2020). Compared to chemical and physical approaches, the biological approach shows more potential owing to its lower price tag, better flexibility and less environmental impact. Green nanotechnology is an area that is growing quickly and has the ability to make small particles while having little effect on the environment. The formation of metal NPs via the utilization of environmentally friendly synthesis has given rise to a wide variety of possible uses in various spheres of everyday life. Viruses, yeast, fungus, bacteria, algae and plant extracts are only some of the kinds that have been used extensively in producing nanoparticles with particular targets during the last several decades. Compared to other microbes, plant extracts have a particular number of advantages because of the occurrence of substances such as flavonoids, phenolic compounds, alkaloids, terpenoids and organic acids that serve as excellent capping and reducing agents. The photosynthesis process is characterized by its non-toxic nature and capacity to accumulate heavy metals, as indicated by previous research (Bindhu et al., 2020; Mohammadinejad *et al.*, 2016).

The inflammation is a natural defence mechanism of the body against a tissue injury, an infection or any other harmful stimuli. However, chronic inflammation can cause various diseases including cancer, arthritis, cardiovascular diseases and neurodegenerative disorders. NSAIDs or non-steroidal anti-inflammatory drugs, are often employed to cure conditions caused by inflammation. However, long-term use of the NSAIDs can result in harmful side effects like gastric ulceration, renal toxicity and cardiovascular complications. Therefore, there is a need for the improvement of novel as well as safer agents of anti-inflammatory (Bindu *et al.*, 2021).

In recent years, silver oxide nanoparticles (AgONPs) gained attention as a potential therapy candidate for treating inflammation-related illnesses due to their anti-inflammatory activities. AgONPs have shown significant anti-inflammatory activity by modulating various pro-inflammatory cytokines, enzymes, and signalling pathways. Furthermore, AgONPs are safer than NSAIDs, with fewer side effects. Green NPs production utilizing plant extracts has gained significant interest owing to its eco-friendliness and potential therapeutic benefits. Various plants extract have also been exploited for the AgONPs fabrication, containing those from *Prosopis juliflora*, *Citrus limon Zest*, *Alhagi graecorum*, *Eupatorium adenophorum*, *Uvaria narum* and *Solanum khasianum* (Ajaykumar *et al.*, 2023; Anwar *et al.*, 2019; Chirumamilla *et al.*, 2023; Dua *et al.*, 2023; Hawar *et al.*, 2022; Khane *et al.*, 2022; Mohammed & Hawar, 2022). The phytonutrients in these plant products include polyphenols, flavonoids, terpenoids, and alkaloids. These are used for producing nanoparticles because they might act as both stabilizing and reducing agents. Several experimental studies have shown that biosynthesized AgONPs can reduce inflammation. For instance, AgONPs synthesized using extract of *Panicum miliaceum grains* showed significant anti-inflammatory activity (Velsankar *et al.*, 2022). AgONPs synthesized using extract of *Holoptelea integrifolia* had potent anti-inflammatory activity (Kumar *et al.*, 2019). Root extract of *Astragalus tribuloides Delile* employed for the formation of AgONPs, which exhibited significant anti-inflammatory activity (Sharifi-Rad *et al.*, 2020). In vitro, spherical AgONPs produced from *Prunus serrulata* fruit extract showed significant anti-inflammatory action (P. Singh *et al.*, 2018).

The biosynthesized AgONPs via *Moringa oleifera* stem extract and its anti-inflammatory activity is a novel research area with potential therapeutic applications. Although several studies have reported the production of AgONPs via several plant extracts, there are limited examinations on the exploitation of *Moringa oleifera* stem extract for the fabrication of AgONPs and its anti-inflammatory activity. Also, the *Moringa oleifera* stem is often called the horseradish tree or the drumstick tree. The stem of *Moringa oleifera* is rich in bioactive compounds that have been demonstrated to be effective in medicinal treatment and function like reducing agents in NPs formation. Nonetheless, using *Moringa oleifera* stem extract for the fabrication of AgONPs and its anti-inflammatory activity is a novel approach that has not been comprehensively studied. Thus, the current study aims to examine the bioinspired AgONPs via *Moringa oleifera* stem extract as well as evaluate its anti-inflammatory activity. The plant material's phytochemical characteristics were assessed through the utilization of various spectroscopic techniques, comprising UV-Visible, FTIR, XRD, SEM, and EDX. The examinations hypothesized that the utilization of stem extract of *Moringa oleifera* for the bio-fabrication of AgONPs would result in the production of NPs with potent anti-inflammatory activity. The anti-inflammatory properties of green prepared AgONPs through a heat-induced hemolysis assay compared to conventional pharmaceutical agent.

2. Literature review

Velsankar *et al.* (2022) synthesized silver oxide NPs utilizing *Panicum miliaceum grains* extract in a quick and environmentally friendly manner to characterize the silver oxide NPs and assess their anti-inflammatory efficacy (Velsankar *et al.*, 2022). Maheshwaran *et al.* (2020) biosynthesized silver oxide nanoparticles via an extract of *Zephyranthes rosea* flower. The NPs that prepared were analyzed as well as characterized. Ag_2O NPs exhibit excellent anti-inflammatory activity. Using Aspergillus terreus FC36AY1, Vellingiri *et al.* (2021) combine microfabrication with the synthesis of AgONPs and performed a thorough evaluation of their anti-angiogenesis and anti-microbial properties.

Alsareii *et al.* (2022) prepared and characterized green-AgNPs by means of *Rhizophora apiculate* extract. AgNPs inhibited protein denaturation at a rate close to that of the anti-inflammatory medication diclofenac (94.24%), with an average percentage of 71.65%. The results suggested that *R. apiculata* AgNPs have significant potential as anti-inflammatory agents (Alsareii *et al.*, 2022). P. Das *et al.* (2019) synthesized and characterized AgNPs via *belladonna mother tincture*. The silver oxide nanoparticles exhibited excellent anti-inflammatory efficacy. Rajput *et al.* (2020) synthesized AgNPs via leaves extract of *Atropa acuminata* in an environmentally friendliness and sustainable manner. Leaves extract of *A. acuminata* biosynthesized silver nanoparticles were characterized and exhibited strong anti-inflammatory activity. Jan *et al.* (2021) synthesized silver nanoparticles by means of an aqueous extract of *Aquilegia pubiflora* via a green approach. The biosynthesized Ag-NPs were characterized. The prepared silver nanoparticles showed good anti-inflammatory potential. Manikandan *et al.* (2017) studied and characterized AgNPs via fruit extract of *Phyllanthus acidus L.* by a green approach**.** The findings suggest that *P. acidus*-mediated biosynthesized AgNPs have therapeutic potential for managing inflammatory problems.

Alkhalaf *et al.* (2020) biosynthesized AgNPs utilizing *Nigella sativa* extract. The biosynthesized silver nanoparticles were characterized and exhibited strong anti-inflammatory activity. Younis *et al.* (2021) fabricated AgNPs using *Cyanobacteria phormidium sp.* Ag nanoparticles were characterized utilizing UV-Visible plus IR spectroscopy. Green silver nanoparticles prepared using *Phormidium sp.* exhibited excellent anti-inflammatory ability. Chakravarty *et al.* (2022) used *Syzygium cumini* fruit extracts to prepare AgNPs. The manufactured AgNPs were characterize via UV–Visible, FTIR, XRD and SEM. The aforementioned medicinal plant extract effectively mediated green synthesized silver nanoparticles, which possessed excellent anti-inflammatory ability in vitro. Ahsan *et al.* (2020) fabricated Ag-NPs utilizing leaves extract of *Parthenium hysterophorus* as a green chemistry method. The manufactured Ag-NPs were characterized and revealed excellent anti-inflammatory activity. Thatoi *et al.* (2016) green prepared AgNPs via the aqueous extracts of *H. fomes* plus *S. apetala* and characterized them. The synthesized nanoparticles possess strong anti-inflammatory potential, which the biomedical sectors may use in a broad range. S. K Das *et al.* (2019) prepared silver NPs via *Avicennia officinalis* and *Xylocarpus granatum* extracts via green approach. The characterisations of the prepared Ag-NPs were characterized using UV–Visible, XRD, SEM, and FTIR. The study's findings indicated that the synthesized silver nanoparticle showed outstanding pharmacological characteristics, including anti-inflammatory efficacy.

Irfan *et al.* (2021) successfully synthesized silver nitrate and ZnO NPs using a biogenic fabrication method and tested the anti-bacterial activities of the fabricated NPs in contrast to multidrug-resistant bacteria called methicillin-resistant *S. aureus*. This was the first time that the gum *Moringa oleifera* was employed both as a capping and reducing agent in the successful formation of nanoparticles. Mohammed *et al.* (2022) used response surface methodology for the biosynthesis of AgNPs from *Moringa oleifera*, evaluating their antioxidant and antibacterial properties and optimizing synthesis conditions. These results help to advance the fabrication of long-lasting nanomaterials with potential uses in antioxidant and antibacterial therapy.

3. Materials and methods

3.1. Chemicals used

A diverse range of substances, including silver nitrate ($AgNO_3$), methanol and distilled water were employed alongside an extract derived from the *Moringa oleifera* stem to synthesise silver oxide nanoparticles.

3.2. Collection of plant

The *Moringa oleifera* stem *plant* was collected near Takkar district Mardan, KPK, Pakistan. Selected healthy and mature *Moringa oleifera* stems. Cut the stems into small pieces using a sharp knife. Rinse the stem pieces thoroughly with distilled water to remove any dirt or debris.

3.3. Isolation and extraction

After the washing process, the *Moringa oleifera* stem plant was subjected to a drying period of 8-10 days in a cool and shaded environment. The plant stem was pulverized to fine particles using a mortar and pestle to grind the stems manually and subsequent to drying. The plant stem was pulverized and yielded a mass of roughly 100 grams. The resulting powder was then placed into a 500-millilitre beaker. Subsequently, it was immersed in a solution containing 300 millilitres of methanol for seven days. Next for a period of seven days, the plant stem was subjected to filtration through filter paper before their utilization.

3.4. Synthesis of silver oxide nanoparticles

We prepared a solution via dissolving 1 mM of AgNO3 in 100 mL of distilled water. Upon dissolving 0.0169 grams of AgNO3 in 100 mL of distilled water, the resultant mixture attains 1 molarity. Upon preparation of the silver nitrate solution, it is recommended to transfer a volume of 10 mL from a 1 millimolar solution into a beaker and subject it to magnetic stirring for 30 minutes. Subsequent to the stirring process, it is recommended to gradually introduce 10 ml of extract water into the AgNO3 solution and subsequently preserve it in a dimly lit location. Following an hour-long duration, the liquid undergoes a transition from a light brown colour to a darker brown colour, thereby signifying the presence of AgONPs. Spectroscopic techniques were employed to accomplish the formation of AgONPs (Jalab *et al.*, 2021).

3.5. Characterizations

The formation of AgONPs was characterized using the UV-Shimadzu-800 equipment (Japan) by measuring the UV-Visible absorption spectra ranging between 200 to 800 nm. The study involved the utilization of *Moringa oleifera* stem extract-mediated AgONPs, which were subjected to analysis using a spectrometer named Perkin Elmer ranging from 400-4000 cm^{-1}. The identification of phytochemicals was carried out through the implementation of FT-IR analysis.

During this investigation, X-ray diffraction (XRD) measurements were obtained using a JEOL Japan JDX-3532 X-ray generator equipped with Copper Kα radiation (40 kV and 1.5406 A°). These measurements aimed to analyze the crystal structure of AgONPs. The morphology of AgONPs was examined utilizing a Hitachi scanning electron microscope (model S 4800, Tokyo, Japan) operating at 10 kV. The surface of the sample was subjected to an electron beam to carry out the EDX procedure, which was utilized to analyze the elemental composition of AgONPs using SEM. The energy range for the beam was from 0-14 keV. The phenomenon of X-ray emission was detected as a result of radiation experience. The energy of X-rays was contingent upon the materials under examination within the X-ray tube. X-rays were produced at a depth of 2 micrometres. A two-dimensional image of each constituent was obtained after subjecting the samples to the electron beam.

3.6. Biological activity of AgONPs

3.6.1. Anti-inflammatory analysis of silver oxide nanoparticles and plant extract

The anti-inflammatory effect of AgONPs was investigated utilizing the heat-induced hemolysis method, utilizing human red blood cells (RBCs) as the subject of study, as reported (Okoli & Akah, 2004). The present study employed diclofenac sodium (DS) (10 mg/10 mL) in phosphate- buffered saline (PBS) with a pH of 7.4 for the experimental procedure. A participant in good health contributed a blood specimen of roughly 5 millilitres devoid of NSAIDs. EDTA was used as an anticoagulant to facilitate the centrifugation of blood in the flacon tube at a speed of 3000 revolutions per minute for around 20 min. The residual constituents were partitioned in order to eliminate the supernatant. Isotonic saline solutions with a weight/volume ratio were utilized for the purpose of removing any remaining residue. The process of centrifugation and washing was iterated three times until a transparent supernatant was obtained. In a solution, a 10% suspension of RBC pellets was produced using an isotonic saline solution.

The control, standard and test samples were prepared through the execution of the subsequent reactions. The control reaction mixtures comprised 100-microlitre of a 10% blood suspension, along with 900-microlitre of a solution containing 20-microlitre of distilled water and 800-microlitre of PBS. The experiment utilized standard reaction mixtures to create a 100-microlitre blood suspension containing 10%. Different diclofenac sodium to PBS solution ratios were used, including 20-microlitres of diclofenac sodium combined with 880-microlitres of PBS µL, 40 µL of diclofenac sodium combined with 860 µL of PBS, 60 µL of diclofenac sodium combined with 840-microlitre of PBS, as well as 80 microlitres of diclofenac sodium combined with 820-microlitre of PBS. The experiment involved the utilization of different ratios of EGE in conjunction with PBS solution. Specifically, the reaction mixture consisted of 100 µL of 10% blood suspension and varying amounts of EGE and Phosphate-Buffered Saline solution, including 20 µL of MGE and 880 µL of PBS, 40 µL of MGE and 860 µL of PBS, 60 µL of MGE and 840 µL of PBS, as well as 80 µL of MGE and 820 µL of PBS. The process of incubation at a temperature of 54°C for 30 minutes was subsequently pursued by subjecting each test to centrifugation at a rate of 6000 revolutions per minute for an approximate 9 minutes. Each sample was read three times. The absorbance of all samples was measured using spectrophotometers.

The formula utilized to compute the %age of inhibition of HRBC lysis is given below:

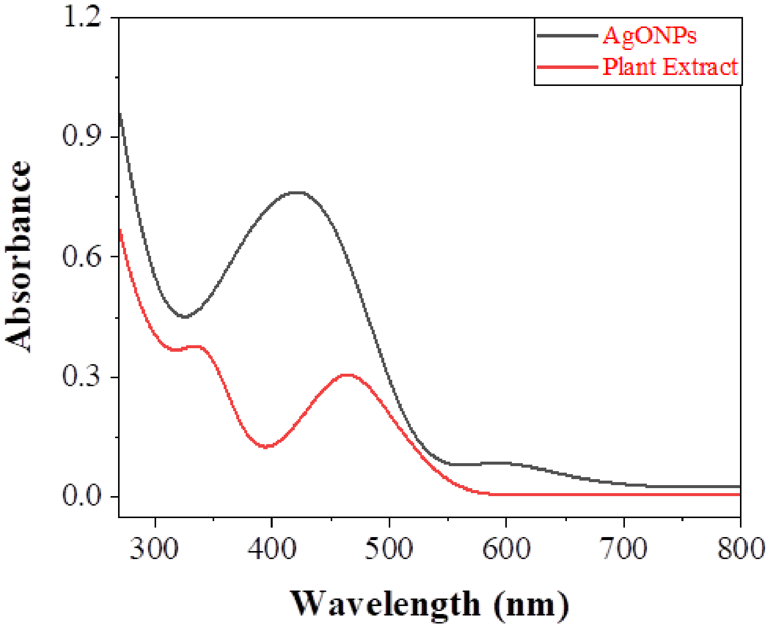
$$\% \, Inhibition = \frac{Control\ Abs - Sample\ Abs}{Absorbance\ of\ control} * 100 \qquad (1)$$

4. Results

4.1. Characterization of prepared silver oxide NPs

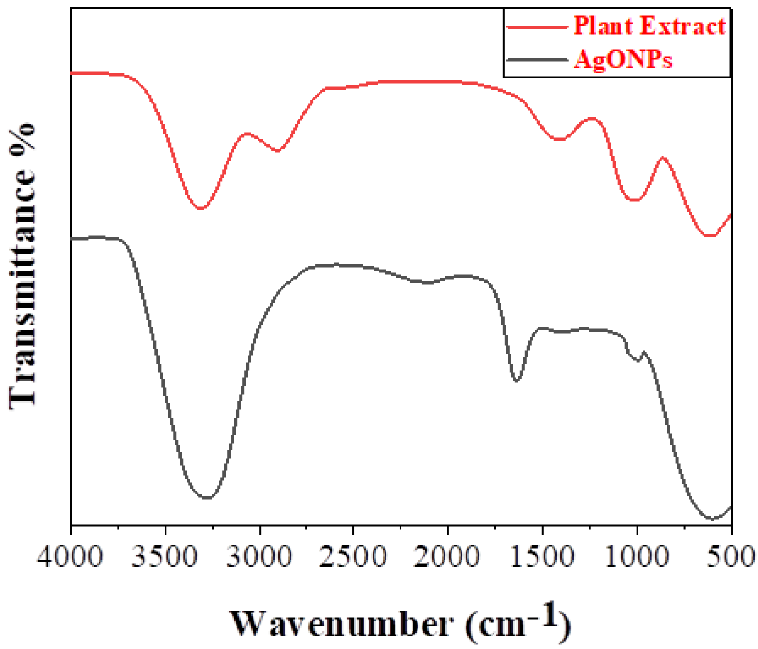
Silver oxide nanoparticles demonstrate intriguing optical characteristics closely associated with surface plasmon resonance. The optical characteristics of the samples were significantly influenced by their morphology. Typically, researchers choose a UV-visible absorption spectrophotometer as their preferred instrument when investigating SPR. The synthesized samples underwent a sequence of measurements to determine their UV-visible absorption properties; the outcomes are shown in Figure 1. The spectra of the UV-Visible for the production of AgONPs and *Moringa oleifera* stem extracts were found to be different. The spectral analysis of the product was conducted within the wavelength ranging from 200-800 nm. The UV-Visible analysis data revealed the display of an SPR within the wavelength range of 419 nm. This discovery aligns with the generation of silver oxide nanoparticles, as reported (Dhasarathan *et al.*, 2018). Hence, the reaction mixture exhibited proof of the formation of AgONPs. Additionally, UV-Visible analysis of the plant extract was performed, and the results showed two peaks at different wavelengths between 300 and 500 nm, which was due to some biomolecules exists in the extract. The research investigated the silver oxide nanoparticles' stability in response to variations in temperature, salinity levels and pH. The stability of silver oxide NPs was observed to be significantly high at 60°C and within a pH range of 3-11.

Figure 1: UV-Visible spectra of green synthesized silver oxide nanoparticles



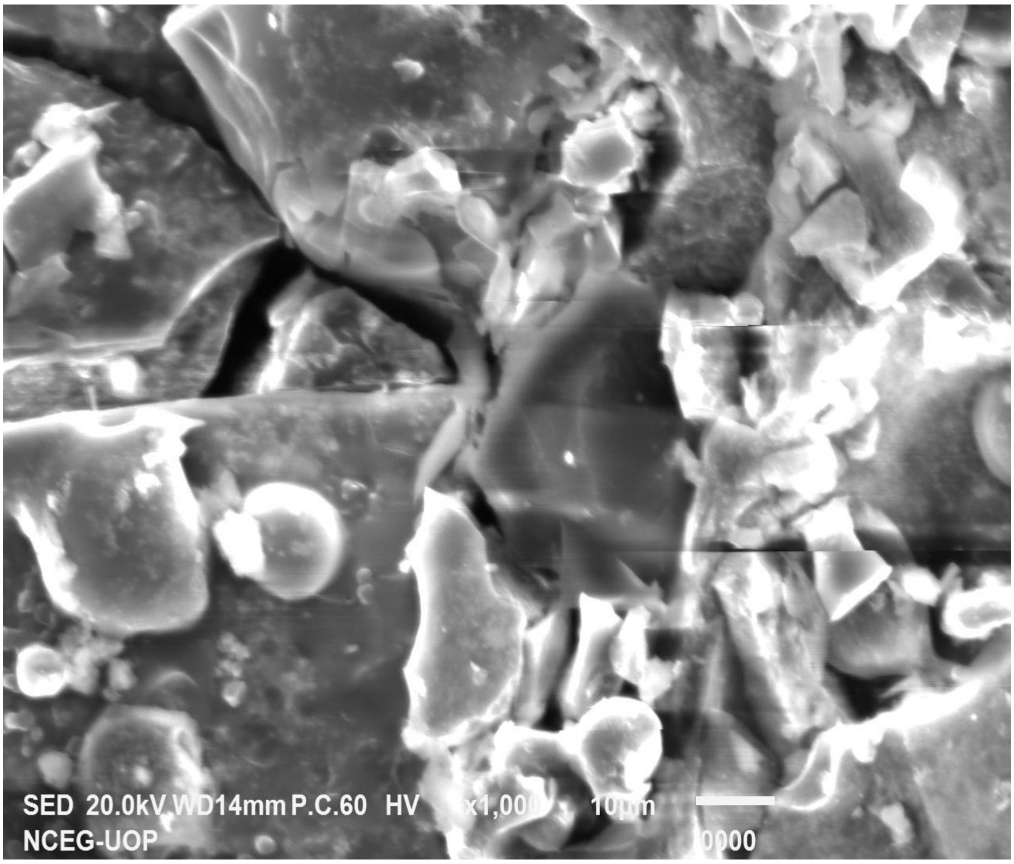
The functional groups of diverse chemical substances were identified using FTIR based on peak values ranging from 400-4000 cm^{-1}. AgO NPs in the *Moringa oleifera* stem appeared to have a distinct spectrum, as shown in Figure 2. In order to determine which biomolecules in plant extracts are essential to their stability and decline, nanoparticles can be examined in this way (El Ouardy *et al.*, 2023). FT-IR study of AgONPs proved that Moringa oleifera stem extracts have capping and reducing properties. There were broad absorption peaks at 3323, 2910, 1424, 1023 and 616 cm^{-1} for *Moringa Oleifera* stem extracts. The spectra of FTIR for silver oxide nanoparticles displayed several absorption peaks at 3294, 1642, 1001 and 609 cm^{-1}.

Figure 2: FTIR spectrum of as-synthesized AgONPs using *Moringa oleifera* stem



The size and form of fabricated green silver oxide NPs using *Moringa oleifera* stem were determined using feedback from microscopy. It may be determined using the XRD data, Scherrer's equation, that silver oxide nanoparticles which had synthesized *Moringa oleifera* stem has irregular as well as agglomerated shape and particle size is around 15-25 nm as demonstrated in Figure 3.

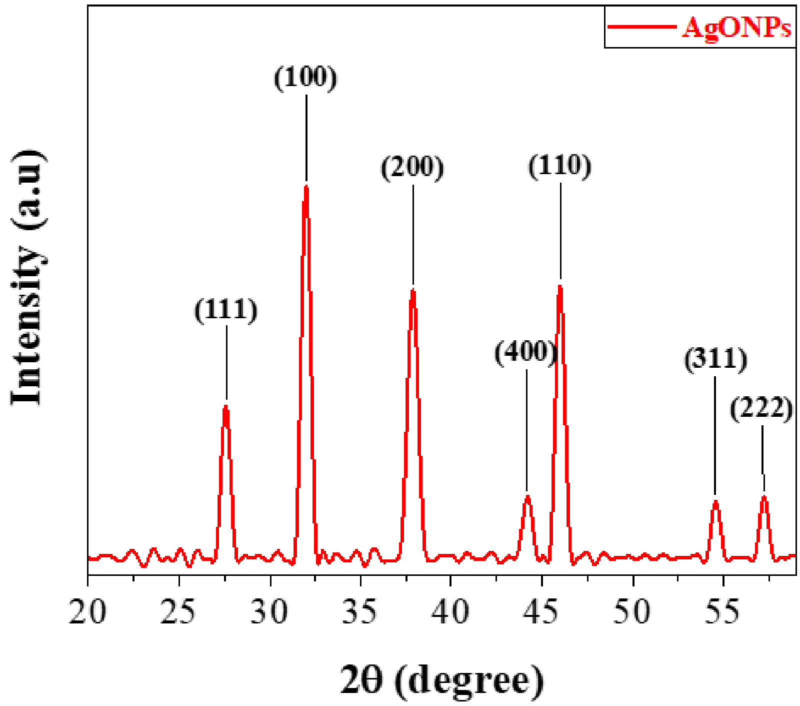
Figure 3: SEM image of green synthesized silver oxide NPs using *Moringa oleifera* stem



The utilization of non-destructive XRD is a highly effective technique in identifying crystalline substances. A plethora of data pertaining to preferred crystal orientations, structures and phases and other structural features like strain, crystalline nature, average size and crystal defects is available. XRD peaks were generated through the dispersion of a monochromatic X-ray beam at predetermined angles from every pair of lattice planes present in a given sample. Peaks intensity were ascertained via the positions of atoms within the lattice planes (Kohli & Mittal, 2019).

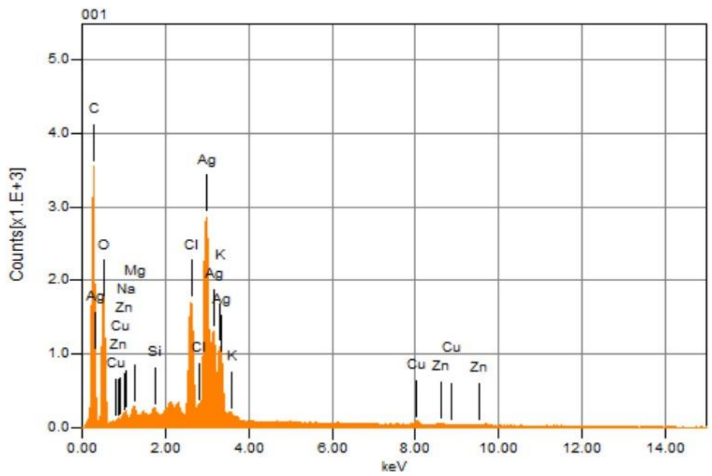
Applying the Scherer formula, crystal size might be determined from XRD patterns that exhibit line broadening. In particular, the fine structure of extended X-ray absorption can be used to identify small particles (Rashidi, 2021). Silver oxide NPs made via green were found to have X-ray diffraction in the 20° 2ϴ > 60° range. The XRD pattern for silver oxide NPs as prepared exhibited strong peaks at 2ϴ =27.56^o, 31.98^o, 37.85^o, 44.19^o, 46^o, 54.57^o and 57.21^o which might be indexed to the 111, 100, 200, 400, 110, 311 as well as 222 planes of bio-synthesized silver oxide NPs by *Moringa Oleifera* stem (Figure 4). XRD studies have confirmed the silver oxide NPs crystals seen in the produced nanoparticles.

Figure 4: Green synthesized AgONPs XRD spectra



The EDX technique, which is extensively utilized, can be employed to ascertain and scrutinize the elemental composition of specimens that are as minuscule as a few cubic micrometres. The EDX data as well as elemental composition of the green-produced AgO NPs by means of *Moringa oleifera* stem extract are shown in Figure 5. The formed silver oxide nanoparticles made from *Moringa oleifera* stem have an elemental composition of 57 percent and 43 percent carbon, magnesium, chlorine, potassium, sodium, zinc, silicon and copper.

Figure 5: EDX of as-synthesized AgONPs utilizing *Moringa oleifera* stem extract



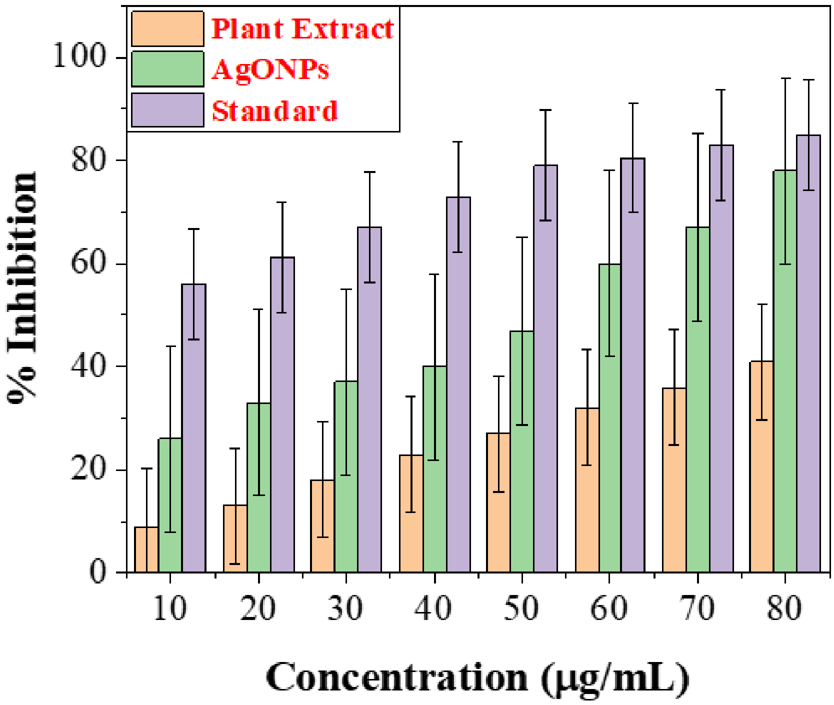
Formula	mass%	Atom%	Sigma	Net	K ratio	Line
C	32.50	51.34	0.02	87735	0.0110662	K
O	33.22	39.39	0.11	47243	0.0272309	K
Na	0.37	0.30	0.03	2422	0.0005672	K
Mg	0.38	0.29	0.02	3980	0.0008958	K
Si	0.20	0.14	0.02	2936	0.0005101	K
Cl	4.29	2.30	0.02	71251	0.0155703	K
K	2.90	1.41	0.03	40622	0.0108107	K
Cu	1.17	0.35	0.05	3858	0.0037745	K
Zn	0.88	0.26	0.05	2443	0.0028505	K
Ag	24.08	4.24	0.09	232279	0.0727665	L
Total	100.00	100.00				

4.2. Anti-inflammatory activity of silver oxide NPs using *Moringa oleifera* stem

4.2.1. Heat-induced hemolysis assay

The body's immune system automatically responds to a diverse array of microorganisms, damaged cells, irritants and toxic stimuli by manifesting inflammation. It has been proven that several secondary metabolites and a range of metallic NPs have anti-inflammatory activities in both vitro and in vivo (Zahoor *et al.*, 2023). Heat-induced hemolysis was exploited to test the anti-inflammatory potency of silver oxide NPs in vitro. The methodology of this test was based on the concept of investigation being the RBC membrane stability/lysis. During the experiment conducted at high temperatures, the RBCs' capacity for MGE was evaluated with the assistance of RBC membrane lysis inhibition.

Figure 6: Silver oxide NPs heat-induced hemolysis assay



Various concentrations of AgONPs by means of *Moringa oleifera* stem extract were used. These concentrations included 10, 20, 30, 40, 50, 60, 70 and 80 µg/mL. The absorption of *Moringa Oleifera* stem was 9, 13, 18, 23, 27, 32, 36 and 41%, while the absorption of AgONPs was 26%, 33%, 37.06%, 40%, 46.9%, 60%, 67% and 78%. Diclofenac sodium of 10 µg/mL was used as a standard group; 10, 20, 30, 40, 50, 60, 70 and 80µg/mL which have exhibited 56, 61.3, 67, 73, 79, 80.54, 83 and 85% of inhibition correspondingly shown in the Figure 6. The lowest inhibition rate of *Moringa oleifera* stem as well as silver oxide NPs is 9 and 26%, respectively, whereas the standard were at 10 µg/mL had 56%, while the maximum absorption at 80 µg/mL was 41% and 78%, respectively, whereas the drug had 85%. Since the study of Landois (1875) has recognised that temperatures over 50 degrees Celsius are harmful to the blood. The present study findings demonstrate that the quantity of haemolysis rises linearly with the length of time that the sample is left to incubate at a temperature of 50 degrees Celsius. The conclusion drawn from these findings is that AgONPs possess beneficial anti-inflammatory agent properties.

5. Discussion

The utilization of extracts from plants in the production of nanoparticles has garnered significant interest as a substitute for conventional physical and chemical methodologies. The use of bioactive compounds derived from plants allows for the removal of expensive and hazardous substances. Biological components from plants, either extracellular or intracellular, might be utilized to produce NPs. Once added to an extract produced from the *Moringa oleifera* stem plant in the current work, the Ag+ ions were converted to silver oxide nanoparticles (NPs). It was proposed that the reduction of Ag+ to AgONPs was brought about by biological components released into the reaction mixture by *Moringa oleifera* stem extract with their multifunctional groups. The UV-Visible spectroscopy performed at 419 nm gave compelling evidence that silver oxide nanoparticles were produced. In a similar vein, earlier studies on the formation of silver oxide NPs utilizing plant extracts (including an ethanol extract of *eucalyptus*) revealed the same range of absorption peaks (Okafor *et al.*, 2013). The capping and reducing properties of *Moringa oleifera* stem extracts and silver oxide nanoparticles were confirmed using FT-IR analysis in Figure 2.

The infrared spectra obtained through FTIR analysis for the plant extract indicate a prominent peak at 3323 cm^{-1}, which might be associated with the vibrational modes of O-H stretching. The 2910 cm^{-1} range is typically associated with the presence of the C-H stretching vibrations of alkanes and alkyl groups. While the 1424 cm^{-1} range is ascribed to the bending vibration of the methyl ($-CH_3$) group. C-O stretching amino acids make a band at 1023 cm^{-1}. The peak at 616 cm^{-1} is typically associated with the out-of-plan bending vibrations of substituted benzenes and mono-substituted alkenes. There is a slight change in the silver oxide NPs FTIR compared to extract FTIR. The FTIR spectra for silver oxide NPs, a band at 3294 cm^{-1} associated with –OH. The absorption band at 1642 cm^{-1} indicates the C=O stretching of either a ketone or an aldehyde. The band observed at 1001 cm^{-1} corresponds to the C-H bending vibrations of aromatic compounds. The FT-IR spectrum results indicate that the biomolecules have a significant impact as reducing and stabilizing agents for the formation of NPs. The presence of an additional peak at 594 cm^{-1} in the spectrum revealed the successful synthesis of silver oxide nanoparticles, as it corresponds to the vibration of silver molecules.

The FTIR spectrum reveals the presence of biomolecules that encompass silver oxide nanoparticles and are characterized by functional groups, including amide, carbonyls and hydroxyl. These groups also aim to decrease the concentration of Ag^+ ions and maintain their stability. Silver oxide nanoparticles are versatile components that find widespread use in numerous applications. The biocompatibility of the material with biological drugs is a significant concern. The produced silver oxide nanoparticles were analyzed for their structural characteristics, sizes and elemental composition by the utilization of SEM, XRD and EDX. The SEM image revealed that the green silver oxide nanoparticles produced possessed an irregular and agglomerated shape morphology. This observation suggests that the extracts utilized in the process exhibited effective capping and stability capabilities. The solution's functional groups exhibit intermolecular attraction and undergo chemical reactions, leading to an increase in particle size. The duration of stirring time, the ratio of salt solution plus reducing agent concentrations are additional factors that impact particle size, as noted by Ismail *et al.* (2019). The agglomerated appearance of particles is attributed to the intermolecular bonding of hydrogen that arises between the molecules present in the vicinity of the particle. The X-ray diffraction (XRD) spectrum exhibits a distinct and clear peak, representing high quality and purity of the produced NPs. The determination of the crystalline size of the synthesized NPs can be achieved through the utilization of Debye-Scherrer's formula. The Scherrer formula is expressed as below:

$$D = 0.9\lambda/\beta\cos\theta \quad (2)$$

The notation D represents the crystallite size, while λ represents the wavelength of the X-ray utilized, specifically 1.5406 Å. The symbol β indicates the full width measured at half of its maximum width, also known as FWHM, and θ represents Bragg's angle. The synthesized silver oxide NPs have a crystalline size of 23 nm. The XRD pattern of AgONPs in their prepared state exhibits prominent peaks at 2θ = 27.56°, 31.98°, 37.85°, 44.19°, 46°, 54.57° and 57.21°. These peaks can be attributed to the (111), (100), (200), (400), (110), (311) and (222) crystallographic planes of silver oxide NPs that were synthesized using *Moringa oleifera* stem. The presence of distinct peaks in the material's structure indicates its crystalline properties. Based on EDX analysis, silver oxide nanoparticles made from *Moringa oleifera* stem have an elemental composition of 57 percent, and 43 percent carbon, magnesium, chlorine, potassium, sodium, zinc, silicon, and copper due to salt and plant extract. The prepared AgONPs showed promising anti-inflammatory properties. Moreover, an important bioactive compound found in the stem of *Moringa Oleifera* is quercetin, which is a flavonoid with antioxidant and anti-inflammatory properties. The quercetin has been revealed to have potential in the treatment of various circumstances, containing cardiovascular disease, cancer, and inflammation-related disorders.

In addition to niazimicin and quercetin, the stem of *Moringa oleifera* also contains other bioactive compounds, including kaempferol, rutin and chlorogenic acid. Kaempferol is a flavonol compound that has been identified to possess antioxidative, anti-inflammatory and anticancer attributes. Rutin, a flavonoid has demonstrated antioxidative and anti-inflammatory properties and holds promise in the management of cardiovascular ailments. Chlorogenic acid, a type of polyphenol, has been discovered to exhibit antioxidative, anti-inflammatory and hypoglycemic properties. *Moringa oleifera* stem extract contains bioactive chemicals with reducing characteristics useful for nanoparticle formation and possible medicinal uses. To stabilize as well as reduce the size of the silver oxide NPs, quercetin has been utilized in the formation process. Developing gold nanoparticles has shown that Kaempferol can be used as a reducing agent.

6. Conclusion

The study synthesized silver oxide nanoparticles using a *Moringa oleifera* stem extract. Synthesizing nanoparticles of silver oxide was done successfully using a plant-mediated method that involved reducing silver nitrate. The method used to produce AgONPs may be extrapolated from the colour change that was observed. The stem of the *Moringa oleifera* plant can be used to prepare AgONPs, as shown by the results of the UV-Visible study. The FT-IR investigation of the *Moringa oleifera* stem showed that there were functional groups, which confirmed the preparation of NPs and the subsequent loss of silver atoms, which was necessary for developing AgONPs. The research utilizing SEM successfully ascertained the particulate matter's size. The size of particles can be influenced by various factors such as the duration of stirring, the amount of reducing agent and the salt solution and stabilizing agent. The EDX analysis revealed the occurrence of Agions within the nanoparticles. According to the results of an XRD analysis, the average crystallite size of the NPs that were produced via this method was 23 nm.

Regarding its anti-inflammatory capacity, heightened levels of silver oxide nanoparticles exhibited greater efficacy compared to the conventional diclofenac sodium. According to the results, it has been determined that the silver oxide NPs produced through green methods exhibit significant anti-inflammatory properties. It can be posited that the process of extracting silver oxide nanoparticles from the stem of *Moringa oleifera* can be feasibly executed on a large scale, is cost-effective and is environmentally sustainable. Nanoparticles exhibited commendable performance as biological activity agents. *Moringa oleifera's* stem generally comprises various bioactive compounds that exhibit therapeutic potential in medical treatment and serve as reducing agents in nanoparticle synthesis. Nevertheless, additional investigation is required to comprehensively comprehend these substances' therapeutic capacity and modes of operation.

Declaration of conflict of interest

The author(s) declared no potential conflicts of interest(s) with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) received no financial support for the research, authorship and/or publication of this article.

ORCID iD

Sheraz Muhammad https://orcid.org/0009-0009-6719-7286

Asif Ali https://orcid.org/0009-0002-6646-2403

Jehangir Shah https://orcid.org/0009-0003-9984-2063

Muhammad Hamza https://orcid.org/0009-0007-7935-810X

Muhammad Kashif https://orcid.org/0009-0003-2656-1092

Behram Khan Ajat Khel https://orcid.org/0009-0005-9978-8196

Arshad Iqbal https://orcid.org/0009-0006-7774-4341

References

Ahsan, A., Farooq, M. A., Bajwa, A. A., & Parveen, A. (2020). Green synthesis of silver nanoparticles using Parthenium hysterophorus: optimization, characterization and in vitro therapeutic evaluation. *Molecules, 25*(15), 3324. https://doi.org/10.3390/molecules25153324

Ajaykumar, A. P., Mathew, A., Chandni, A. P., Varma, S. R., Jayaraj, K. N., Sabira, O., . . . Swaminathan. T. R. (2023). Green synthesis of silver nanoparticles using the leaf extract of the medicinal plant, uvaria narum and its antibacterial, antiangiogenic, anticancer and catalytic properties. *Antibiotics, 12*(3), 564. https://doi.org/10.3390/antibiotics12030564

Alkhalaf, M. I., Hussein, R. H., & Hamza, A. (2020). Green synthesis of silver nanoparticles by Nigella sativa extract alleviates diabetic neuropathy through anti-inflammatory and antioxidant effects. *Saudi Journal of Biological Sciences, 27*(9), 2410-2419. https://doi.org/10.1016/j.sjbs.2020.05.005

Alsareii, S. A., Manaa Alamri, A., Al Asmari, M. Y., Bawahab, M. A., Mahnashi, M. H., Shaikh, I. A., . . . Kumbar, V. (2022). Synthesis and characterization of silver nanoparticles from Rhizophora apiculata and studies on their wound healing, antioxidant, anti-inflammatory, and cytotoxic activity. *Molecules, 27*(19), 6306. https://doi.org/10.3390/molecules27196306

Anwar, Y., Fakieh, M., Ullah, I., Alkenani, N., & Shareef, M. A. (2019). Synthesis of silver nanoparticles using Prosopis juliflora extract: potential of antimicrobial and pollutants degradation performance. *Desalination and Water Treatment, 167*, 105-112. https://doi.org/10.5004/dwt.2019.24571

Bindu, S., Mazumder, S., & Bandyopadhyay, U. (2020). Non-steroidal anti-inflammatory drugs (NSAIDs) and organ damage: A current perspective. *Biochemical Pharmacology, 180*, 114147. https://doi.org/10.1016/j.bcp.2020.114147

Bindhu, M. R., Umadevi, M., Esmail, G. A., Al-Dhabi, N. A., & Arasu, M. V. (2020). Green synthesis and characterization of silver nanoparticles from Moringa oleifera flower and assessment of antimicrobial and sensing properties. *Journal of Photochemistry and Photobiology B: Biology*, 205, 111836. https://doi.org/10.1016/j.jphotobiol.2020.111836

Chakravarty, A., Ahmad, I., Singh, P., Sheikh, M. U. D., Aalam, G., Sagadevan, S., & Ikram, S. (2022). Green synthesis of silver nanoparticles using fruits extracts of Syzygium cumini and their bioactivity. *Chemical Physics Letters, 795*, 139493. https://doi.org/10.1016/j.cplett.2022.139493

Chirumamilla, P., Dharavath, S. B., & Taduri, S. (2023). Eco-friendly green synthesis of silver nanoparticles from leaf extract of Solanum khasianum: optical properties and biological applications. *Applied Biochemistry and Biotechnology, 195*(1), 353-368. https://doi.org/10.1007/s12010-022-04156-4

Das, P., Ghosal, K., Jana, N. K., Mukherjee, A., & Basak, P. (2019). Green synthesis and characterization of silver nanoparticles using belladonna mother tincture and its efficacy as a potential antibacterial and anti-inflammatory agent. *Materials Chemistry and Physics, 228*, 310-317. https://doi.org/10.1016/j.matchemphys.2019.02.064

Das, S. K., Behera, S., Patra, J. K., & Thatoi, H. (2019). Green synthesis of sliver nanoparticles using Avicennia officinalis and Xylocarpus granatum extracts and in vitro evaluation of antioxidant, antidiabetic and anti-inflammatory activities. *Journal of Cluster Science, 30*, 1103-1113. https://doi.org/10.1007/s10876-019-01571-2

Dhasarathan, P., Devi, N. R., Sangeetha, P., Navaraj, S. M. G., Ranjitsingh, A., & Padmalatha, C. (2018). Utilisation of green synthesised silver nanoparticles for water quality management. *Advances in Nanoparticles, 7*(4), 77-84. https://doi.org/10.4236/anp.2018.74007

Dua, T. K., Giri, S., Nandi, G., Sahu, R., Shaw, T. K., & Paul, P. (2023). Green synthesis of silver nanoparticles using Eupatorium adenophorum leaf extract: characterizations, antioxidant, antibacterial and photocatalytic activities. *Chemical Papers, 77*, 1-10. https://doi.org/10.1007/s11696-023-02676-9

El Ouardy, K., Lbouhmadi, R., Attaoui, H., Mouzaki, M., Mouine, H., Lemkhente, Z., & Mir, Y. (2023). Biosynthesis and characterization of silver nanoparticles produced by Parachlorella kessleri and Cyclotella spp., and the evaluation of their antibacterial activity. *International Journal of Molecular Sciences, 24*(13), 10599. https://doi.org/10.3390/ijms241310599

Hawar, S. N., Al-Shmgani, H. S., Al-Kubaisi, Z. A., Sulaiman, G. M., Dewir, Y. H., & Rikisahedew, J. J. (2022). Green synthesis of silver nanoparticles from Alhagi graecorum leaf extract and evaluation of their cytotoxicity and antifungal activity. *Journal of Nanomaterials, 2022*, 1-8. https://doi.org/10.1155/2022/1058119

Irfan, M., Munir, H., & Ismail, H. (2021). Moringa oleifera gum based silver and zinc oxide nanoparticles: green synthesis, characterization and their antibacterial potential against MRSA. *Biomaterials Research, 25*(1), 17. https://doi.org/10.1186/s40824-021-00219-5

Ismail, M., Gul, S., Khan, M., Khan, M. A., Asiri, A. M., Khan, S. B. (2019). Green synthesis of zerovalent copper nanoparticles for efficient reduction of toxic azo dyes congo red and methyl orange. *Green Processing and Synthesis, 8*(1), 135-143. https://doi.org/10.1515/gps-2018-0038

Jalab, J., Abdelwahed, W., Kitaz, A., & Al-Kayali, R. (2021). Green synthesis of silver nanoparticles using aqueous extract of Acacia cyanophylla and its antibacterial activity. *Heliyon, 7*(9), 1-9. https://doi.org/10.1016/j.heliyon.2021.e08033

Jan, H., Zaman, G., Usman, H., Ansir, R., Drouet, S., Gigliolo-Guivarc'h, N., Hano, C., & Abbasi, B. H. (2021). Biogenically proficient synthesis and characterization of silver nanoparticles (Ag-NPs) employing aqueous extract of Aquilegia pubiflora along with their in vitro antimicrobial, anti-cancer and other biological applications. *Journal of Materials Research and Technology, 15*, 950-968. https://doi.org/10.1016/j.jmrt.2021.08.048

Khane, Y., Benouis, K., Albukhaty, S., Sulaiman, G. M., Abomughaid, M. M., Al Ali, A., . . . Dizge, N. (2022). Green synthesis of silver nanoparticles using aqueous Citrus limon zest extract: Characterization and evaluation of their antioxidant and antimicrobial properties. *Nanomaterials, 12*(12), 2013. https://doi.org/10.3390/nano12122013

Kohli, R., Mittal, K. J. D. (2019). Methods for assessing surface cleanliness. *Developments in Surface Contamination and Cleaning, 12*, 23-105. https://doi.org/10.1016/B978-0-12-816081-7.00003-6

Kumar, V., Singh, S., Srivastava, B., Bhadouria, R., & Singh, R. (2019). 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, 7*(3), 103094. https://doi.org/10.1016/j.jece.2019.103094

Maheshwaran, G., Bharathi, A. N., Selvi, M. M., Kumar, M. K., Kumar, R. M., & Sudhahar, S. (2020). Green synthesis of Silver oxide nanoparticles using Zephyranthes Rosea flower extract and evaluation of biological activities. *Journal of Environmental Chemical Engineering, 8*(5), 104137. https://doi.org/10.1016/j.jece.2020.104137

Manikandan, R., Beulaja, M., Thiagarajan, R., Palanisamy, S., Goutham, G., Koodalingam, A., . . . Arumugam, M. (2017). Biosynthesis of silver nanoparticles using aqueous extract of Phyllanthus acidus L. fruits and characterization of its anti-inflammatory effect against H2O2 exposed rat peritoneal macrophages. *Process Biochemistry, 55*, 172-181. https://doi.org/10.1016/j.procbio.2017.01.023

Mohammadinejad, R., Karimi, S., Iravani, S., & Varma, R. S. (2016). Plant-derived nanostructures: types and applications. *Green Chemistry, 18*(1), 20-52. https://doi.org/10.1039/C5GC01403D

Mohammed, A. A., Mohamed, A., El-Naggar, N. E.-A., Mahrous, H., Nasr, G. M., Abdella, A., . . . Selim, S. J. (2022). Antioxidant and antibacterial activities of silver nanoparticles biosynthesized by Moringa Oleifera through response surface methodology. *Journal of Nanomaterials, 2022*, 1-15. https://doi.org/10.1155/2022/9984308

Mohammed, G. M. & Hawar, S. N. (2022). Green Biosynthesis of Silver Nanoparticles from Moringa oleifera Leaves and Its Antimicrobial and Cytotoxicity Activities. *International Journal of Biomaterials, V.2022,* 4136641. https://doi.org/10.1155/2022/4136641

Okafor, F., Janen, A., Kukhtareva, T., Edwards, V., & Curley. M. (2013). Green synthesis of silver nanoparticles, their characterization, application and antibacterial activity.

International Journal of Environmental Research and Public Health, 10(10), 5221-5238. https://doi.org/10.3390/ijerph10105221

Okoli, C. O., Akah, P. A. (2004). Mechanisms of the anti-inflammatory activity of the leaf extracts of Culcasia scandens P. Beauv (Araceae). *Pharmacology Biochemistry and Behavior, 79*(3), 473-481. https://doi.org/10.1016/j.pbb.2004.08.012

Rajput, S., Kumar, D., & Agrawal, V. (2020). Green synthesis of silver nanoparticles using Indian Belladonna extract and their potential antioxidant, anti-inflammatory, anticancer and larvicidal activities. *Plant Cell Reports, 39,* 921-939. https://doi.org/10.1007/s00299-020-02539-7

Rashidi, L. (2021). Magnetic nanoparticles: synthesis and characterization. *Electronic and Optical Materials*, 3-32. https://doi.org/10.1016/B978-0-12-823688-8.00035-1

Sharifi-Rad, M., Pohl, P., Epifano, F., & Álvarez-Suarez, J. M. (2020). Green synthesis of silver nanoparticles using Astragalus tribuloides delile, root extract: Characterization, antioxidant, antibacterial, and anti-inflammatory activities. *Nanomaterials, 10*(12), 2383. https://doi.org/10.3390/nano10122383

Singh, J., Dutta, T., Kim, K.-H., Rawat, M., Samddar, P., & Kumar, P. (2018). 'Green'synthesis of metals and their oxide nanoparticles: applications for environmental remediation. *Journal of Nanobiotechnology, 16*(1), 1-24. https://doi.org/10.1186/s12951-018-0408-4

Singh, P., Ahn, S., Kang, J.-P., Veronika, S., Huo, Y., Singh, H., . . . Yang. D. (2018). In vitro anti-inflammatory activity of spherical silver nanoparticles and monodisperse hexagonal gold nanoparticles by fruit extract of Prunus serrulata: A green synthetic approach. *Artificial Cells, Nanomedicine, and Biotechnology, 46*(8), 2022-2032. https://doi.org/10.1080/21691401.2017.1408117

Thatoi, P., Kerry, R. G., Gouda, S., Das, G., Pramanik, K., Thatoi, H., . . . Patra, J. K. (2016). Photo-mediated green synthesis of silver and zinc oxide nanoparticles using aqueous extracts of two mangrove plant species, Heritiera fomes and Sonneratia apetala and investigation of their biomedical applications. *Journal of Photochemistry and Photobiology B: Biology, 163,* 311-318. https://doi.org/10.1016/j.jphotobiol.2016.07.029

Vellingiri, M. M., Ashwin, J. K. M., Soundari, A. J. P. G., Sathiskumar, S., Priyadharshini, U., Paramasivam, D., . . . Balasubramanian, B. (2021). Mycofabrication of AgONPs derived from Aspergillus terreus FC36AY1 and its potent antimicrobial, antioxidant, and anti-angiogenesis activities. *Molecular Biology Reports, 48,* 7933-7946. https://doi.org/10.1007/s11033-021-06824-w

Velsankar, K., Parvathy, G., Sankaranarayanan, K., Mohandoss, S., & Sudhahar, S. (2022). Green synthesis of silver oxide nanoparticles using Panicum miliaceum grains extract for biological applications. *Advanced Powder Technology, 33*(7), 103645. https://doi.org/10.1016/j.apt.2022.103645

Younis, N., El Semary, N., Mohamed, M. E. (2021). Silver nanoparticles green synthesis via cyanobacterium Phormidium sp.: characterization, wound healing, antioxidant, antibacterial, and anti-inflammatory activities. *European Review for Medical and Pharmacological Sciences*, *25*(7). https://doi.org/10.26355/eurrev_202104_25563

Zahoor, S., Sheraz, S., Shams, D. F., Rehman, G., Nayab, S., Shah, M. I. A., . . . Khan, W. (2023). Biosynthesis and Anti-inflammatory Activity of Zinc Oxide Nanoparticles Using Leaf Extract of Senecio chrysanthemoides. *BioMed Research International*, *2023*. https://doi.org/10.1155/2023/3280708