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Green synthesis of silver nanoparticles (AgNPs) using of *Laurus nobilis* L. leaf extracts and evaluating its antiarthritic activity by *in vitro* protein denaturation and membrane stabilization assays

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Abstract

Arthritis, a systemic autoimmune disease that involves synovial growth and cartilage breakdown produces persistent inflammation of connective tissue, particularly in the joints. Arthritis is more common in persons over 65, but it can strike anyone at any age (including children). Antiarthritic medicines can help with arthritis treatment, but they can also cause dizziness, drowsiness, headaches, swollen or painful gums, hair loss, decreased appetite, mouth sores, rash, diarrhoea and several adverse side effects. Thus, there is a need for the development of novel antiarthritic drugs. Therefore, the present study focuses on the green synthesis of silver nanoparticles using leaf extracts of *Laurus nobilis* L. and evaluating its antiarthritic activity. The silver nanoparticles were synthesized and characterized using UV-Vis spectroscopy and dynamic light scattering. The *in vitro* antiarthritic activity of the green synthesized AgNPs was determined using protein denaturation and RBC membrane stabilization methods. The UV-Vis spectrum showed the nanoparticle peak at 396 nm, and the average size of the nanoparticle was found to be 78.1 nm. Five concentrations (50 µg, 100 µg, 250 µg, 500 µg and 1000 µg) of AgNPs were used to compare with the standard drug (aspirin). AgNPs showed a significant inhibition percentage compared with the standard drug. An increase in concentration increases the inhibition percentage. Maximum inhibition of protein denaturation and membrane stabilization of AgNPs was observed to be 53.47 ± 0.33% and 62.43 ± 0.25% at 1000 µg concentration. Therefore, AgNPs synthesized from *L. nobilis* can be used for the development of novel antiarthritic drugs.

1. Introduction

Autoimmune illnesses are pathological conditions characterised by autoantibodies and T-cell responses to self-molecules due to immune system reactivity. The immune system destroys or disrupts the body's own tissues as a result of a complex combination of hereditary and environmental factors. Both of these factors influence autoimmunity susceptibility on three levels: the immune system's overall reactivity, the individual antigen and its presentation, and the target tissue. Most autoimmune diseases are thought to be polygenic, involving more than one gene. For clinicians, autoimmune diseases appear either systemic (*e.g.*, systemic lupus erythema-tosus) or organ-specific (*e.g.*, type 1 diabetes mellitus). This classification, although clinically useful, does not necessarily correspond to a difference in causation (Van Delft and Huizinga, 2020).

Arthritis is a disease that causes one or more joints to swell and become painful. Joint pain and stiffness are the most common symptoms of arthritis, which normally worsen with age. Rheumatoid arthritis and osteoarthritis are the two most frequent kinds of arthritis. When the body's natural defence mechanism can not distinguish the difference between our own cells and foreign cells, the body assaults them by mistake. This is how arthritis, which is a type of autoimmune illness, works. The autoimmune mechanism of arthritis is characterised by the immune system's attack on joints, which results in inflammation (Pandey *et al.*, 2018).

Arthritis is more common in age 65 and older, according to the Centre for Disease Control and Prevention (CDC), but it can affect people of all ages (even children). Almost two-thirds of arthritis patients are under 65 years old. In every age group, women (26 per cent) have more arthritis than males (19%), affecting people of all races and ethnicities. Adults who are obese are also more likely to develop arthritis than those who are normal weight or underweight. According to the CDC, 7.1% of adults aged 18 to 44 have been diagnosed with arthritis by a doctor. Doctor-diagnosed arthritis is reported by 29.3% of adults aged 45 to 64. Doctor-diagnosed arthritis is reported by 49.6% of people aged 65 and over (Prothero *et al.*, 2018).

Antiarthritic medicines can help with arthritis treatment, but they can also cause dizziness, drowsiness, headaches, swollen or painful gums, hair loss, decreased appetite, mouth sores, rash, and diarrhoea in humans. The use of these drugs regularly can harm your stomach, kidneys, liver, and heart. Even early in therapy, over-the-counter medicines like ibuprofen (Advil, Motrin IB, and others) and naproxen sodium (Aleve) can induce stomach bleeding and kidney damage, as well as raise your risk of heart attack and stroke. As a result, the

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development of novel antiarthritic medications will assist in lessening the detrimental effects of arthritis (Wang *et al*., 2018).

Herbal drugs are promising for creating effective and novel pharmaceuticals because plants are a rich source of novel phytocompounds. India is projected to have around 47,500 plant species, accounting for more than 11.4% of the world's total plant species. Approximately, 28% of the plants found in India are indigenous to the country. These medicinal herbs have various biological activities, including antibacterial, antidiabetic, antimicrobial, antiulcer, analgesic, and antiamnesic capabilities, among others. Medicinal herbs are commonly utilised and thought to be safe and less expensive than chemically manufactured medications (Umamaheswari *et al*., 2021). Various plants like *A. paniculata, T. chebula, Terminalia bellirica, O. turpethum, etc*., and herbal preparations like Triphala (comprising fruits of *P. emblica* or *E. officinalis, T. chebula* and *T. bellirica*) are also known to have potential antiarthritic and anti-inflammatory activities along with other medicinal properties (Punit *et al.*, 2019; Tamanna Malik *et al.*, 2020; Manubotula D. Sahithya *et al*., 2021).

Nanoparticles are particulate dispersions or stable debris with a length between 10 and 100 nanometers. Nanoparticles have unique capabilities determined by their size, shape, and morphology, allowing them to interact with plants, animals, and bacteria. Silver nanoparticles were one of the metals with enormous biological applications (Mathur *et al*., 2018). Different plants and their extracts have been used for the green synthesis of metal nanoparticls of gold, silver and zinc and have shown wide activities as anti-inflammatory agents (Santwana Palai *et al.*, 2021). Antimicrobial capabilities of silver nanoparticles (AgNPs) have been demonstrated against a wide spectrum of bacteria. AgNPs are environmentally beneficial due to their use in electronics, catalysis, medicines, and pharmaceuticals (Mathur *et al*., 2018).

Therefore, the present study concentrates on the green synthesis of silver nanoparticles using extracts of *L nobilis*. The nanoparticles were characterized using UV-Vis spectroscopy and dynamic light scattering. Further, the green synthesized silver nanoparticles were subjected to *in vitro* antiarthritic analysis.

2. Materials and Methods

2.1 Collection of plant and processing

The dry leaves of *L. nobilis* were procured from a local shop at Tharamani, Chennai and stored at room temperature. The dried leaves were finely grounded to powders and stored in a sterile container for extraction. Figure 1 shows the leaves of *L. nobilis* used in the study.

2.2 Extraction and green synthesis of silver nanoparticles (Ahmed and Mustafa, 2020)

The powders of leaves (20 g) were finely chopped into small pieces and 100 ml of deionized water was added, stirred for 20 min at 60^0C. The leaf extract was cooled at room temperature after boiling and filtered, conferring 75 ml of leaf broth which was stored at 4^0C. 0.1 M (1.69 g in 100 ml distilled water) of $AgNO_3$ (99.99%) was used in the green synthesis of AgNPs. 5 ml of leaf extract was introduced to 45 ml of 0.01M $AgNO_3$ aqueous solution and allowed at suitable conditions to react. After various time intervals, the colour change of the reaction mixture is noticed from colourless to brownish-black designates that the synthesis of AgNPs. The AgNPs precipitates were procured through the centrifugation process. The pellets were collected and dried using a hot air oven at 50^0C for 1 h. The dried powders were collected and stored in sterile tubes.

Figure 1: Laurusnobilis used in the study.

2.3 Characterization of silver nanoparticles

2.3.1 UV-Vis spectroscopy analysis

UV-visible spectroscopy (UV-Vis) is used to evaluate the unique optical properties of nanoparticles synthesized from certain metals like gold and silver, which strongly interacts with a specific wavelength (nm) of light. UV-visible spectroscopy was performed to confirm the reduction of silver ions in the colloidal solution to silver oxide forms using Shimadzu -UV 1800. Double distilled water is used as reference and 1 ml of the colloidal silver solution is poured in a quartz cuvette and subjected for wavelength scanning between 350 and 500 nm.

2.3.2 Dynamic light scattering

Dynamic light scattering is used to identify the average size of the synthesized nanoparticles. Zeta analyser (Malvern Instruments) was used to quantify the size of the green synthesized nanoparticles (AgNPs).

2.4 Antiarthritic activity (Mohamed *et al*., 2014; Saket *et al*., 2010)

The antidenaturation study for analysing antiarthritic activity was carried out by utilising bovine serum albumin (BSA). When BSA is heated, antigens which are realted to to type-III hypersensitivity reactions are released, and are linked to disorders like rheumatoid arthritis.

2.4.1 Inhibition of protein denaturation (Pavithra *et al*., 2015)

The method was implemented from the previous article published by Pavithra *et al*. (2015) with slight alterations. The reaction mixture consisted of the 100 µl of AgNPs (final concentration 50, 100, 250, 500, 1000 µg/ml) and 100 µl of 5% aqueous solution of bovine serum albumin (BSA). The sample was incubated at 37°C for 20 min, and the temperature was increased up to 70°C in a hot water bath for 10 min. The tap water is used to cool the mixture for 10 min after which turbidity and absorbance were calculated at 660 nm. The blank comprised of the distilled water. The findings were compaired to those of the commonly used medication aspirin.

Formula used

Percentage inhibition = Abs control – Abs sample / Abs control x 100

2.4.2 Effect on membrane stabilization (Vallabh *et al.*, 2009)

The technique used was from a procedure adopted by Vallabh Deshpande and his team with some variations. Blood sample from healthy human volunteers who did not consume NSAID for two weeks before this experiment was obtained. The reaction mixtures 4.5 ml consists of 2 ml hypotonic saline (0.25% NaCl) + 1 ml 0.15 M phosphate buffer (pH 7.4) + 1 ml test sample (50-1000 µg/ml) in normal saline + 0.5 ml of 10% HRBC in normal saline. For 30 min, the mixture was incubated at 56°C. All the test tubes were cooled for 20 min under running tap water. After that, reaction mixture was centrifuged at 3000 rpm for 10 min, and the absorbance of the supernatant was calculated at 560 nm.

Formula used

Percentage inhibition = Abs control – Abs sample / Abs control x 100

3. Results

3.1 Green synthesis of silver nanoparticles

Green synthesis involves the usage of plant extracts as the reducing agents. The colour change from colourless to black colour on the addition of plant extract indicates the formation of silver nanoparticles. Figure 2 shows the formation of silver nanoparticles on the addition of plant extracts. The nanoparticles were centrifuged, collected and dried for further analysis.

Figure 2: Green synthesis of silver nanoparticles.

3.2 Characterization of silver nanoparticles

Green synthesized silver nanoparticles were characterized using UV-vis spectroscopy and dynamic light scattering. UV-vis spectroscopy is the basic instrumentation to confirm the successful synthesis of nanoparticles. The UV-vis spectrum of green synthesized silver nanoparticles showed peak at 396 nm. The obtained peak is in the range of the silver nanoparticles. This confirms the effective synthesis of silver nanoparticles. Figure 3 shows the UV-vis spectrum of the green synthesized silver nanoparticles.

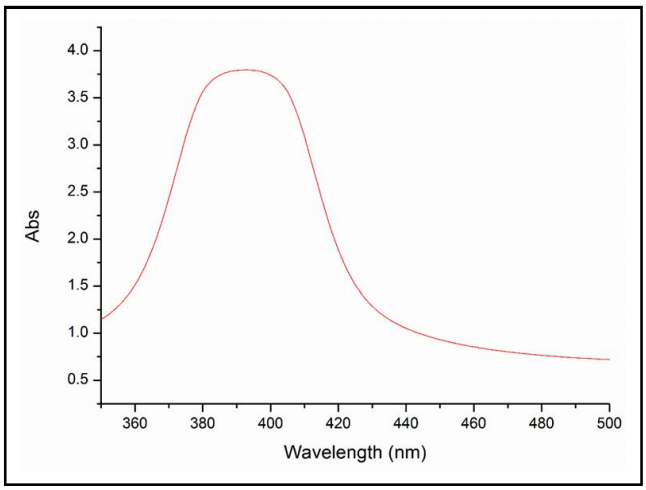

Figure 3: UV-Vis spectrum of the green synthesized AgNPs.

Dynamic light scattering is used to evaluate the average size of the nanoparticles. From the analysis, the average size of the green synthesized nanoparticles was observed to be 78.1nm. The distribution graph based on the intensity is shown in Figure 4.

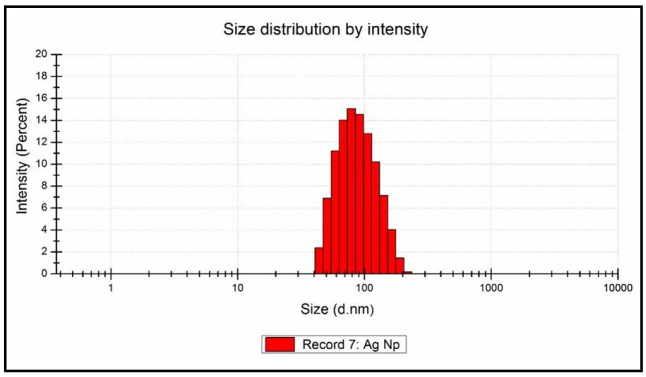

Figure 4: Particle size analysis of the green synthesized AgNPs.

3.3 Inhibition of protein denaturation

About five concentrations of the green synthesized silver nanoparticles were used for the inhibition of protein denaturation assay. 50 µg of AgNPs showed 11.71 ± 0.61%, 19.22 ± 0.18% for 100 µg, 33.41 ± 0.52% for 250 µg, 44.52 ± 0.63% for 500 µg and 53.47 ± 0.33% for 1000 µg. Similarly, 50 µg, 100 µg, 250 µg, 500 µg and 1000 µg of the standard drug (aspirin) showed 15.37 ± 2.54%, 31.9 ± 0.96%, 42.19 ± 1.38%, 68.24 ± 0.88% and 81.49 ± 1.05% of inhibition. Figure 5 shows the graphical representation of protein denaturation inhibition of silver nanoparticles on compared with standard drugs.

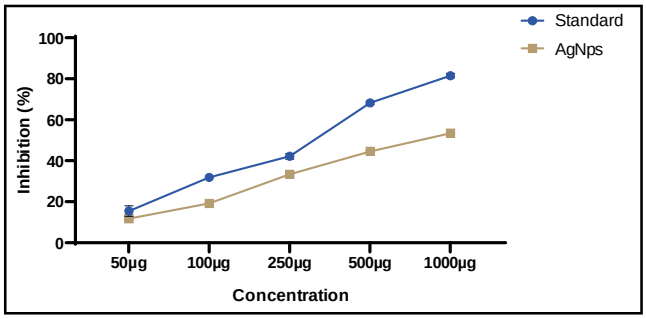

Figure 5: Inhibition of protein denaturation analysis.

3.4 Membrane stabilization assay

RBC's from volunteers who did not consume NSAID for past two weeks were collected and utilized in this study. 50 µg, 100 µg, 250 µg, 500 µg and 1000 µg of the standard drug (aspirin) showed 17.42 ± 0.19%, 22.87 ± 0.34%, 37.61 ± 0.26%, 45.22 ± 0.47% and 62.43 ± 0.25% of inhibition of haemolysis. At the same time, standard drug (aspirin) showed 22.35 ± 1.26%, 35.73 ± 0.99%, 47.07 ± 2.53%, 64.65 ± 1.28% and 86.49 ± 0.23% inhibition of hemolysisfor 50 µg, 100 µg, 250 µg, 500 µg and 1000 µg concentrations. Figure 6 shows the inhibition of AgNPs by RBC membrane stabilization method.

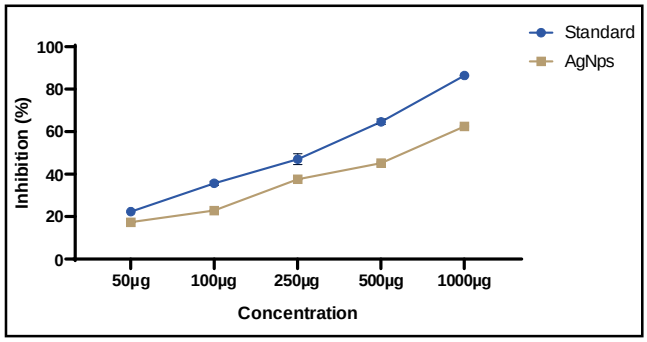

Figure 6: Membrane stabilization potential of AgNPs.

4. Discussion

Arthritis is a systemic autoimmune disease that involves synovial growth and cartilage breakdown and produces persistent inflammation of connective tissue, particularly in the joints. The first joint tissue to be affected is a synovial membrane which lays the joint cavity (Kim *et al*., 2009). As there are several antiarthritic drugs which cause adverse side effects, the present study concentrates on the green synthesis of silver nanoparticles using extracts of *L. nobilis.*

Bay leaf, also known as laurel leaf, is the leaf of the sweet bay tree (*L. nobilis*), an evergreen of the *Lauraceae* family, native to Mediterranean regions. They contain about 2% essential oil, with cineole being the most important component. *L. nobilis*, a flowering plant in the *Lauraceae* family, is an aromatic evergreen tree or big shrub with green, glabrous smooth leaves. It comes from the Mediterranean region and is used to season food as a bay leaf. It grows in full sun to light shade and tolerates a variety of well-drained soil types. It is a slow-growing plant that has been utilised as a hedge or topiary. In places colder than zone 8b, it must be carried indoors throughout the winter to avoid frost damage. *L. nobilis*is an important industrial plant that is used in foods, pharmaceuticals, and cosmetics. Bay is utilised in the food sector as a food preservative because of its antibacterial and insecticidal properties. It has traditionally been used to treat rheumatism and dermatitis, as well as gastrointestinal issues such as epigastric bloating, poor digestion, eructation, and flatulence. Turkish folk medicine uses the aqueous extract as an antihemorrhoidal, antirheumatic, diuretic, antidote for snakebites, and stomachache treatment (Tharun *et al*., 2017; Usmani *et al*., 2021).

The silver nanoparticles were synthesized using the extracts of *L. nobilis* and characterized using UV-vis spectroscopy and dynamic light scattering. The UV-vis spectrum showed the nanoparticle peak at 396 nm, and the average size of the nanoparticle was found to be 78.1 nm. The obtained size is the range of the standard nanoparticle (<100 nm). The characterization analysis showed the effective synthesis of silver nanoparticles from leaf extracts of *L. nobilis.* The *in vitro* antiarthritic activity of the green synthesized AgNPs was determined using protein denaturation and RBC membrane stabilization methods. The inhibition of protein denaturation and inhibition of hypotonicity induced membrane stabilization was considered as a measure of antiarthritic activity. Five concentrations (50 µg, 100 µg, 250 µg, 500 µg and 1000 µg) of AgNPs were used in the study to compare with the standard drug (aspirin). AgNPs showed significant inhibition on both the assay on compared with the standard. An increase in concentration increases the inhibition percentage. This shows that the antiarthritic activity of the AgNPs was concentration dependent.

When live tissue is harmed, protein denaturation occurs, which leads to the inflammation process. Hydrogen, disulphide bonds are formed as a result of the electrostatic disruption, resulting in protein denaturation. The compounds that prevent these alterations tend to possess antiarthritic properties. The stability of the RBC membrane was identical to that of the lysosomal membrane, which regulates the inflammatory process. The inflammatory response of the lysosomal membrane was significant because it aids the inhibition process by preventing the release of activated neutrophil lysosomal components. Thus, the green synthesized AgNPs possibly inhibit the release of lysosomal neutrophil contents at the inflammatory site (Joshi *et al*., 2021).

5. Conclusion

Green synthesis of silver nanoparticles was carried out using the leaf extracts of *L. nobilis.*The silver nanoparticles were synthesized and characterized using UV-vis spectroscopy and dynamic light scattering. The *in vitro* antiarthritic activity of the green synthesized AgNPs was determined using protein denaturation and RBC membrane stabilization methods. AgNPs showed a significant inhibition percentage on compared with the standard drug. Increase in concentration increases the percentage of inhibition. Therefore, AgNPs synthesized from *L. nobilis* can be used for the treatment of arthritis. Further, the green synthesized silver nanoparticles can be subjected to *in vitro* enzymological studies and *in vivo* animal studies for development of novel antiarthritic drugs.

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Conflict of interest

The author declares no conflicts of interest relevant to this article.

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