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Antibacterial, antioxidant, and cytotoxicity analysis of green synthesis silver nanoparticles from Oregano, Rosemary, and Thyme leaf extract

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ABSTRACT

Despite being still in its early phases of development, nanotechnology has grown in popularity over the last few years. This is due to its capacity to transform metals into nano-sized particles, altering their chemical, physical, and visual properties. Nanotechnology is important in a variety of scientific and technological domains because of its unique properties. In this study, silver nanoparticles were successfully synthesized by using oregano, rosemary, and thyme leaf extract through a sustainable and eco-conscious approach. The nanoparticle thus produced has been characterized by using TEM. Further, the antibacterial activity of the synthesized nanoparticles was also assessed by using a disc diffusion assay. Finally, the antioxidant and cytotoxicity were also analyzed. The results indicate the successful synthesis of NPs, which showed potent antibacterial properties. The maximum zone of inhibition against *E.coli* was shown by the nanoparticles synthesized with oregano extract (16.66 mm). Additionally, the cytotoxicity assay conducted on the SW480 cell line confirmed that the nanoparticles are either non-toxic or minimally toxic. However, a dose-dependent cytotoxicity is also seen *in vitro*. Although green synthesis is gaining popularity due to its eco-friendly approach, further clinical analysis needs to be done.

Key words: *Silver nanoparticles, Green synthesis, Antioxidant, Antimicrobial*

Introduction

Nanoparticle-mediated studies have gained significant traction in the interdisciplinary sciences of nanotechnology due to their wider range of potential applications (Kashyap *et al*., 2023; Singh *et al*., 2022). The attributes of NPs (nanoparticles), like optical, electronic, catalytic, and electrochemical, are undeniably influenced by factors like size, shape, and reaction medium (Singh *et al*., 2021; Das *et al*., 2022). These nanoparticles can be synthesized using various methods, including wet chemical methods, polyol methods, microwave synthesis, photochemical approaches, and biogenic synthesis, which have gained popularity in recent times (Hebbalalu *et al.*, 2013). Biogenic synthesis, in particular, is a green chemistry method that utilizes plant origin, edible items, algae, and herbal products (Singh *et al*., 2022a; Raveendran *et al*., 2006; Singh *et al*., 2023). Silver nanoparticles (AgNP) have garnered significant interest in research fields due to their ability to be synthesized in different sizes and shapes, making them suitable for novel biosensors, chemical sensors, electrooptical devices, data storage media, surface-enhanced Raman spectral spectroscopy, and biological imaging (Mishra *et al*., 2010; Singh *et al.,* 2022b). Additionally, AgNP has demonstrated pronounced antimicrobial properties and outperformed other metal nanoparticles (Das *et al*., 2022). Recently, many researchers have been utilizing plant or herbal extracts such as neem, Aloe vera, Phyllanthus, turmeric, seaweeds, coffee, and tea as reducing agents in the synthesis of AgNP (Singh *et al*., 2022c; Kannan *et al*., 2013). Synthesis of nanoparticles using green resources exhibits good biomedical activity due to the surface of the nanoparticle's present functional group. Traditional chemical and physical processes are known for their energy-intensive, hazardous, and costly nature, prompting a shift towards more environmentally friendly alternatives (Das *et al*., 2022). Chemical synthesis of Ag (silver) NPs mostly ended in aggregation as time passed, while biosynthesis of NPs using different plant extracts, also known as green synthesis, is environmentally kind and produces stable nanoparticles (Sharma *et al*., 2014.) The main advantages of using plant resources are that they are cheap, easily available, contain a wide range of components, and are safe to handle. The extracts of plants can act as a stabilizing agent and reductant in the AgNP synthesis and impart its inherent properties, which are contributed by the terpenoids, polysaccharides, flavanones, etc. The use of plant extract for synthesis has emerged as a fast and widely adopted processing technology for various nanostructured materials, resulting in smaller sizes, narrower size distribution, and different shapes such as spherical, octahedron, rod, and tetrahedron (Mittal *et al*., 2013; Singh *et al*., 2022d; Singh *et al*., 2024; Singh *et al.,* 2024a). The main objective of this study is to synthesize the silver NPs from these three important plants.We have also evaluated its antibacterial efficiency in antimicrobial activity. Rosemary, thyme, and oregano herbs are excellent sources of antioxidants with their higher content of phenolic compounds, and their cytotoxicity was analyzed by using the SW480 cell line.

Materials and Methods

Green Synthesis of AgNPs

To synthesize AgNPs, a 0.3 M $AgNO_3$ precursor solution of 10 ml was prepared in a 150 ml round-bottom flask and then stirred on a magnetic stirrer until a clear solution was obtained. Then, 2 ml of Soxhlet extract of each oregano, thyme, and rosemary diluted in acetone at 1:17 V/V was added dropwise to the above 10 ml of $AgNO_3$ precursor solution, adjusting the pH up to 8 by using NaOH and stored at a dark place overnight for the complete reduction of silver NPs. The color of the solution turned into a dark brown color (indicating the AgNPs formation). AgNPs were obtained by centrifuge followed by filtration (Sharma *et al*., 2021).

Antioxidant test

For the antioxidant test, 50 µl of green synthesized nanoparticle extract for all three samples was taken. After that, a 5mL 0.004%(w/v) solution of DPPH was added. The obtained solution was vortexed and incubated for 30 minutes at room temperature in a dark place. Readings were taken on a spectrophotometer at 517 nm (Ali *et al*., 2015).

Antimicrobial Test

Discs were made up of sterilized filter paper and had a diameter of 6mm. The discs were impregnated with the synthesized NPs and were placed on a Petri plate with media.100 µl of bacterial inoculum was used. Plates were kept in an incubator overnight at 37+ 1 ^{0}C. The zone of inhibition (ZOI) around the disc was measured. The antimicrobial activity was tested against *E. coli* (Singh *et al.,* 2021).

Cytotoxicity test

The cytotoxicity was conducted on SW480, which is a human colon cancer cell line maintained in DMEM, which is modified Eagle's media with 10% serum. Antibiotics used are penicillin-streptomycin. This cell line was incubated at 37 °C and 5% CO_2.The cells were harvested and seeded at a density of 1×10^4 cells/wells on 96-well plates, and after 24 h, cells were treated with different concentrations of synthesized nanoparticles, including 5, 10, 20, and 30 µg/ml, and then incubated for 24 h. After 24 hours, the media was removed. The cell viability test was performed by adding an MTT reagent (5 mg in 1 ml of PBS and 10%). In the next step, complete media was removed, and 100 µl DMSO was added to dissolve the formazan crystals. The plates were read on a multi-well spectrophotometer, and OD was recorded at 560 nm (Singh *et al.,* 2021).

Statistical tool

All the experiments were performed in triplets so that the mean values and standard deviation could be calculated wherever required.

Result and Discussion

Synthesis and Characterization

Green synthesis methods are used to produce nanoparticles by utilizing phytochemicals from plants, including flavonoids, phenols, and terpenoids. These methods are both cost-effective and sustainable. Silver nanoparticles have distinct properties, and when combined with therapeutic compounds from plants, they can be applied in various ways. The phytochemicals in the plant extract act as reducing agents, causing the reduction of silver (Ag^{+}) ions to silver atoms (Ag^0). The change in color from white to greenish brown indicates the formation of AgNPs.

The TEM image (Figure 1) strongly supports this; the particles are clearly speared over the image without any agglomeration. The majority of the synthesized particles are homogenous in size and spherical shape. The SEAD pattern (Figure 1d) exhibits that the green synthesized Ag NPs are polycrystalline in nature. The morphology and sizes of AgNPs were determined by TEM micrographs. The sample was prepared by locating a drop of synthesized silver nanoparticles on a carbon-coated Cu grid and dried in air (Gade *et al.,* 2008). The TEM images, as shown in Figure 1, are spherical particles of the biosynthesized AgNPs using rosemary extract with a varying size range from 10 to 32 nm that can be used in several applications.

Antioxidant Test

Antioxidants work by counteracting the harmful effects of free radicals, which are chemical compounds that can cause oxidative damage to biological processes. Free radicals are primarily linked with oxidative stress, which occurs when oxygen combines with certain chemicals, leading to the production of these unstable molecules. Once formed, free radicals pose a potential threat because they can react with essential cellular components like cell membranes, proteins, and DNA, potentially causing damage. Antioxidants interact with free radicals, neutralizing them and preventing potential damage before they occur. The antioxidant activity of the nanoparticle was determined using the DPPH free radical scavenging assay, as discussed by Nithianantham *et al.*, 2011 and Zuraini *et al.*, 2008, with some modifications. In the present study, three solvents have been used for herbal extraction: acetone, ethanol, and water. Further herbal nanoparticles were synthesized, and an antioxidant activity test was performed. The absorbance reading has been shown in Table 1 along with the antioxidant activity calculated. The formula used for calculation is:

DPPH Scavenging Effects (%) = $\{(A_0\text{-}A)/A_0\} \times 100$

A_0: absorbance of blank

A: absorbance in the presence of extract

The highest antioxidant activity was seen in NPs of Thyme with ethanol solvent at 41.30%, Oregano with water solvent at 23.55%, and Rosemary with water solvent at 80.95%.

Antimicrobial

The dimensions of the nanoparticles are also influ-

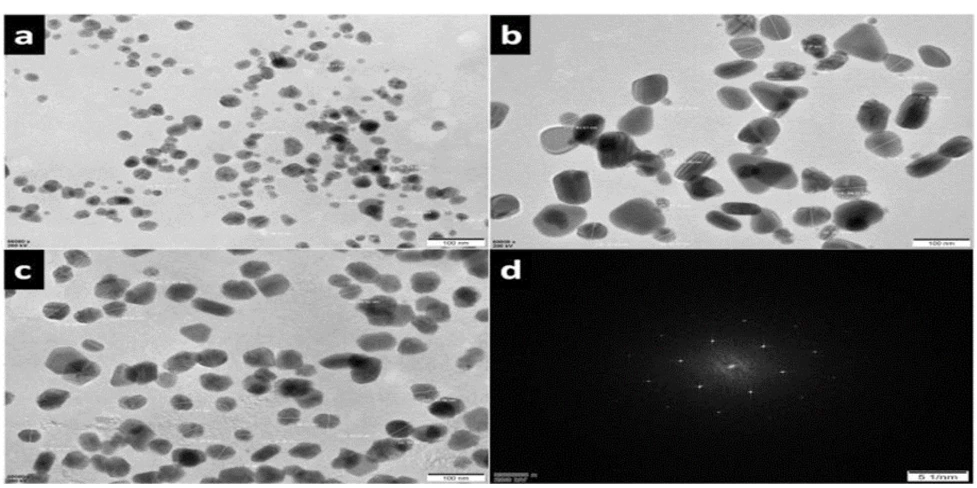

Fig. 1.TEM images of AgNPssynthesized from a) Rosemary, b) Oregano, c) Thyme,and d) SEAD.

Table 1. The results of DPPH free radical scavenging assay

S.No.	Sample	O.D.(517nm)	% activity
1	Blank	0.845	0
2	Thyme NP(acetone)	0.638	24.50
3	Thyme NP(ethanol)	0.496	41.30
4	Thyme NP(water)	0.548	35.18
5	Oregano NP (acetone)	0.770	8.88
6	Oregano NP (ethanol)	0.734	13.14
7	Oregano NP (water)	0.646	23.55
8	Rosemary NP (acetone)	0.560	33.73
9	Rosemary NP (ethanol)	0.454	46.27
10	Rosemary NP (water)	0.161	80.95

enced by the physico-chemical reactions for the antimicrobial action. The dimension of the nanoparticles should be under 50 nm to become an effective antimicrobial factor. In our studies, an increase in antimicrobial activity for nanoparticles can be easily seen. Oregano, rosemary, and thyme extracts were taken and compared with silver nanoparticles imbibed with the plant extracts. The outcomes of antibacterial activity for medicinal herb extracts and nano phytochemicals can be compared by measuring the ZOI (Figure 2) (Singh *et al.*, 2022b; Singh and Agarwal, 2022; Singh and Agarwal, 2021).

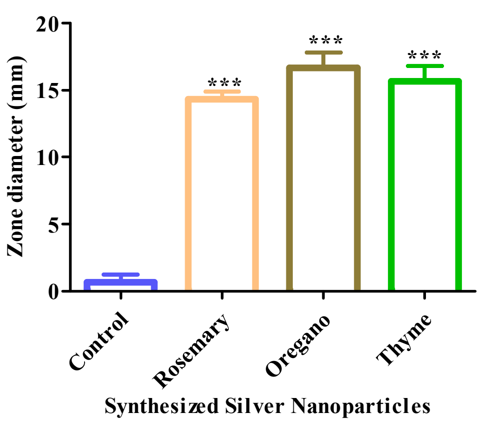

Fig. 2. Shows the ZOI for silver nanoparticles synthesized with rosemary extract (14.33 mm), thyme extract (15.66 mm),oregano extract (16.66 mm), and control (0.66 mm).

Cytotoxicity Test

Analyzing the cytotoxicity of compounds through the use of a cell line is a method that is widely accepted in the scientific community. The cytotoxicity test was performed on human colon cancer cell lines. The results obtained indicated dose-dependent toxicity. Therefore, while considering the biological application, silver nanoparticle dose optimization needs to be considered (Figure 3) (Singh *et al.*, 2022d; Singh and Agarwal, 2021).

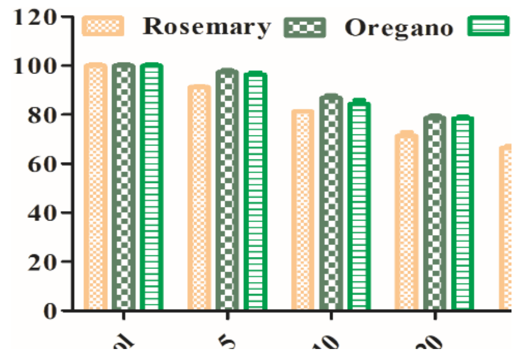

Fig.3. The cell viability (%) of synthesized NPs at various concentrations is shown.

Conclusion

In recent times, herbal extracts have gained popularity due to their lesser side effects. The most recent method is found to be imbibing them with nanoparticles. So, in the present study, the green synthesis of herbal NPs has been carried out by the use of plant extract. This method is not only cost-effective but also an eco-friendly approach. In our research, TEM characterization results indicate the formation of AgNPs and leaf extract as the reducing and stabilizing agent for nanoparticle formation. The synthesized AgNPs showed enhanced antibacterial properties like antimicrobial and antioxidants, which suggests possible bio-medical applications. However, the cytotoxicity analysis indicates that a dose-dependent toxicity is seen.

Conflict of Interest

No conflict of interest exists.

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