

## EFFECT OF VARIOUS ENVIRONMENTALLY FRIENDLY REDUCING AGENTS ON THE ANTIOXIDANT ACTIVITY OF GREEN SYNTHESIZED SILVER NANOPARTICLES

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**Abstract.** This study's objective was to assess the antioxidant properties of green produced silver nanoparticles (AgNPs) using ecologically friendly reducing agents such as extracts of parsley (*Petroselinum crispum*), rosemary (*Rosmarinus officinalis*), and cinnamon (*Cinnamomum zeylanicum*). The ultrasonic-assisted extract of plant material served as a reducing agent for silver ions. Spectrophotometric assessment with a resolution of 1 nm and a wavelength range of 300–600 nm confirmed the synthesis of silver nanoparticles. On the basis of the observed Surface Plasmon Resonance (SPR) peak, the size of the AgNPs was determined using correlation equations. The hydrogen peroxide scavenging activity was used to evaluate the antioxidant activity of the green nanoparticles that were synthesized. Cinnamon-derived silver nanoparticles displayed the highest scavenging activity. The results shown that environmentally friendly reducing agents can be employed to generate AgNPs, which can be utilized for antioxidant activity in several domains.

**Keywords:** antioxidant activity, eco-friendly agents, green synthesis, nanoparticles, ultrasound-assisted extraction.

### INTRODUCTION

Green nanotechnology refers to the use of biotechnological instruments to produce nanoparticles or nanomaterials via biological mechanisms, such as by utilizing bacteria, plants, and viruses, or their byproducts such as lipids and proteins. For a number of reasons, nanoparticles produced using green technology are vastly superior to those created using physical and chemical processes.

For example, green techniques eliminate the use of expensive chemicals, consume less energy, and generate environmentally benign products and by-products. The 12 green chemistry principles are becoming a reference guide for researchers, scientists, chemical engineers, and pharmacists all around the world who are working to manufacture less harmful chemical products and byproducts (Pleisner et al., 2020). As a result, green nanobiotechnology offers a possible alternative way to manufacture biocompatible stable nanoparticles (de Marco et al., 2019).

The conventional way of producing metallic nanoparticles from plants uses dried plant biomass as a bio-reducing agent and metallic salt as a precursor, respectively. Nanoparticle manufacturing through the biological route uses a bottom-up approach, requiring reducing and stabilizing chemicals to complete the process. The selection of a solvent medium, identification of an eco-friendly and ecologically safe reduction agent, and selection of a harmless substance as a capping agent to stabilize the manufactured nanoparticles are the three primary phases of biological nanoparticle production (Ahmed et al., 2019). The greater number of components made available by the biological system for the synthesis of nanoparticles gives "Green synthesis" a significant advantage over more conventional methods. The optical, conductive, antioxidant, anticancer, and antibacterial characteristics of silver nanoparticles have led to their usage in a variety of disciplines. Compared to chemical, physical, and mechanical approaches, green synthesis is an eco-friendly and reliable approach that uses environmentally benign reducing agents for obtaining silver nanoparticles.

Bionanomaterials, which are environmentally friendly and may be employed in a variety of medicinal applications, have been synthesized using the vast biodiversity of such biological components (Mousavi et al., 2018).

The purpose of this study was to evaluate the antioxidant capabilities of green-manufactured silver nanoparticles utilizing environmentally friendly reducing agents such as parsley (*Petroselinum crispum*), rosemary (*Rosmarinus officinalis*), and cinnamon (*Cinnamomum zeylanicum*) extracts.

### MATERIAL AND METHODS

The reagents and plant materials used in the study were purchased from Chemical Company Iasi, respectively Top Ingrediente Company. The vegetal material was processed with a coffee grinder to obtain powder. Subsequently, 10 g of each plant, respectively parsley (*Petroselinum crispum*), rosemary (*Rosmarinus officinalis*), and cinnamon (*Cinnamomum zeylanicum*) were mixed with 100 ml of distilled water and submitted to ultrasound-assisted extraction, at 60 °C for 30 min. The obtained extracts were left to chill at room temperature, filtrated and the pH was adjusted to 9 using sodium hydroxide (NaOH, 0.1N). The silver nanoparticles were manufactured by blending the extract with silver nitrate solution (1mM), in a 1:9 ratio (v/v), subsequently applying heat treatment at 90 °C for 10 minutes). After cooling at room temperature, the

absorbance was measured using a spectrophotometer in the range of 300-600 nm. The equation developed by Dalal et al., (2019) was used to determine the size of the nanoparticles:

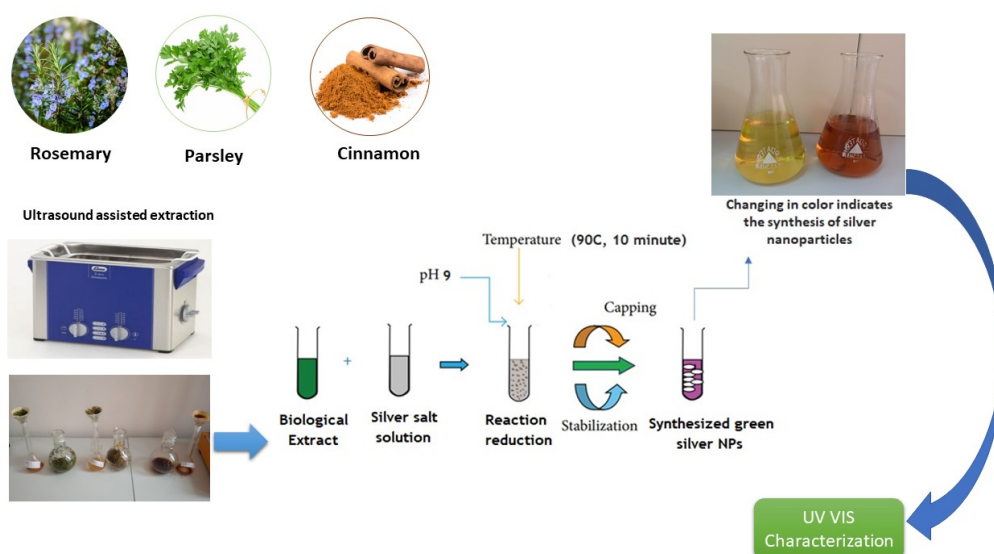
The hydrogen peroxide scavenging activity was assessed according to an approach presented by Bhatti et al., (2015). Briefly, 0.1 ml AgNPs with concentrations ranging from 50 to 1000 ug/ml, in phosphate buffer (50 mM, pH = 7.4) was mixed with 0.3 mL phosphate buffer (50 mM, pH = 7.4) and 0.6 ml hydrogen peroxide solution (30% stock). The mixture was vortexed and incubated at room temperature for 10 min. Subsequently, the absorbance was recorded at 230 nm using UV-Vis spectrophotometer and compared to a blank solution consisting of phosphate buffer and no hydrogen peroxide. Ascorbic acid served as the benchmark. The following equation was used to calculate the hydrogen peroxide scavenging activity percentage:

$$\text{Scavenging activity (\%)} = \left[ \frac{A_c - A_s}{A_c} \right] \times 100$$

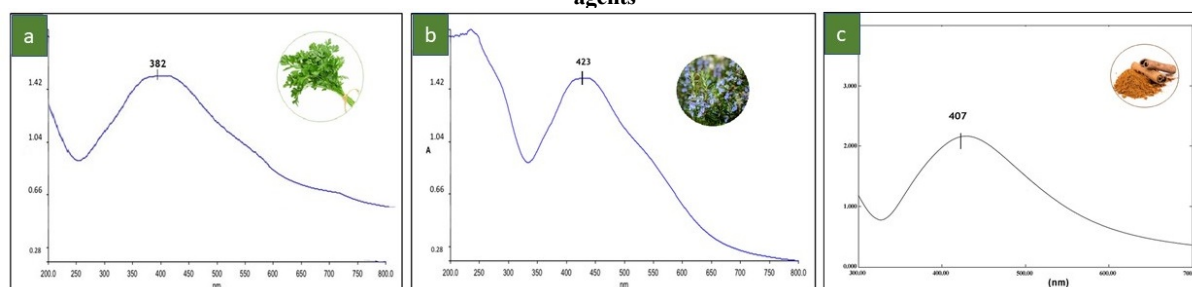
where,  $A_c$  is the absorbance of the control, and  $A_s$  is the absorbance of the sample or standard. The experiment was replicated three times for each type of nanoparticles and the hydrogen peroxide scavenging activity (%) was calculated as mean.

## RESULTS AND DISCUSSIONS

In the current study, we investigated the antioxidant activity of the silver nanoparticles synthesized using eco-friendly reducing agents using the hydrogen peroxide scavenging assay. Silver nanoparticles were manufactured using plant extract as both capping and reducing agents. The color shift from light yellow to reddish brown indicated the synthesis of the nanoparticles (Figure 1). UV-Vis analysis was carried out to confirm the presence of the green synthesized silver nanoparticles. The surface plasmon resonance peak for each mixture is presented in Figure 2.



**Figure 1. Schematic representation of the green synthesis of silver nanoparticles using environmentally friendly reducing agents**



**Figure 2. UV-Vis absorption spectra of 'Green' Silver nanoparticles synthesized using Parsley (A), Rosemary (B) and Cinnamon (C)**

Normally, for the characterization of nanoparticles, a combination of approaches is used to determine the size, shape, and density. The size and morphology of the samples may be determined using Transmission Electron Microscopy (TEM) and (Scanning Electron Microscopy) SEM, respectively. Atomic force microscopy (AFM), on the other hand, offers information regarding the shape. Notwithstanding their importance, these techniques are rather expensive, may take longer time, require specially trained personnel, and are accessible to all laboratories or research centers. According to a study conducted by Dalal et al., (2019), there is a correlation among the SPR (surface plasmon resonance) wavelength, size, shape, and density of metallic nanoparticles, which may be exploited for a much accessible characterization of the nanoparticles.

In our study, the size of the green-manufactured silver nanoparticles was determined using the equation proposed by Dalal et al., 2019. The smallest nanoparticles were obtained using parsley (*Petroselinum crispum*), extract, while rosemary (*Rosmarinus officinalis*), rendered the largest nanoparticles. (Table 1).

**Table 1. Surface Plasmon Resonance peak and size of green synthesized silver nanoparticles**

| Type of Silver NPs | Peak | Size (nm) |
|--------------------|------|-----------|
| Parsley            | 382  | 10.88     |
| Rosemary           | 423  | 45.32     |
| Cinnamon           | 407  | 31.88     |

The quest for effective, non-toxic, natural substances with antioxidative action has accelerated in recent years. Green chemistry has revolutionized the manufacturing of nanomaterials because it utilizes green and environmentally friendly approaches for the synthesis of silver nanoparticles. Because green synthesis excludes the use of expensive instruments, technical skills, and excessive use of harmful chemicals, it has been shown to be more cost-effective than others.

The resulting AgNPs have a wide range of applications, including catalysis, biosensing, water purification, antibacterial formulations, wound dressings, pharmaceuticals, surgical equipment, and optoelectronics (Vijay Kumar et al., 2015; Vijay Kumar et al., 2016). The green synthesis of noble metal nanoparticles (NMNPs) such as silver (Vijay Kumar et al., 2017), gold (Vijay Kumar et al., 2019), or platinum (Najlaa S.Al-Radadi, 2019) is currently receiving more and more popularity. Due to the significant benefits of biological applications (Akintola et al., 2020) such as imaging, diagnosis, drug administration, cancer therapy, or infectious disease prevention, nanomaterials have recently begun to play a vital role in human and animal life and health.

The antioxidant properties of diverse biological samples, pure chemicals, and isolated molecules are well known. Aside from the use of AgNPs in a variety of applications, a considerable number of studies dealing with the antioxidant properties of silver nanoparticles have been published in the past years (Ahmed et al., 2019; Bedlovičová et al., 2020; Keshari et al., 2020; Vijayan et al., 2019; Yousaf et al., 2020; Wang et al., 2018).

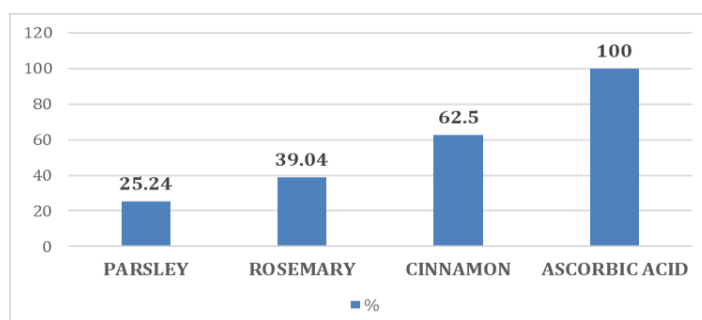
According to previous studies, 24 compounds were identified in parsley, the main compounds being myristicin, apiole,  $\alpha$ -pinene, and  $\beta$ -pinene. Other relevant compounds found in parsley were ally-tetramethoxybenzene, limonene, and elemicin (Marin et al., 2016). Rosmary on the other hand is considered to exhibit a more significant free radical scavenging activity, its antioxidant capacity is linked mostly to its principal compounds such as diterpenes, carnosol, and carnosic acid, as well as the essential oil components (Nieto et al., 2018).

Cinnamon (*Cinnamomum zeylanicum*), the other plant used in this study, for the synthesis of silver nanoparticles, is a multifaceted medicinal plant used worldwide for its well-known properties such as antioxidant, anti-inflammatory, antidiabetic as well as antimicrobial. Cinnamon is comprised of multiple resinous compounds, including cinnamaldehyde, cinnamate, cinnamic acid, and a wide range of essential oils (Rao and Gan, 2014). Cinnamon extracts, both aqueous and alcoholic, effectively reduce fatty acid oxidation and lipid peroxidation in vitro, while flavonoids possess anti-oxidant and free-radical-scavenging effects (Okawa et al., 2001). Furthermore, a study conducted by Kumaravel and Srinivasan, (2017), showed that green synthesized AgNPs from *Cinnamomum zeylanicum* exhibited superior scavenging activity as compared to the extract itself.

The concomitant activity of polyphenols from the vegetal matrix, acting as an antioxidant, with silver, that works as a catalyst, might explain the higher antioxidant capacity of silver nanoparticles (Danila et al., 2011; Xixi et al., 2017; Besliu and Meghea, 2021; Milinčić et al., 2019; Dănilă et al., 2019; Panzella et al., 2020; Zaharia et al., 2020; De Matteis et al., 2021). Furthermore, a study conducted by Demirbas et al., (2016), showed that AgNPs exhibited antioxidant activity equivalent to ascorbic acid and superior to the capacity of the leaf extract itself.

According to Journal et al., 2018 the enhanced antioxidant capacity of nanoparticles may be due to the adsorption of bioactive chemicals from leaf extract onto the spherical-shaped nanoparticles. Other authors have attributed the antioxidant ability of AgNPs to the presence of phenolic compounds, terpenoids, and flavonoids from the plant matrixes used for synthesis, nanoparticles acting as singlet oxygen quenchers, hydrogen donors, or reducing agents (Elemike et al., 2017).

In the present study, ascorbic acid was used as a control, to compare the antioxidant activity of the green synthesized silver nanoparticles. AgNPs synthesized from cinnamon exhibited the highest scavenging activity (62.5%), while the ones synthesized from parsley presented the lowest hydrogen peroxide scavenging activity (25.24%) (Figure 3).



**Figure 3. Hydrogen Peroxide Scavenging Activity of Green Synthesized Silver Nanoparticles**

The lower antioxidant activity of silver nanoparticles synthesized from parsley may be attributed to the main components myristicin, respectively apiole, which according to previous studies are pro-oxidant (Marin et al., 2016). Due to their already proven antibacterial effect, this type of silver nanoparticles may be used for biomedical applications, in which both their antibacterial and antioxidative effect may be exploited.

## CONCLUSIONS

The results confirmed that environmentally friendly reducing agents may be used for the production of AgNPs which can be exploited for their antioxidant activity. Further research is needed to refine protocols for the large-scale green manufacture of silver nanoparticles with antioxidant activity.

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