

(RESEARCH ARTICLE)



Preparation and evaluation of anti-arthritic activity of *Moringa oleifera* leaves extract loaded silver nanoparticles

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Abstract

Silver Nanoparticles (Ag-NPs) are increasingly used in various fields including medical, food, healthcare consumer and industrial purposes due to their unique physical and chemical properties. The conventional physical and chemical methods of synthesis of Ag-NPs seem to be very expensive and hazardous. Interestingly, biologically prepared Ag-NPs show high yield, solubility and high stability. The biologically Ag-NPs was characterized by UV-visible spectroscopy and SEM with EDAX. The appearance of specific absorbance peak at 440 nm indicates the presence of Ag-NPs. The development of Ag-NPs as anti-arthritic molecules is one of the most interesting approaches for arthritic treatment as it can overcome poor delivery and the problem of drug resistance. The anti-arthritic activity of developed MO-AgNPs was determined by albumin denaturation assay. MO-AgNPs (Moringa Oleifera leaves extract loaded silver nanoparticles) exhibit 25% higher anti-arthritic activity when compared with standard Aspirin drug.

Keywords: Silver Nanoparticles; *Moringa Oleifera*; Rheumatoid arthritis; Anti-arthritic activity

1 Introduction

1.1 Silver nanoparticles (Ag-NPs)

Silver Nanoparticles (Ag-NPs), nanodots or nano-powder are spherical or nano flake high surface area metal particles with their unique physical and chemical properties. Nanoscale Silver Particles are available in the size range of 10-200 nm, with specific surface area (SSA) in the 30-60 m²/g range and also available as flakes with an average particle size of 2-10 micron range with a specific surface area of approximately 40-80 m²/g^[3].

Silver nanoparticles (Ag-NPs) are increasingly used in various fields, including medical, food, health care, consumer, and industrial purposes, due to their unique physical and chemical properties. Used as antibacterial agents, in industrial, household, and healthcare related products, in consumer products, medical device coatings, optical sensors, and cosmetics, in the pharmaceutical industry, the food industry, in diagnostics, orthopedics, drug delivery^[3].

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1.2 Properties Of Silver Nanoparticles (Ag-NPs)

1.2.1 Theoretical properties

Table 1 Theoretical Properties of Silver Nanoparticles

Molecular Weight	107.87
Appearance	Powder
melting Point	961.78 °C
Boiling Point	2162 °C
Density	N/A
Bulk Density	0.312 g/cm ³
True Density	~10.5 g/cm ³
Size Range	80-100 nm
Average Particle Size	<100 nm
Specific Surface Area	5.37 m ² /g
Morphology	Spherical
Solubility in H ₂ O	N/A
Crystal Phase / Structure	Cubic
Poisson's Ratio	0.37
Thermal Expansion	(25 °C) 18.9 μm·m ⁻¹ ·K ⁻¹
Vickers Hardness	251 MPa
Young's Modulus	83 GPa

1.2.2 Optical properties

When silver nanoparticles are exposed to a specific wavelength of light, the oscillating electromagnetic field of the light induces a collective coherent oscillation of the free electrons, which causes a charge separation with respect to the ionic lattice, forming a dipole oscillation along the direction of the electric field of the light. The amplitude of the oscillation reaches maximum at a specific frequency, called surface plasmon resonance (SPR). The absorption and scattering properties of silver nanoparticles can be changed by controlling the particle size, shape and refractive index near the particle surface. For example, smaller nanoparticles mostly absorb light and have peaks near 400 nm, while larger nanoparticles exhibit increased scattering and have peaks that broaden and shift towards longer wavelengths^[35].

1.2.3 Antibacterial effects

It is well known that silver ions and silver-based compounds are highly toxic to microorganisms. Introduction of silver nanoparticles into bacterial cells can induce a high degree of structural and morphological changes, which can lead to cell death. The antibacterial effects of silver nanoparticles have been used to control bacterial growth in a variety of applications, including dental work, surgery applications, wounds and burns treatment, and biomedical devices^[36].

1.2.4 Synthesis of ag-nps

Physical methods

Evaporation-condensation and laser ablation are the most important physical approaches. The absence of solvent contamination in the prepared thin films and the uniformity of NPs distribution are the advantages of physical synthesis methods. Physical synthesis of silver NPs using a tube furnace at atmospheric pressure has some disadvantages, for example, tube furnace occupies a large space, consumes a great amount of energy while raising the environmental temperature around the source material, and requires a lot of time to achieve thermal stability. It was demonstrated that silver NPs could be synthesized via a small ceramic heater with a local heating area. The small ceramic heater was

used to evaporate source materials. The evaporated vapor can cool at a suitable rapid rate, because the temperature gradient in the vicinity of the heater surface is very steep in comparison with that of a tube furnace.

This makes possible the formation of small NPs in high concentration. Silver NPs could be synthesized by laser ablation of metallic bulk materials in solution. The ablation efficiency and the characteristics of produced nano-silver particles depend upon many parameters, including the wavelength of the laser impinging the metallic target, the duration of the laser pulses (in the femto-, pico- and nanosecond regime), the laser fluence, the ablation time duration and the effective liquid medium, with or without the presence of surfactants^[37,38].

Chemical methods

In this chemical process basically 3 components are there; metal precursors, reducing agent and capping agents and there are two steps in reduction of metal and i.e., Nucleation and growth of subsequent. By “top-down” method and “bottom-up” method Ag-NPs can be prepared. In “top-down” method, with the use of colloidal protecting agent there is using of mechanical grinding of large size metals with consequent stabilization. In second method i.e., “bottom-up” methods chemical reduction, Sono-decomposition and electrochemical methods are included^[39].

Green synthesis approach (biological method)

This method is environmental, commercial and single step method and doesn't need elevated temperature, pressure, force and deadly chemicals^[36]. Different materials like leaf extract, bark, root, stem, leaf, fungi etc., are used for the synthesis of nanoparticles. Biological synthesis includes main 3 components these are solvent, reducing agent and non-toxic material. In the biological method we are able to synthesize nano-particle of controlled size and shape, which is one of the most important requirements for preparation of nanoparticle. Availability of amino acids and proteins is the main advantage of biological method as in this ecofriendly material is used which is less toxic towards both environment and humans.

1.3 Characterization

1.3.1 UV-Visible Spectroscopy

UV-vis spectroscopy is a very useful and reliable technique for the primary characterization of synthesized nanoparticles which is also used to monitor its stability. A sample is placed between a light source and a photodetector. The intensity of a beam of UV- visible light is calculated before and after the transitory through the sample. These measurements are compared at every wavelength to specify the sample's wavelength-dependent spectrum. The data is classically plotted as absorbance as a function of wavelength^[35].

1.3.2 Scanning Electron Microscopy with EDAX

Among various electron microscopy techniques, SEM is a surface imaging method, fully capable of resolving different particle sizes, size distributions, nanomaterial shapes, and the surface morphology of the synthesized particles at the micro and nanoscales. Using SEM, we can probe the morphology of particles and derive a histogram from the images by either by measuring and counting the particles manually, or by using specific software. The combination of SEM with energy- dispersive X-ray spectroscopy (EDAX) can be used to examine silver powder morphology and also conduct chemical composition analysis. The modern high- resolution SEM is able to identify the morphology of nanoparticles below the level of 10 nm^[35].

1.3.3 Applications of silver nanoparticles (Ag-NPs)

The applications of Ag-NPs in various biological and biomedical applications, such as antibacterial, antifungal, antiviral, anti-inflammatory, anti-cancer, and anti-angiogenic.

Antimicrobial Agents.

Ag-NPs exerts antibacterial effects by attaching to the bacterial cell wall and subsequently penetrating it, and thus, result in a structural alteration of the cell membrane. Thus, a compromise in the permeability of the cell membrane leads to cell death. Ag-NPs are used as coating materials for medical devices because of their unique antimicrobial properties. Ag-NPs protect the outer and inner surfaces of the devices and facilitate continuous release of silver ions to induce antibacterial activity.

Wound Healing Agents

Ag-NPs are used as excellent healing wound dressings because they accelerate re-epithelialization and increase the bacterial clearance from infected wounds. These effects of Ag-NPs are because of a decrease in the activity of local matrix metalloproteinase (MMP) and an increase in the apoptosis of neutrophils within the wound. In addition, Ag-NPs inhibit the activities of proinflammatory cytokines interferon gamma and tumor necrosis factor alpha.

Drug Delivery

Nanoparticles have advantages such as small particle size, high stability, and specific targeting ability through surface functionalization; thus, nanoparticles can be used as versatile drug delivery systems. The surface of Ag-NPs was functionalized with a targeting ligand, for example, folic acid. Then, the Ag-NPs were conjugated with diminazene aceturate, a drug used to treat animal trypanosomiasis. In addition, Ag-NPs have been developed as vehicles for various drugs and biomolecules, e.g., oligodeoxynucleotides and interleukin-10, for treating cancers and inflammatory diseases, respectively^[44].

1.4 Rheumatoid arthritis

Rheumatoid Arthritis (RA) is a chronic, systemic inflammatory disease predominantly affecting the joints and periarticular tissue. RA still remains a formidable disease, being capable of producing severe crippling deformities and functional disabilities and cartilage destruction and commonly leads to significant disability, caused by a number of pro-inflammatory molecules released by macrophages including reactive oxygen species and eicosanoids such as prostaglandins, leukotrienes and cytokines. The regulation of these mediators secreted by macrophages and other immune cells and modulation of arachidonic acid metabolism by inhibiting enzymes like Cox and LOX are the potential target for chronic inflammatory conditions.

Recently, it has been reported that microorganism including bacteria, viruses, fungi, parasites, bacterial DNA, and bacterial toxin may exacerbate the inflammatory response at the joint and bone. Population studies have shown that 10–20% of all people who are 65 years or older either are currently receiving or have recently received a prescription for non-steroidal anti-rheumatic drugs. Prospective 2 decade will record people over 65 is expected to increase from 380 million to 600 million^[46].

RA is a complex process, which is mediated by an interdependent network of cytokines, proteinoids and proteolytic enzymes. Pro-inflammatory cytokines, such as interleukin-1 (IL-1) and tumor necrosis factor (TNF- α), are central mediators in RA. The patients with RA, who experience an initial cell-mediated response that leads to the presence of elevated levels of IL-1 in the synovial fluid. Furthermore, IL-1 concentrations in the plasma have been reported to correlate with disease activity. It has also been demonstrated that patients with erosive RA have higher synovial and circulating levels of IL-1 than patients without erosions.

Interleukin-6 (IL-6) is an inflammatory cytokine that is characterized by pleiotropy and redundancy of action, involved in inflammation, bone metabolism, immunity, endocrine functions and in particular it is a major regulator of the synthesis of acute phase reactants by the liver. IL-6 is produced by many different cells in the body including lymphocytes, monocyte, fibroblasts and endothelial cells. Adipose tissue is another major source of IL-6, accounting for about 30% of total circulating concentrations of IL-6 in healthy subjects. Excessive adipose tissue deposition leads to excessive production of IL-6, a high risk factor to the RA^[47].

1.5 *Moringa oleifera*

Moringa oleifera (*M. oleifera*), known as a miracle tree is a small plant cultivated and grown in many countries including India, Bangladesh, and Pakistan, and due to its multiple medicinal uses, is one of the promising plants because it contains several compounds that have anti-oxidants and anti-inflammatory and immunomodulatory effects. It is cultivated for its nutritious pods, edible leaves, and flowers which are very helpful as food, medicine, cosmetic oil, and forage for livestock. It is a good source of protein, oils, vitamins, fatty acids, micro-macro mineral elements, and various phenolics. Its roots, bark, gum, leaf, fruit (pods), flowers, seed, and seed oil possess various biological activities. The main flavonoids found in its leaves are myricetin, quercetin, and kaempferol^[48,49].

It is widely used in traditional medicine against the denaturation of proteins, which shows that it could be used as an antiarthritic agent. Aqueous extract of moringa is widely used for the therapy of arthritis as it can reduce the level of rheumatoid factor, TNF-alpha, and IL-1 in arthritic experimental animals. This fact meets a critical need for in vitro evaluation of plant-mediated nanocarriers by using laboratory methods. Therefore, the present study was carried out to study the anti-arthritis, and of *Moringa oleifera* medicated silver-nanocarriers^[50].

Each part of the *Moringa oleifera* tree is used for a variety of nutritional and medicinal purposes. The tree has anti-inflammatory, antimicrobial, antioxidant, anticancer, antihypertensive, hepatoprotective, anti-ulcer, antifertility, and diuretic properties. Its many pharmacological benefits are exploited as therapeutic remedies in the traditional medicinal system for various diseases^[48,51].



Figure 1 Leaves of *Moringa oleifera*



Figure 2 Powder of *Moringa oleifera* Leaves

1.6 Medicinal uses

1.6.1 Treating edema

Edema is a painful condition where fluid builds up in specific tissues in the body. The anti-inflammatory properties of moringa may be effective in preventing edema from developing.

1.6.2 Preventing and treating cancer

Moringa extracts contain properties that might help prevent cancer developing. It also contains niazimicin, which is a compound that suppresses the development of cancer cells.

1.6.3 Fighting against bacterial diseases

Due to its antibacterial, antifungal, and antimicrobial properties, moringa extracts might combat infections caused by Salmonella, Rhizopus, and E.coli.

1.6.4 Making bones healthier

Moringa also contains calcium and phosphorous, which help keep bones healthy and strong. Along with its anti-inflammatory properties moringa extract might help to treat conditions such as arthritis and may also heal damaged bones.

1.6.5 *Protecting the cardiovascular system*

The powerful antioxidants found in Moringa extract might help prevent cardiac damage and has also been shown to maintain a healthy heart.

1.6.6 *Treating diabetes*

Moringa helps to reduce the amount of glucose in the blood, as well as sugar and protein in the urine. This improved the haemoglobin levels and overall protein content in those tested.

1.6.7 *Reducing high blood pressure*

Moringa contains isothiocyanate and niaziminin, compounds that help to stop arteries from thickening, which can cause blood pressure to rise.

1.6.8 *Treating anaemia and sickle cell disease*

Moringa might help a person's body absorb more iron, therefore increasing their red blood cell count. It is thought the plant extract is very helpful in treating and preventing anaemia and sickle cell disease^[52,53].

1.7 **Preliminary phyto-chemical screening of moringa oleifera**

The crude hydro-alcoholic extract of Moringa Oleifera was subjected to qualitative phyto-chemical screening to detect the presence of various chemical constituents. The chemical constituents such as alkaloids, glycosides, tannins, flavonoids, proteins and carbohydrates were present.

2 **Materials and methods**

2.1 **List of chemicals**

99.9% Ethanol, Silver Nitrate, Distilled Water.

2.2 **List of equipments**

Electronic Balance, UV Visible Spectrophotometer, FTIR, SEM with EDAX

2.3 **Collection of *Moringa oleifera* leaves**

The Moringa Oleifera leaves were collected from in and around Perambalur, collected leaves are authenticated by P. G. Assistant in Botany, Government Higher Secondary School, Reddiyur, Cuddalore. Then the leaves are cleaned properly and shade dried at room temperature.

2.4 **Drying of *Moringa oleifera* leaves**

The fresh Moringa Oleifera leaves were collected and shade dried and powdered to coarse form.

2.5 **Maceration process of moringa oleifera**

The collected, cleaned and shade dried leaves are subjected to the size reduction and sieved. Then the Moringa oleifera leaves extract are prepared by maceration process. About 40gm of dry powdered Moringa oleifera leaves are taken with 170ml of 99.9% Ethanol and 80ml distilled water used for maceration process. This process is done for a week in a round bottom flask with occasional shaking. The flask was kept in the dark to avoid effect of the light on the active constituents of the Moringa Oleifera. Then the extract are filtered through a muslin cloth after a week of maceration. The extract is collected and stored.

2.6 **Preparation of 0.1mM silver nitrate solution**

About 0.084g of 0.1mM Silver Nitrate was taken in standard flask, with 500 ml of distilled water was added to the silver nitrate and continuous stirring until dissolved.



Figure 3 Preparation of 0.1 mM Silver nitrate Solution

2.7 Preparation of moringa oleifera leaves extract loaded silver nanoparticles

An aliquot of aqueous plant extract sample was added to 1mM aqueous AgNO_3 . To drive silver nanoparticles formation, the reaction mixtures were exposed to direct sunlight. The color change of the reaction mixtures were monitored to determine nanoparticle formation which is indicated by a dark brown color. Once the color intensities of the solutions reached a maximum, the vessels were removed from sunlight and stored in darkness at room temperature to prevent agglomeration of the nanoparticles.

2.8 Preparation Table of Moringa Oleifera Loaded Silver Nanoparticles



Figure 4 Formed Silver Nanoparticles

Table 2 Preparation table

S.no	Ingredients	Quantity required
01	Moringa Extract	5ml
02	0.1mM Silver Nitrate	50ml

2.9 Evaluation

2.9.1 UV visible spectroscopy

Silver nanoparticle solutions were diluted 1:2 with distilled water and distilled water served as a blank. Nanoparticle solutions and the control were simultaneously scanned from 190-900nm using a UV-vis spectrometer.

2.9.2 Scanning electron microscopy with edax

It is based on electron scanning principle. It is used to determine shape, morphology, and dispersion of nanoparticles in the bulk or matrix.

2.9.3 Sample Preparation

- The dried powder was placed on carbon tape.
- It is coated with gold palladium.
- The images were performed at certain voltage and pressure after palladium coating at different magnification.

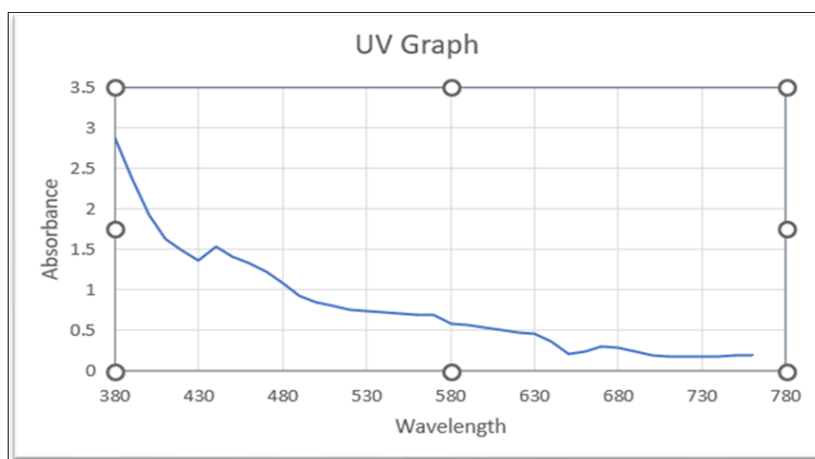
2.10 Anti-arthritis assay

The anti-arthritis assay was performed by using the 30, 40, 50, 60, 70 µg/ml of Aspirin, 50, 100, 150, 200, 250 µg/ml of MO-AgNPs with various concentrations were added to 0.45ml of bovine serum albumin to bring the final volume to 0.5 ml. All the samples were kept in the incubator for 20 minutes at 37°C, and later the temperature was raised to keep the sample at 72°C for 5 minutes. After cooling, 2.5ml of phosphate buffer was added to all the samples. The absorbance was measured at a wavelength of 600nm using a visible spectrophotometer. The control represents complete protein denaturation. The result of the sample was compared with the standard value of Aspirin. The inhibition percentage was determined using the following formula:

$$\% \text{ Inhibition of denaturation} = 100 \times (1 - A_2/A_1)$$

Where, A1 = absorption of the control sample, A2 = absorption of the test sample

3 Results and discussion

**Figure 5** UV Graph

3.1 UV visible spectroscopy

This is the primary characterization of synthesized Ag-NPs. It is one of the easiest and reliable method, as the stability of Ag-NPs can also be checked by this technique. The appearance of specific absorbance peak at 440 nm in the UV-visible region of the spectrum confirmed the presence of Ag-NPs.

3.2 Scanning electron microscopy with Edax

SEM is a technique which is basically used to determine the particle size, particle size distribution, surface morphology, a shape of the nanoparticles. In this, Ag-NPs were rod shaped the particle size ranged from 108.5 to 130.5 nm. The width of the particles is 9.5 nm. To determine the chemical composition of developed Ag-NPs, SEM is used along with EDAX. The EDAX shows the presence of 57.98 % w/w of silver in its total composition. This indicates the presence of silver nanoparticles.

3.3 Anti-arthritis assay

Table 3 Percentage of Inhibition

Test	Concentration of Silver nanoparticles (µg/ml)	% of Inhibition	Aspirin
Albumin denaturation assay	50	25	30
	100	35	40
	150	45	50
	200	55	60
	250	65	70
IC 50 VALUE		175	150

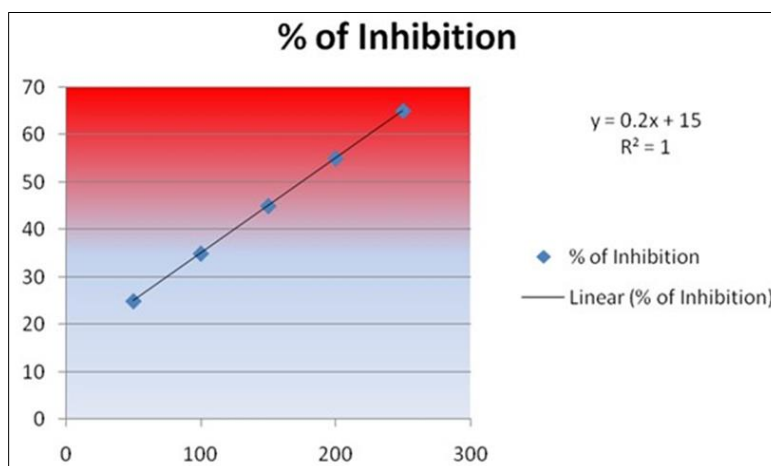


Figure 6 Percentage of Inhibition

The anti-arthritis activity of developed MO-AgNPs was determined by albumin denaturation assay. MO-AgNPs (Moringa Olifera leaves extract loaded silver nanoparticles) exhibit 25% higher anti-arthritis activity when compared with standard Aspirin drug.

4 Conclusion

This review comprehensively addressed synthesis, characterization, and applications of *Moringa Oleifera* leaves extract loaded Silver nanoparticles, with special emphasis on anti-arthritis activity with its mechanisms and also therapeutic approaches for arthritis using Ag-NPs. Although Ag-NPs play an important role in clinical research, several factors need to be considered, including the source of raw materials, the method of production, stability, bio-distribution, controlled release, accumulation, cell-specific targeting, and finally toxicological issues to human beings.

The development of Ag-NPs as anti-arthritic molecules is one of the most interesting approaches for arthritic treatment and other arthritic-related disorder; it can overcome poor delivery and the problem of drug resistance.

The conclusion of the Research was to study the Anti-arthritic activity of *Moringa Oleifera* leaves extract loaded Silver Nanoparticles. The developed Silver Nanoparticles was characterized by UV-visible spectroscopy, SEM with EDAX. The Anti-arthritic activity of Ag-NPs was evaluated by Bovine Serum Albumin Methods that shows better results.

Compliance with ethical standards

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Disclosure of conflict of interest

No conflict of interest.

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