

Research Article

Green Synthesis of *Citrus sinensis* Peel (Orange Peel) Extract Silver Nanoparticle and its Various Pharmacological Activities

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Abstract

Citrus sinensis is a rich source of bioactive compounds and has attracted attention due to its medicinal benefits. Historically regarded as agricultural waste, orange peel is rich in flavonoids, polyphenols, tannins, and essential oils with antibacterial, anti-inflammatory, and antioxidant qualities. The phytochemicals in *Citrus sinensis* peel were used as natural reducing and stabilizing agents in the green synthesis method used in this work to create silver nanoparticles (AgNPs). This method is an environmentally friendly alternative to conventional nanoparticle production, eliminating the need for hazardous chemicals. Based on the study's results, green-synthesized silver nanoparticles derived from *Citrus sinensis* peel extract offer a sustainable and biocompatible substitute for biomedical applications. The pharmaceutical and healthcare industries may find therapeutic uses for them due to their exceptional antibacterial, antioxidant, and anticancer properties.

Introduction

The green synthesis of nanoparticles is acknowledged by the international scientific community because of its environmentally benign methodology. As an effective antibacterial agent, silver is widely known to possess strong antibacterial action against bacteria, viruses, and fungi. The ability to control silver nanoparticles' size, shape, and stability is essential for a variety of uses.

Silver nanoparticles, highly studied nanostructures ranging from 1 to 100 nm, are primarily used in novel and advanced biomedical applications, including tissue scaffolding, drug administration, wound dressings, and protective coatings. Regarding the surface functionalization of silver nanoparticles, it opens amazing possibilities. For its antibacterial properties, Silver is often used in the form of silver nitrate (AgNO₃). Furthermore, the larger surface area of silver nanoparticles makes microorganisms more exposed, making them more advantageous than free silver. Silver nanoparticles (AgNPs) exhibit high yield, solubility, and stability.

Due to their special physical and chemical characteristics,

silver nanoparticles (AgNP) are being employed more and more in a variety of industries, including food, pharmaceuticals, consumer products, and healthcare. These consist of strong electrical conductivity, optical, electrical, and thermal qualities, as well as biological characteristics. Due to their unique properties, AgNPs are used in a variety of industries and products, such as antibacterial agents, consumer goods, medical device coatings, optical sensors, and cosmetics; the pharmaceutical and food industries; orthopaedics, diagnostics, drug delivery; and, in the end, they have improved the tumour-killing capabilities of anticancer drugs [1].

Synthesis of silver nanoparticles AgNP

Synthesis of AgNP using physical methods: The physical methods are categorized into "top-down" and "bottom-up" approaches. In the "top down" approach, the larger materials are pulverized into smaller particles by the mechanical milling technique. A major drawback of this method is the difficulty in achieving the desired particle size and shape. When compared to regular particles of the same size, the deviation of magnetic characteristics of the prepared samples by milling process

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is observed due to the defects of lattice parameters which were developed due to the milling process. In the “bottom-up” method either liquid or gaseous phase, nanoparticles are condensed in which the larger materials are formed by the chemical combination of the smaller ions [2].

Synthesis of AgNP using chemical methods: Chemical methods use water or organic solvents to prepare the silver nanoparticles. This process usually employs three main components, such as metal precursors, reducing agents, and stabilizing/capping agents. Basically, the reduction of silver salts involves two stages (1) nucleation; and (2) subsequent growth. In general, silver nanomaterials can be obtained by two methods, classified as “top-down” and “bottom-up.” The “top-down” method is the mechanical grinding of bulk metals with subsequent stabilization using colloidal protecting agents. The “bottom-up” methods include chemical reduction, electrochemical methods, and Sono-decomposition. The major advantage of chemical methods is high yield, contrary to physical methods, which have low yield. The above-mentioned methods are extremely expensive. Moreover, common AgNP synthesis materials such as citrate, borohydride, thioglycerol, and 2-mercaptoethanol are considered toxic and hazardous [3].

***Citrus sinensis* peel was used to a greater extent:** [4]. One of the most prized and extensively consumed fruits in the world is *Citrus sinensis*. From the tender age of childhood to the advanced age of old age, sweet consumption when it is ripe has appealed as patterned after many, creating high market demand and simultaneous increasing commodity value of the plant worldwide production of *Citrus sinensis* is projected to be 115 million tons per year [5]. *Citrus sinensis* bioactive components, including anthraquinone, tannins, terpenoids, flavonoids, and saponins, are among the main causes of its high production [6]. The *Citrus sinensis* peels contain many phytochemicals, they are rich in nutrients, and have been used in various pharmaceutical and food formulations [7]. The plant extract is commonly used to treat antioxidant, antitumor, antimicrobial, anti-inflammatory and gastroprotective [8].

***Citrus sinensis* belongs to the Rutaceae family:** This plant grows to a height of 3 - 10 meters, has short thorns and has leaf stalks from 0.5 cm – 3.5 cm long. They come in a range of forms, such as egg-shaped, elliptic, and extended with sharply pointed extremities, the fruits are green while unripe and become yellow or orange when mature.

Plant profile

Kingdom: Plantae
Subkingdom: Tracheobionta
Super division: Spermatophyta
Division: Magnoliophyta
Class: Magnoliopsida
Subclass: Rosidae

Order: Sapindales

Family: Rutaceae

Genus: Citrus

Species: *Citrus sinensis* [5]

Materials and methods

Plant material collection and processing

The *Citrus sinensis* peels were collected, following a thorough cleaning with distilled water, the peels were cut into small pieces, let to dry in the shade, and then ground into a fine powder using a mechanical blender. After that, the powder was stored in an airtight container [4].

Extract preparation

300 mL of water and 50g of powdered *Citrus sinensis* peel were combined. The extraction was allowed to stand at 27 °C for two days before being filtered using Whatmann filter paper. The extracts were stored at 4 °C for future analysis [9].

Photochemical analysis

Evaluating carbohydrates: A few drops of concentrated H_2SO_4 were added to 2 millilitres of extract with Molish's reagent, and the mixture was allowed to stand for some time. The presence of a carbohydrate is indicated by the formation of a purple precipitate ring.

Alkaloid evaluation: After adding a few drops of diluted H_2SO_4 to 0.5 mL of extract, boiling, and filtering: Therefore, the presence of alkaloids is indicated by a white or yellowish precipitate.

Saponin evaluation: After boiling and filtering half a milliliter of the extract, ten millilitres of distilled water were added to the mixture. Frothing or honey comb formation indicated saponin.

Tannin evaluation: A dropwise addition of $FeCl_3$ solution at a concentration of 10% on 3mL of the extract was performed to develop a deep blue Color for tannin identification.

Steroid evaluation: To the extract of 0.5 mL add 3 mL of chloroform and 2mL of concentrated H_2SO_4 - appearance of a blue-green color indicates the presence of steroids.

Phenol evaluation: Five millilitres of Folin-Ciocalteu reagent and four millilitres of Na_2CO_3 were added to one millilitre of extract, the presence of phenols is as indicated by the development of a blue color.

Flavonoid evaluation: Ten millilitres of ethyl acetate and a small amount of the extract are boiling in water for three minutes. The mixture was filtered and 1 millilitre of diluted 1% ammonia solution was agitated with the filtrate and the layers drift apart. This Color is yellow at the ammonia layer, indicating the presence of flavonoids.



Proteins and amino acid evaluation: Tyrosine residue, which is mostly found in proteins, is present when a few drops of Millions reagent are added to the extract and heated; this results in the production of a reddish-brown hue or precipitation [9].

Silver nanoparticles synthesis

Stock solution preparation: One milligram of an aqueous extract was weighed and diluted in ten millilitres of distilled water.

Preparation of 1 mM silver nitrate aqueous solution: 0.017 g of silver nitrate was dissolved in 100 mL of distilled water to create a 1 mM solution, which was stored in an amber-colored container until further use [2].

Synthesis of silver nanoparticles: Aliquots of aqueous plant extract sample (1, 2, 3, 4, and 5 mL) were combined with 10 mL of 1 mM aqueous AgNO_3 to create nanoparticles. To facilitate nanoparticle formation, the reaction mixtures were then continuously stirred at 120 rpm using a magnetic stirrer. Monitoring the color changes of the reaction mixtures allowed for the identification of the formation of silver nanoparticles, which have a colloidal brown color [2,10].

Purification of silver nanoparticles: Finally, AgNPs were extracted from the reaction mixture. Next, two equal quantities of the reaction mixture (10 mL AgNO_3 , 5 mL leaf extract sample) were transferred to a sterile 15 mL centrifuge tube that had been previously weighed. Following that, the mixtures were centrifuged for 20 minutes at 4000 rpm. After discarding the supernatants, the pellets were gathered and kept in storage. To get rid of any contaminating plant debris, the pellets were rinsed in 10 millilitres of distilled water before being centrifuged for an hour. Once water-soluble biomolecules, such as proteins and cellular metabolites had been removed twice, the washing process was dried for an hour at 37 °C in an oven [2,10].

Pharmacological activity of *Citrus sinensis* peel extract and green synthesized *Citrus sinensis* peel extract silver nanoparticles.

Pharmacological activity of *Citrus sinensis* peel extract

Antioxidant activity: The antioxidant activity of *Citrus sinensis* peel was evaluated through bioactive component extraction and integrated reactivity toward selective free radicals. It involves a DPPH radical scavenging assay, which focuses on how well the peels can neutralize free radicals; cleaning, drying, and grinding into a fine powder were done first. The results obtained revealed that the peel demonstrates strong antioxidant potential due to the presence of flavonoids, polyphenols, carotenoids, and vitamin C. Because of these substances, the peel of *Citrus sinensis* can efficiently scavenge reactive oxygen species, lowering oxidative stress. The study found that *Citrus sinensis* peel has a high degree of free radical

scavenging activity and protective benefits against oxidative damage, making it a rich natural source of antioxidants with potential uses in pharmaceutical and nutraceutical formulations [11].

Antimicrobial activity: *Citrus sinensis* peel contains a variety of secondary metabolites that give it its pharmacological antibacterial properties, including tannins, flavonoids, phenolic compounds, saponins, and essential oils. Such bioactive substances impart their antimicrobial property by rupture of microbial cell walls, inhibition of enzyme function, and interference with bacterial metabolism. *Citrus sinensis* extracts were actively demonstrated against both Gram-positive and Gram-negative bacteria, with effective inhibition of bacterial strains. The main components responsible for the antibacterial actions are tannins, which bind to microbial enzymes irreversibly, and flavonoids, which can combine with bacterial proteins to create complexes. Through their ability to penetrate bacterial membranes and cause cell leakage and death, the essential oils found in *Citrus sinensis* peel also aid in the antibacterial function of the plant. These results suggest that *Citrus sinensis* may be utilized as a natural antibacterial agent with pharmacological applications, such as developing alternative therapies for bacterial infections [12].

Gastro protective effect: The in vitro investigation of *Citrus sinensis* aqueous extract's gastroprotective function examined its effects on inflammatory markers and stomach mucosal protection in order to highlight its anti-inflammatory qualities. The extract of dried *Citrus sinensis* peels was analyzed using High-Performance Liquid Chromatography (HPLC). Significant bioactive chemicals with pharmacological effects, including gallic acid, naringenin, and hesperidin, were found to be present. The anti-inflammatory potential was evaluated via the measured levels of pro-inflammatory cytokines, mainly C-reactive protein (CRP) and interleukin beta-6 (IL β 6), linked to gastric mucosal damage. These inflammatory markers were significantly suppressed by the extract, indicating that it may be able to control the inflammatory response in gastric cells. Cell viability assays further confirmed the extract's potential as a safe therapeutic agent by demonstrating that it had no detrimental effects on gastric epithelial cells. According to the results, *Citrus sinensis* aqueous extract has a potent gastroprotective action by means of anti-inflammatory mechanisms, which makes it a viable option for the development of natural remedies for illnesses connected to ulcers and stomach inflammation [7].

Pharmacological activity for an green synthesis *Citrus sinensis* peel extract silver nanoparticle

Antimicrobial activity: The antimicrobial activity of the *Citrus sinensis* (orange) peel extract was evaluated using the preparation of AgNPs through the green synthesis route from the aqueous extract. The synthesis was confirmed through UV-Visible and FT-IR spectroscopy, showing characteristic absorption peaks indicative of AgNPs formation. The agar well

diffusion technique was used to conduct antibacterial tests against gram-positive *Bacillus subtilis* and gram-negative *E. coli* using treatments with different doses of AgNPs, ranging from 100 to 400 µg/mL. The findings showed dose-dependent antibacterial activity, with the maximum dosage (400 µg/mL) generating inhibition zones of 20 mm for *B. subtilis* and 21 mm for *E. coli*. The study found that AgNPs made from the peel extract of *Citrus sinensis* had strong antibacterial qualities, making them a potential natural antibacterial agent for use in medicine [2,13,14].

Antioxidant activity: Antioxidant characteristics of silver nanoparticles (AgNPs) made environmentally using peel extract from *Citrus sinensis* were assessed using tests for total antioxidant capacity, reducing power, and DPPH free radical-scavenging. In the DPPH assay, free radical-scavenging characteristics of AgNPs were determined based on the capability to reduce DHPPH stable free radical, hence resulting in a shift in the color from violet to yellow. AgNPs were found to have a moderate capability to scavenge free radicals with an IC₅₀ value of 7 µg/mL. By converting Fe³⁺ to Fe²⁺, the reducing power test evaluated AgNPs' capacity to donate electrons. The results confirmed their function in decreasing oxidative stress, showing a reducing power of 0.177 mg AAE/mL.

The phosphomolybdenum method-based assay for total antioxidant capacity showed an antioxidant activity of 0.325 mg AAE/mL, indicating the overall antioxidant potential of AgNPs to inhibit oxidative processes. According to these results, green-synthesized AgNPs have antioxidant qualities, which makes them promising for use in biomedical, pharmacological, and cosmetic domains where controlling oxidative stress is crucial [14,15].

Antitumor activity: The anticancer activity of *Citrus sinensis* peel extract and green-synthesized AgNPs was evaluated through the MTT assay against colon (HCT-116) and liver (HepG2) cancer cell lines. The metabolic activity of viable cells is measured in this experiment to determine cell viability. The extract alone exhibited no significant cytotoxic effects on both of the cell lines. However, when the nanoparticles were made using AgNPs, they showed substantial cytotoxicity against cancer cells; with IC₅₀ values of 1.6 µg/mL for HepG2 and 16 µg/mL for HCT-116, indicating that HepG2 cells were more susceptible to AgNPs [16-23].

AgNPs' strong anticancer activity may be explained by mechanisms including cell cycle arrest, oxidative stress, and apoptosis induction. These findings demonstrate that although the extract alone has no cytotoxic effects, its AgNPs have strong anticancer activity, which makes them promising cancer treatment options [14].

Conclusion

The use of *Citrus sinensis* orange peel extract for

synthesizing silver nanoparticles (AgNPs) represents a sustainable and eco-friendly method of nanoparticle production. The synthesis of nanoparticles does not require toxic chemicals, as the bioactive compounds in the peel act as natural reducing and stabilizing agents. These AgNPs show promise for pharmaceutical and biomedical applications, the study shows that the green synthesized *Citrus sinensis* AgNPs exhibit potent pharmacological activities, including antimicrobial, antioxidant, and anticancer effects. The results show that AgNPs derived from *Citrus sinensis* have the potential to be natural therapeutic agents and provide a sustainable substitute for pharmaceutical development and other biomedical uses.

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