Comprehensive Research and Reviews in
Chemistry and Pharmacy
Journal homepage: https://crrjournals.com/crrcp/
ISSN: 2961-3604 (Online)

(RESEARCH ARTICLE)

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Plant mediated green synthesis, characterization and biological study of silver nanoparticles from *Ocimum gratissimum* aqueous leaf extract

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Comprehensive Research and Reviews in Chemistry and Pharmacy, 2022, 01(01), 034–040

Publication history: Received on 01 August 2022; revised on 07 September 2022; accepted on 10 September 2022

Article DOI: https://doi.org/10.57219/crrcp.2022.1.1.0004

Abstract

Ocimum gratissimum is traditionally used as antibacterial medicine and accumulates many antioxidant phytochemicals. The study here, expanded this traditional usage with the green biosynthesis of silver nanoparticles achieved using *Ocimum gratissimum* leaf extract as a reducing and capping agent. The green synthesis of Ag NPs reaction was carried out using 2mL of *Ocimum gratissimum* leaves extract added to 50mL aqueous solution comprising 85 mg of silver nitrate The effect of temperature on the synthesis of Ag NPs was examined using room temperature (25 ^{0}C) and 60 ^{0}C. The silver nanoparticles were formed in 20 min by stirring at room temperature. In this case, a deep brown color was developed. The successful formation of silver nanoparticles was further confirmed by UV–Vis and SEM analysis. The characteristic peaks of the UV-vis spectrum and SEM confirmed the synthesis of Ag NPs as the UV spectrum revealed that the maximum absorption peak was at absorbance of 0.99 with corresponding wavelength (λ max) at 500 nm and the SEM micrograph of biosynthesized Ag NPs showed relatively face centered cubic structure, well distributed without aggregation and an average size of about 28nm with 10% of *Ocimum gratissimum* leaf extract in 1.5 mM Ag nitrate concentration. The biosynthesized Ag NPs exhibited potential antibacterial activity against human pathogenic bacteria. The result clearly suggest that the green biosynthesized Ag NPs can constitute an effective antibacterial agent.

Keywords: Plant mediated; Green synthesis; Characterization; Biological study; Silver nanoparticles; *Ocimum gratissimum*

1 Introduction

In modern materials science, the field of nanotechnology is one of the most active areas of research, as nanoparticles exhibit novel properties depending upon their size, shape, and morphology, thereby effectively enabling them to interact with plants, microbes and animals [1-2]. Their unique optical and physical properties such as high surface to volume ratio, Surface Plasmon Resonance (SPR), as well as Surface Enhance Raman scattering (SERS) have resulted in the metal nanoparticles recent development [3]. These unique features result in increased applications of metal nanoparticles in the field of medicine, cosmetics, agriculture, sensing & bio imaging, textile waste treatment, purification and treatment of water [4-11]. Research based on advanced nanomaterials of noble metals like silver has conquered a lot of interest among scientists during the past decades for its physiochemical properties such as size, distribution and morphology, they have been studied for catalytic activity, optical properties, electronic properties, antibacterial properties and magnetic properties [12-16**]**.The plant mediated synthesis of nanoparticles is an emerging branch of nanotechnology as it has many merits over chemical and physical methods of nanoparticle synthesis. The advantages of this approach are but not limited to; It is environmentally friendly, simple, cost-effective, eco-friendly, easily scaled up for mass-scale synthesis, relatively reproducible, more so, there is no need of: high pressure, energy, toxic chemicals,

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high temperature, and often results in more stable materials. In recent years, the integration of the principles of green chemistry with nanotechnology has become a key area in nanoscience and has received great attention [17-18]. Nowadays, biological methods are being utilized in the synthesis of metal and metal oxide nanoparticles since the particles obtained are of desirable size and morphology and the properties of the particles are enhanced in a greener way. Owing to the rich biodiversity of plants and their potential secondary metabolites, plants and plant parts have been well exploited in recent years in the bio fabrication of a variety of nanoparticles. Since plant extracts can act as both reducing and stabilizing agents for the formation of nanoparticles, hence the use of chemical as reductants and stabilizers can be avoided. These plants and their parts help to produce metal nanoparticles that are much stable as compared to the other organisms and can reduce the metal ions faster than that of fungi and bacteria [19]. Furthermore, they equally reduce the cost of isolation and culturing bacteria and fungi, hence, increasing their cost-competitive feasibility of nanoparticle production [20]. Additionally, it is worthy to note that, *Ocimum gratissimum* (Scent leaf) is a relatively well-studied herb, with research that has demonstrated that it can radically and speedily improve anxiety and depression, and reduce stress both physical and emotional. Traditionally, *Ocimum gratissimum* (figure 1) has been used for the treatment of headache, diarrhoea, wart worms and kidney infections. The leaves of the African varieties of *Ocimum gratissimum* are said to contain thymol oil, which has been found to be highly antiseptic and also used to prevent mosquito bite.

In this study, silver nanoparticles have been successfully synthesized by using *Ocimum gratissimum* (cent leaf) *aqueous* leaf extract. The synthesized Ag NPs were characterized using UV-Visible Spectroscopy and Scanning Electron Microscopy (SEM. These Ag NPs were further used for various antibacterial applications.

Figure 1 *Ocimum gratissimum* (scent leaf)

2 Material and methods

The main materials employed during this work were but not limited to; *Ocimum gratissimum* (scent leaf) leaves, deionized water, $AgNO_3$, magnetic stirrer, hot plate, whatman no. 1 filter paper, crucible, beaker. All the reagents used during this work were of analytical grade, hence were used without further purification.

2.1 Sample Collection

The *Ocimum gratissimum* leaves were collected from a compound in Tunfure and were transported to Federal University of Kashere for further identification. The collected *Ocimum gratissimum* leaves were thoroughly washed under running tap water and rinsed severally with distilled water followed by sun drying to remove residual moisture. The dried materials were cut into smaller sizes and ground with the aid of motar and pistle.

2.2 Preparation of the leaf Extract

In the preparation of plant extract, Mela *et al.,*2022 method [21] was adopted with slight modifications as follows: 10g of *Ocimum gratissimum* leaves powder was weighed and dispersed in 100 ml of deionized water in a 250 ml glass beaker. The mixture was heated on hot plate at 60^0C for 20 min. After 20 min, the extract was filtered using 0.45 mm Millipore membrane filter and followed by 0.2 mm Millipore membrane filter and the filtrates collected (figure 2a). This was used immediately for the synthesis of silver nanoparticles.

2.3 Green Synthesis of Silver Nanoparticles

The silver NPs were prepared according to Jabna and Meera, 2017 method [22] with little modifications outlined as follows: 2mL of *Ocimum gratissimum* leaves extract was added to 50mL aqueous solution comprising 85 mg of silver nitrate (0.5 mM) in a 100 mL round-bottom flask. The round-bottom flask was equipped with a magnetic stir bar and fixed with a cooling condenser. The reaction mixture was stirred for 1h at 60^0C. The reaction mixture color transformed instantaneously and the observation recorded; afterwards, no color transformation was noticed up to the end of the reaction. The reaction mixture was allowed to cool down, and the reaction mixture was then centrifuged for 30 min at 9000 rpm. Subsequently, the product obtained was washed numerous times with deionized water. Lastly, a black precipitate was formed, which was dried out for 12 h at 60^0C in an oven.

2.4 Characterization of the Sample Synthesized

2.4.1 UV-visible spectral analysis

The silver nanoparticles were confirmed by measuring the wavelength of reaction mixture in the UV-Vis spectrum at a resolution of 1 nm from 200 to 800 nm.

2.4.2 SEM Analysis

The surface morphology of the nanomaterial (Ag NPs) was characterized by scanning electron microscope (SEM).

2.5 Antimicrobial Analysis

Agar well diffusion method was used for the antimicrobial susceptibility assay here. The test organisms for this study were: *Staphylococcus aureus and Streptococcus pyogene* (Gram-Positive bacteria), *Helicobacter pylori, Chlamydia trachomatis* (Gram-negative bacteria). The pure clinical isolates were obtained from the Pathology Laboratory of Gombe State University. All the clinical isolates were checked for purity and were maintained on nutrient broth at 4^0C in the refrigerator for further use. Into sterile Petri dishes, nutrient agar was poured and allowed to solidify. On the solidified agar, 1ml of the test culture was dropped and the organism was spread all over the surface of the agar using a spreader. Using a sterile cork borer, wells of approximately 5 mm in diameter were made on the surface of the agar medium. The plates were turned upside down and the wells labelled with a marker. Each well was filled with 0.2 ml of the solution of silver nanoparticles. At 37^0C, the plates were incubated aerobically for a period of 24 hours and the sensitivity of the organisms to the nanoparticles was noted. The minimum inhibitory concentration (MIC) was determined by comparing the different concentrations of the nanoparticles having different zones as well as selecting the lowest concentration.

3 Results and discussion

3.1 Silver Nanoparticles' Formation and UV- Visible Spectrophotometric Study

Figure 2 (a) leaf extract (b) $AgNO_3$ solution (c) Ag NPs

The formation of Ag NPs was first noticed based on the visual change in color of the reaction mixture at room temperature from dark yellow to deep brown (figure 2a and 2c respectively) within 20 min. The change in color of the reaction mixture was possibly due to surface Plasmon resonance phenomenon which provides a convenient indication of the formation of Ag NPs. This was however, followed by UV-Vis spectroscopy frequently used to characterize the

nanoparticles. The reduction of Ag^{+} to Ag^{0} was measured periodically at 200-800nm, using distilled water as the blank. A spectrum of Ag NPs was plotted with wavelength on x-axis and absorbance on y-axis. The maximum absorption peak was observed at absorbance of 0.99 with corresponding wavelength (λ max) at 500 nm indicating the formation of Ag NPs due to the excitation of the surface Plasmon vibration in the Ag NPs. The UV result is in agreement with the one reported from the literatures [23-24]. The UV–Vis absorption spectrum of the synthesized Ag NPs is shown in Figure 3.

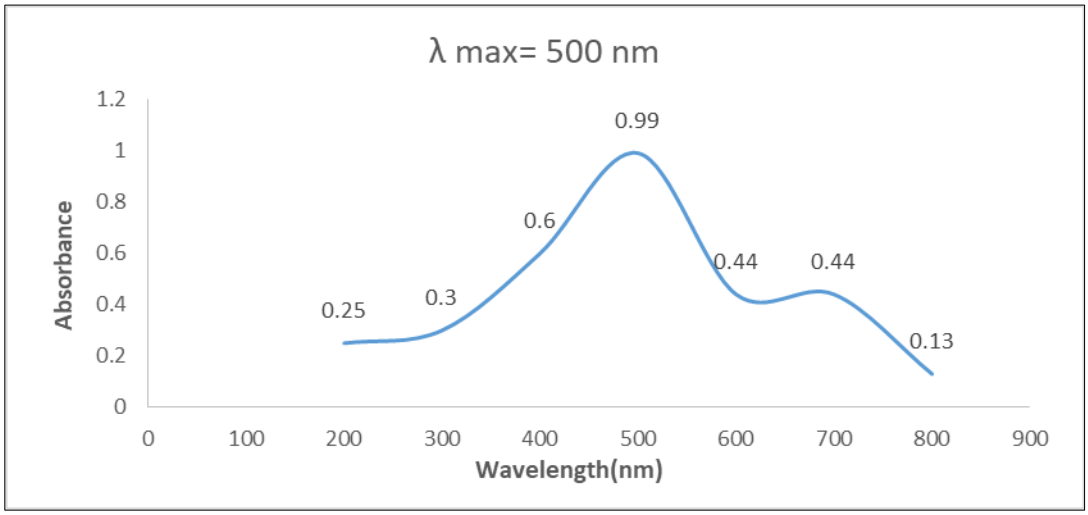

Figure 3 UV spectrum of Ag NPs from *Ocimum gratissimum* leaf extract

3.2 SEM Investigation

Shown in Figure 4 is a SEM spectrum recorded from the Ag NPs bio fabricated. The SEM micrograph of biosynthesized Ag NPs showed relatively face centered cubic structure, well distributed without aggregation and an average size of about 28nm with 10% of *Ocimum gratissimum* leaf extract in 1.5 mM Ag nitrate concentration. The biosynthesized Ag NPs had been evenly distributed throughout the solution. Furthermore, the Scherer rings characteristic of face centered cubic Ag nanoparticles is evidently observed, showing that the structure seen in the SEM image are Nano crystalline in nature The findings of this research are consistent with prior research that found face centered cubic Ag NPs when synthesis was carried out by plant extract [25].

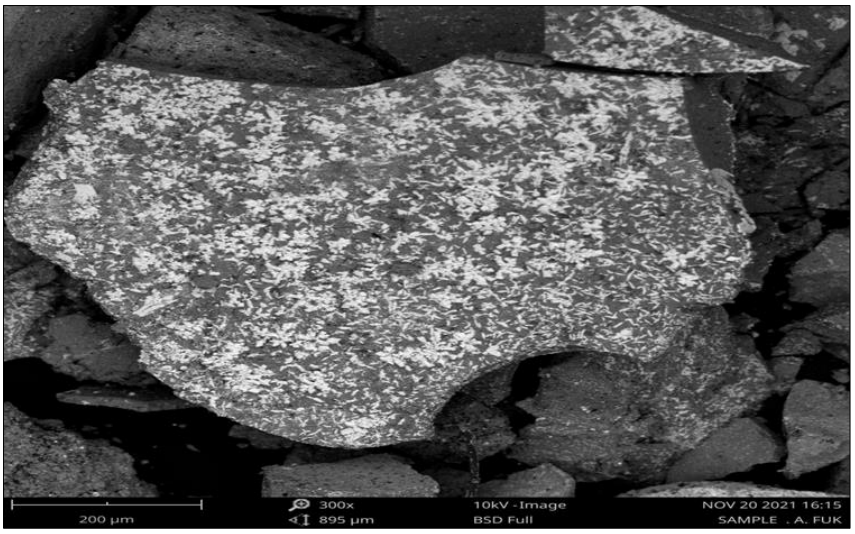

Figure 4 SEM image of biologically synthesized silver nanoparticles by *Ocimum gratissimum* leaf extract

3.3 Antibacterial susceptibility assay

In this current work, flagyl was used as a positive control in the experiment and the zone of inhibition of silver nanoparticles bio fabricated from the leaf extract of *Ocimum gratissimum* against four pathogenic microbes is shown in Table 1. Two each of Gram-negative and Gram-positive bacteria microbes were used for the study. These are human

pathogens capable of causing diseases ranging from anaemia, osteomyelitis, endocarditis, diarrhoea, pneumonia, meningitis, skin infections, bacteraemia, sepsis, toxic shock syndrome, urinary tract infections, vomiting, lung infection, kidney infections to wound infections. The cobalt nanoparticles' surfaces might have interacted directly with the microbes' outer membrane, causing the membrane to rupture thereby killing the pathogens. It therefore implies that, the antibacterial activity demonstrated by the silver nanoparticles in the present study is attributed to their small size and high surface to volume ratio, which therefore enables them to interact closely with bacterial membranes. The minimum inhibitory concentration (MIC) of the silver nanoparticles is shown in Table 2. Hence, it could be deduced that, the silver nanoparticles synthesized inhibited the growth of the selected organisms, the result that is in consonance with the earlier reports [26].

Table 1 Zone of inhibition (mm) of silver nanoparticles against pathogens

Concentration of silver nanoparticles (mg/ml)	***S. aureus***	***S. pyogene***	***H. pylori***	***C. trachomatis***	**Positive control (mm)**
25.00	4.01	3.10	-	-	19.20
50.00	6.50	4.78	4.80	5.20	25.50
100.00	8.90	6.10	6.70	6.52	36.80

Table 2 Minimum inhibitory concentration of silver nanoparticles against pathogens

Concentration of silver nanoparticles (mg/ml)	***S. aureus***	***S. pyogene***	***H. pylori***	***C. trachomatis***	**Positive control (Flagyl)**
5.5	-	-	-	-	+
10.25	-	-	-	-	+
20.5	+	+	-	-	+
41.0	+	+	+	+	+

From the MIC, the degree of inhibition is summarized as: *H. pylori = C. trachomatis< S. aureus = S. pyogene. This implies that, S. aureus = S. pyogene > H. pylori = C. trachomatis.*

4 Conclusion

The present research investigation examined the antimicrobial efficacy of silver nanoparticles fabricated using green method utilizing *Ocimum gratissimum* leaf extract. The formation of silver nanoparticles was initially monitored based on color change of the reaction mixture at room temperature from dark yellow to deep brown within a space of 20 min. UV analysis of the sample revealed that the maximum absorption peak was at absorbance of 0.99 with corresponding wavelength (λ max) at 500 nm. The sample was subjected to SEM analysis to determine the surface morphology. Antibacterial study conducted revealed that silver nanoparticles inhibited the growth of *H. pylori, C. trachomatis, S. aureus* and *S. pyogene* thereby rendering it a potential antibacterial agent.

Compliance with ethical standards

Acknowledgments

Authors wish to thank Federal University of Kashere for the work space.

Disclosure of conflict of interest

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

Authors' Contributions

This work was carried out in collaboration among all authors. Author MY conceived and designed the study, performed the statistical analysis, wrote the protocol, wrote the first draft of the manuscript and managed the analyses of the study. Authors JWKJ, JDS, JJ, PDB, DGA and NU managed literature searches. All authors read and approved the final manuscript.

Funding

This research received no external funding.

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