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Green synthesis, Characterization and Antimicrobial potential of Selenium Nanoparticles from *Ocimum gratissimum*[#]

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ABSTRACT

Ocimum gratissimum L. is a perennial herbaceous plant used in the treatment of fungal and bacterial infections. Green synthesis has provided cost effective, environment friendly procedure and raising safe strategies for the synthesis of nanoparticles. This study was aimed at investigating the potential of *O. gratissimum* for the synthesis of selenium nanoparticles (SeNPs) and their antimicrobial activities. Phytochemical screening on aqueous extract was carried out using standard procedures. Selenium nanoparticles was biosynthesized by *O. gratissimum* and characterized using Visual detection, UV-Visible spectroscopy, Scanning Electron Microscope, Transmission Electron Microscope, Energy dispersive X-ray, Fourier Transform Infra-red spectroscopy and X-ray diffraction spectroscopy. Antimicrobial activity of the biosynthesized selenium nanoparticles by *O. gratissimum* was done using agar well diffusion method. Saponins, tannins, cardiac glycosides, terpenoids and phenols were present. The biosynthesized SeNPs had a strong plasmon resonance band at 300 nm, changes in colour from dark brown to ruby red. The SeNPs were spherical and aggregated with varying shapes and size ranged from 20 – 50 nm. Strong signal of selenium element was observed. Hydroxyl, esters, aldehyde, alkane and amine are present and responsible for the efficient stabilization and bioreduction of Selenium nanoparticle. Furthermore, biosynthesized SeNPs by *O. gratissimum* (OGSeNPs) exhibited higher antimicrobial activity against both Gram ositive and Gram negative bacteria. Green synthesis of nanoparticles is a promising method in the biomedical field, due to its high bioactive components.

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Introduction

Nanotechnology is an all-round disciplinary field in science that involves materials at the atomic level or nanoscale level. Nanoparticles are the fundamental components of nanotechnology that differs from various dimensions, to shapes and sizes apart from their material (Minakshi et al., 2020). They are nanometer-sized (<100 nm) atomic or molecular scale solid particles having some excellent physical properties on their size and morphology (Stadler et al., 2019). Metal and metal oxide nanoparticles have been thoroughly examined using science and technology due to their excellent properties such as high surface to volume ratio, high dispersion in solution, etc. Metal and metal oxide nanoparticles displays enhanced antimicrobial properties (Ingle et al., 2020). Among all the metal nanoparticles, Selenium nanoparticles (SeNPs) have attracted numerous research interests due to its nutritional supplementation value and low toxicity (Bhattacharjee et al., 2019). Selenium can be present in nature showing various polymorphic structures, either amorphous or crystalline. It is a trace element present in both plants and mammals (Skalickova et al., 2017). It is effective against different types of cancer and neurodegenerative diseases (Santos et al., 2019) and its deficiency is associated with disease in man (Rajeshkumar et al., 2019). Selenium nanoparticles have been studied for the treatment of several diseases, such as diabetes, Alzheimer's disease (AD) and inflammation-related diseases such as rheumatoid arthritis (Gupta et al., 2019). Also, they are being explored as a protector against potential toxic agents, such as chromium, cadmium, and chemotherapeutics agents, which side effects can be very harmful (Khurana et al., 2019).

Three main methods have been applied for the synthesis of selenium nanoparticles: physical, chemical, and biological methods. In the physical approach for SeNPs synthesis, pulsed laser ablation, vapour deposition, hydrothermal and solvent thermal methods were used. The pulsed laser ablation method has the advantage over other methods due to the lack of contamination with chemical reagents, easy collection of nanoparticles by centrifugation, and their high stability (Tzeng et al., 2020). In chemical approach, chemical reduction of inorganic selenium forms as the precursors is the most commonly used method for the preparation of SeNPs. Ascorbic acid, glucose, fructose, cysteine, glutathione, sodium metasulfite and ionic liquid 1-ethyl-3-methylimidazolium thiocyanate have been used as reducing agents, usually in the presence of stabilizing agent to prevent aggregation of nanoparticles (Guleria et al., 2020). Water-soluble polymers, natural polysaccharides, carboxymethyl cellulose or bovine serum albumin have been used for this purpose (Chung et al., 2020). However, some residuals of these chemicals limit the applications of the formed SeNPs in the pharmaceutical and medicinal areas.

The biological approach involves the use of microorganisms and plants for the synthesis of nanoparticles. Different groups of bacterial strains and fungi have the ability to reduce selenite or selenate to nano selenium as a method of detoxification. SeNPs may be formed both within the bacterial cells and/or extracellularly. The utilization of water plant extracts is also a better alternative to chemical methods for the synthesis of selenium nanoparticles. This approach requires non-toxic solvents, mild temperatures, and application of the reducing agents that are easily accessible, cheap, biodegradable, and not harmful to the environment. It also reduces the high cost of microorganism's isolation and a final SeNPs purification, when applied in biomedical sectors. Such greenly synthesized methods of SeNPs are becoming preferred over the conventional chemical and physical methods due to their reduced toxicity towards the environment which uses living organisms such as plants, microalgae, and other microorganisms. Selenium nanoparticles produced via the green synthesis method can be an alternative to antibiotics (Ingle et al., 2020). Plants are considered to be more suitable compared to microbes for green synthesis of nanoparticles as they are non-pathogenic and various pathways are thoroughly researched. A wide spectrum of metal nanoparticles has been produced using components (including leaves, fruits, roots, stems, and seeds) of different plants (Das et al., 2017). Plant extracts have the ability to produce nanoparticles with defined size, shape, and composition. Furthermore, the presence of a wide array of phytochemicals in their extract may function as natural stabilizing and/or reducing agents for nanoparticles production. It is accepted that plant-derived nanoparticles are less likely to cause harmful side effects in humans when compared to chemically synthesized nanoparticles, and exhibit a high biological potential with applications in agriculture, food science and technology, bioengineering, cosmetic or nanomedicine, and human health protection (Geoffrion et al., 2020).

Ocimum gratissimum is a herbaceous plant popularly known as scent leaf. It is highly cultivated due to its culinary and medicinal importance (Shittu et al., 2016). It belongs to the family of Lamiaceae and is found in Africa, Asia, and South America. It is used as a natural flavouring agent, condiment, or vegetable in the preparation of fish, meat, soup, and stew. It is also used in traditional medicine for the treatment of several ailments such as cough, pneumonia, fever, inflammation, anaemia, diarrhoea, pains, and fungal and bacterial infections (Akara et al., 2021). Scientific reports have shown that *O. gratissimum* has a wide range of bioactive compounds such as flavonoids and polyphenol and essential oils with several beneficial effects (Melo et al., 2019). Furthermore, several studies have also shown that this plant possesses numerous pharmacological properties such as antioxidant, antimicrobial, anti-inflammatory, anthelmintic, cardiovascular, anti-mutagenic and antidiarrheal. However, there have been numerous reports on the use of several members of the *Ocimum* family in the synthesis of Selenium nanoparticles with *Ocimum sanctum* being the most used. (Aderibigbe and Idowu, 2020). The rise in antimicrobial resistance has emerged as a big crisis and a serious threat in every region of the world (Krupodorova et al., 2022; Unal et al., 2022). It is becoming a worsening problem in recent decades not only in public health, but also in economic and social impacts, which requires the development of new drugs for more effective treatments (Sevindik et al., 2017; Mohammed et al., 2022). Selenium nanoparticles produced via the green synthesis method can be an alternative to antibiotics. SeNPs showed an antibacterial effect toward standard and antibiotic-resistant phenotypes of Gram-negative and Gram-positive bacteria in a dose-dependent manner (Geoffrion et al., 2020). This study is aimed at phytochemical screening, synthesis, characterization, and antimicrobial activity of selenium nanoparticles synthesized from *O. gratissimum*.

Materials and Method

Collection of Plant

Fresh *O. gratissimum* leaves were obtained from Bodija market in Ibadan, Oyo state, South-west, Nigeria. The leaves were identified at the Department of Botany, University of Ibadan, Nigeria. The leaves were labeled and transported to the laboratory for immediate microbiological analysis.

Culture collection

The test organisms used to determine the antimicrobial activity were collected from the department of medical microbiology University College Hospital (UCH), Ibadan. The test organisms were *Escherichia coli*, *Paenibacillus alvei*, *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Candida albicans*. They were revived on nutrient agar (LabM, UK) before subsequently used.

Preparation of O. gratissimum extract

O. gratissimum leaves were washed with sterile distilled water to remove the particles and later air dried. 20 g of the leaf was finely cut, put in 250 mL glass beaker along with 100 mL distilled water, crushed and boiled for 5 mins at 90^{o}C. The extract was cooled at room temperature and filtered with Whatman No.1 filter paper. The filtrate was centrifuged for 10 minutes at 10000 rpm. The supernatant was collected and stored at 4°C (Radha et al., 2014).

Phytochemical screening of O. gratissimum leaf extract

The phytochemical tests to detect the presence of saponins, tannins, flavonoids, cardiac glycosides, anthraquinones, steroids, terpenoids, alkaloids and phenols was performed according to the procedure described by Harbourne, (1998) and the quantitative analysis was determined according to the method of Dohou et al. (2003).

Green synthesis of Selenium Nanoparticles using O. gratissimum leaf extract

The synthesis of Selenium nanoparticles (SeNPs) using *O. gratissimum* was done using the modified method of Alagesan and Venugopal, (2019). 10 mLs of *O. gratissimum* leaf extract was added to 90 mLs of 5 mM sodium selenite solution and heated up to 90°C for 5 minutes, the immediate colour change indicate the formation of Selenium nanoparticles. The Selenium nanoparticles solution thus obtained were purified by repeated centrifugation at 10000 rpm for 15 minutes. The supernatant was transferred to a clean dry beaker for further settlement of particles and repeated centrifugation was carried out. The particles obtained were used for further characterization.

Characterization of Greenly synthesized SeNPs

Visual Detection and UV –Visible spectrophotometric analysis of SeNPs

The SeNPs synthesized using *O. gratissimum* was observed visually for the change in colour from the initial colour (brown) of the sample to ruby red colour indicating the formation of selenium nanoparticles.

The bioreduction of Sodium selenite to selenium nanoparticles was monitored using UV-visible spectrophotometer (752 UV - Vis Spectrophotometer) with a resolution of 0.5 nm. 1 mL of each sample was taken and the absorbance was read at the wavelength ranging from 200 – 700 nm (Alagesan and Venugopal, 2019).

Scanning Electron Microscopic (SEM) and Transmission Electron Microscopic (TEM) analysis of SeNPs

The shape and morphology of the synthesized SeNPs was determined using Scanning Electron Microscopic (SEM) machine. The biosynthesized samples were freeze dried and mounted on a cover slip, coated with gold using a coater. Images of the selenium nanoparticles were taken in a SEM (JEOL - JSM - 7600F US). The details of the applied voltage and magnification used were also implanted on the images (Wadhwani et al., 2017).

A thin film of the sample was prepared on a carbon coated copper grid by dropping a very small amount of the sample on the grid. The biosynthesized SeNPs were subjected to centrifugation at 13000 rpm for 10 min. The pellet thus recovered was subjected to washing by its re-suspension in de-ionized water followed by centrifugation at 13000 rpm for 10 min, to remove possible organic contamination present in nanoparticles. Finally, the pellets were freeze dried using a lyophilizer. Transmission electron micrographs and recorded was done using TEM-ARM200F-G Verios 460L, USA instrument operated at an accelerating voltage of 15kV with a resolution of 0.23 nm(Kumar et al., 2014).

Energy Dispersive X-ray (EDX) and X-Ray Diffraction analysis of SeNPs

The elemental composition of the sample was determined using Energy Dispersive X-ray (EDX) (JEOL - JSM - 7600F, US). The centrifuged sample was drop coated on a carbon film and a spot-profile mode was done by focusing the electron beam onto a region on the surface of the biosynthesized selenium nanoparticles sample. It was done at a voltage of 40.0 Kv and current of 350μA (Kumar et al., 2014).

The crystalline size and purity were characterized by an X-ray diffractometer (Rigaku D/ Max111c, pw1800, China) using Cu K α radiation of wavelength $\lambda = 1.541$Å. The crystalline size of the prepared SeNPs nanoparticles was determined by using Scherrer's equation as follows $D \approx 0.9\lambda/\beta\cos\theta$, where D is the crystal size, λ is the wavelength of X-ray, θ is the Bragg angle in degree, and β is the full width at half maximum of the peak in radians (Alagesan and Venugopal, 2019).

Fourier Transform Infra-red (FTIR) analysis of SeNPs

FTIR was used to determine the functional groups in the synthesized SeNPs. 2 mg of the nanoparticles were encapsulated in 100 mg of KBr pellet in order to prepare translucent sample discs. The pelleted samples were subjected to Fourier transform infrared (FT-IR) spectroscopy in the range of wavelength 350 to 4400 cm^{-1}. The FT-IR spectrum was recorded using Nicolet iS10 FT-IR Spectrophotometer, China (Alagesan and Venugopal, 2019).

Effects of Physicochemical Parameters on the greenly synthesized SeNPs

Effect of incubation time, temperature, sodium selenite concentration and pH on the biosynthesis of SeNPs was investigated. The effect of temperature (27°C, 37°C and 45°C), incubation time (24, 48 and 72 hrs), different concentrations of Na_2SeO_3 (2 mM, 4 mM, 6 mM, and 8 mM) and pH (2, 5, 7, 9 and 11) on SeNPs synthesis was evaluated and characterized using UV-visible spectrophotometer at wavelengths ranging from 200 - 700 nm.

Antimicrobial Potential of the synthesized SeNPs using O. gratissimum leaf extract

The antimicrobial activity of the synthesized SeNPs was tested against selected pathogenic organisms using agar well diffusion method. Mueller Hinton agar was used as the growth media and was prepared and sterilized at 121°C for 15 minutes. The sterile media was allowed to cool to about 45°C, poured into sterile Petri dishes. Standardized inoculum of the test organisms were swabbed onto the surface of the sterile and solidified Mueller Hinton agar in separate Petri dishes. The plates were then allowed to dry. A 5 mm standard cork borer was used to bore uniform wells in the agar. SeNPs were introduced into each well on the agar. The plates were incubated at 37°C for 24 hrs after which the plates were observed for zone of inhibition of growth. The zones of inhibition were measured and recorded excluding the wells (Yusuf et al., 2021).

Results and Discussion

O. gratissimum is one of the discovered medicinal plants with the potential to serve as an alternative therapy for the treatment of various ailments. Scientific reports have shown that *O. gratissimum* has a wide range of bioactive compounds which possess unique antibacterial

activities against the plant and human bacterial pathogens. The qualitative and quantitative phytochemical analysis of *O. gratissimum* leaf extract is shown in Table 1. The result revealed that Saponins, Tannins, Cardiac glycosides, Terpenoids and Phenols are present in the leaf extract. Flavonoids and Alkaloids are abundantly present while Anthraquinones and Steroids were absent. These phytochemicals rich in polysaccharide galactomannan, act as good reducing and capping agents for the stability of selenium nanoparticles and may have played a role in reducing sodium selenite to SeNPs. Similar studies have also been reported by Olamilosoye et al. (2018) that the aqueous extract of *O. gratissimum* leaves contains alkaloids, flavonoid, tannins, saponins, cardiac glycoside and terpenoid. Melo et al. (2019) reported that aqueous extract of *O. gratissimum* is a rich source of flavonoids and phenolics. This is in line with the work of Usunomena and Eseosa, (2016) who reported the presence of tannins, saponins, flavonoids and alkaloids in *O. gratissimum* leaves.

Table 1. Qualitative and quantitative phytochemical composition of *O. gratissimum*

S/N	Phytochemical parameters	Qualitative	Quantitative (mg/100g)
1	Alkaloids	++	875
2	Flavonoids	++	615
3	Saponins	+	65
4	Tannins	+	122
5	Phenols	+	70
6	Cardiac glycosides	+	-
7	Terpenoids	+	90
8	Anthraquinones	-	-
9	Steroids	-	-

Key: ++: Abundantly present; +: Present; -: Absent

Plate 1. Visual characterization of the synthesized SeNPs using *O. gratissimum* leaf extract

Key: Na_2SeO_3: Sodium selenite; SeNPs: Biosynthesized SeNPs using *O. gratissimum;* Extract*: O. gratissimum* leaf extract

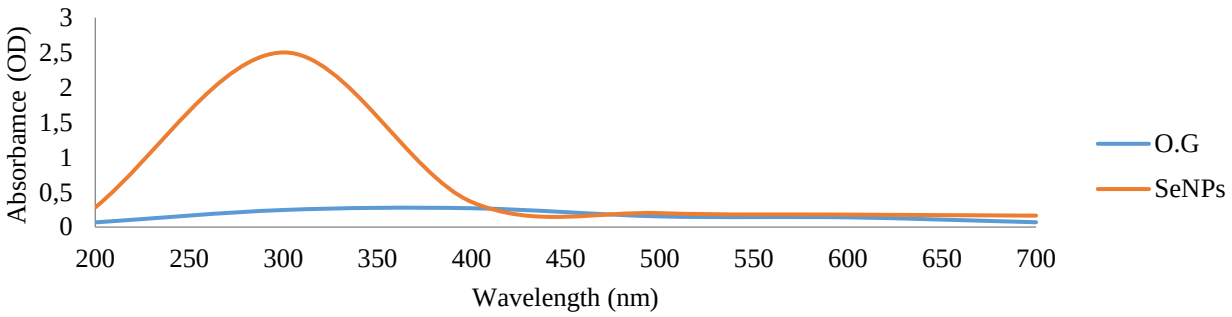

Figure 1. UV-Visible absorption spectrum of synthesized SeNPs using *O. gratissimum*

Key: O.G: *O. gratissimum* extract; SeNPs: Greenly synthesized SeNPs using *O. gratissimum*

Green synthesis and Characterization of SeNPs

O. gratissimum leaf extract used for the synthesis of selenium nanoparticles produced nanoparticles which were evident in the change of colour from dark brown to ruby red indicating the formation of SeNPs in the reaction mixture. Plate1 shows the visual characterization of SeNPs produced. The changes in colour from dark brown to ruby red may be due to the excitation of surface plasmon vibrations of selenium nanoparticles. This is in accordance with the work of Alagesan and Venugopal, (2019) who reported that the color change observed after adding *Withania somnifera* leaf extract to selenious acid solution is ruby red. Similarly, coloration changes from the colourless solution of sodium selenite to brick red have also been reported in the phytofabrication of Selenium nanoparticles using *Emblica officinalis* fruit extract (Gunti et al., 2019).

The greenly synthesized SeNPs was further characterized using the UV-Visible spectrophotometer. The spectra obtained from the synthesized SeNPs using *O. gratissimum* is shown in Figure 1. A strong surface plasma resonance peak (SPR) was obtained at 300 nm for the synthesized SeNPs which indicated the formation of the nanoparticles with a broad band at 200 - 400 nm while *O. gratissimum* extract did not show any characteristic SPR

peak. Similar studies have been reported by Ramamurthy et al. (2013) who stated that the UV-Visible spectra of their SeNPs showed absorption maximum at 200 nm – 400 nm with small peak observed at 300 nm in the UV region which may be due to the small organic molecules present in the reaction mixture.

The scanning electron microscopic analysis was carried out to determine the morphology of the synthesized SeNPs. Plate 2 shows the SEM micrograph of the synthesized SeNPs using *O. gratissimum* extract. The SeNPs in this study presented a monodispersed spherical morphology without forming aggregates. This result may be due to the presence of capping agents in the extract that act as stabilizers or binding molecules to provide colloidal stability along with preventing agglomeration and inhibit uncontrolled growth of nanoparticles. Similar sizes and morphologies of SeNPs have been reported by Fardsadegh et al. (2019) using *Pelargonium zonale* extract. Ojeda et al. (2020) also obtained spherical shaped particles with the synthesis of selenium using *Juglans regia* On the contrary, Alagesan and Venugopa (2019) reported that the SEM image of SeNPs showed greater numbers of agglomerated spherical particles.

The TEM analysis was further used to characterize the SeNPs produced by determining the size of the nanoparticle. The TEM analysis of synthesized SeNPs using *O. gratissimum* extract is shown in Plate 3. The particles size ranged from 20 nm - 100 nm. Changes in the size of SeNPs may be due to different nucleation and growth mechanisms related to factors such as temperature and reaction. This is in accordance with the work of Alagesan and Venugopa (2019) who reported that the nanoparticles produced were spherical particles within the diameter range 45 – 90 nm. Gunti et al. (2019) reported that the size of the biosynthesized SeNPs ranged from 15 – 40 nm.

The SeNPs was characterized by EDX to determine the elemental composition of the sample. The EDX analysis of synthesized SeNPs using *O. gratissimum* extract is shown in Figure 2. The presence of a strong selenium signal was observed and it was as a result of the surface plasmon resonance indicating that the SeNPs obtained were of high purity. The weak signal from Silicon, Carbon, and Oxygen may be due to mixed precipitate present in the extract. This is similar to the work of Miglnai and Tani-ishii (2021) who reported that there were strong signals from selenium atoms in the selenium nanoparticles. Vyas and Rana, (2017) reported that Selenium had the highest proportion in nanoparticles followed by Oxygen, Carbon, Sodium, Phosphorus and Chlorine.

To evaluate the crystalline nature of the synthesized SeNPs, it was characterized by the XRD technique. Figure 3 shows the X-ray diffraction analysis for the synthesized SeNPs using *O. gratissimum*. The XRD analysis for the biosynthesized SeNPs indicated five diffraction peaks in the whole spectrum of 2θ values ranging from 35 to 80. All diffraction peaks corresponded to the characteristic crystalline face-centered cubic (FCC). Sharp peaks were observed indicating the formation of crystalline SeNPs. All the peaks could be indexed according to the trigonal phase of elemental selenium. This corresponds to the work of El-Deeba et al., (2018) who reported the pure crystalline form of selenium. This result contradicts the work of Filipovic et al., (2021) who reported that the diffraction peaks which indicate the crystalline phase of selenium were not observed.

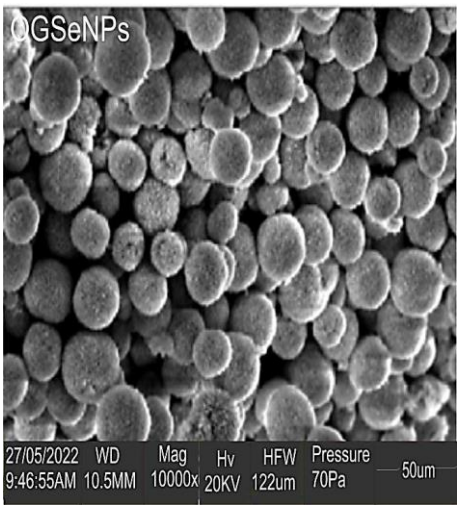

Plate 2. SEM micrograph of synthesized SeNPs using *O. gratissimum* extract

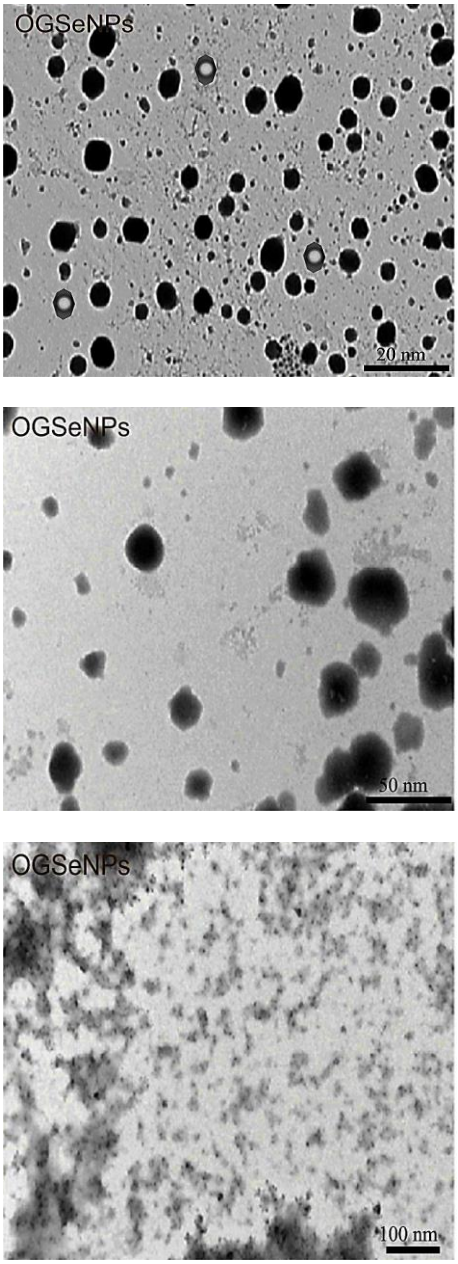

Plate 3. TEM micrograph of synthesized SeNPs using *O. gratissimum* extract

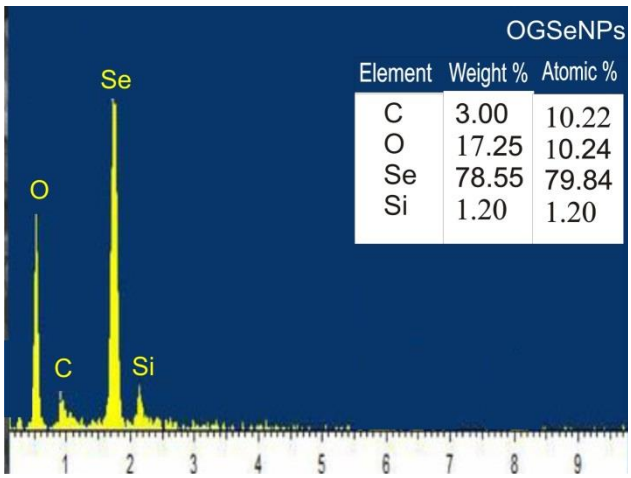

Figure 2. EDX analysis of synthesized SeNPs using *O. gratissimum* extract

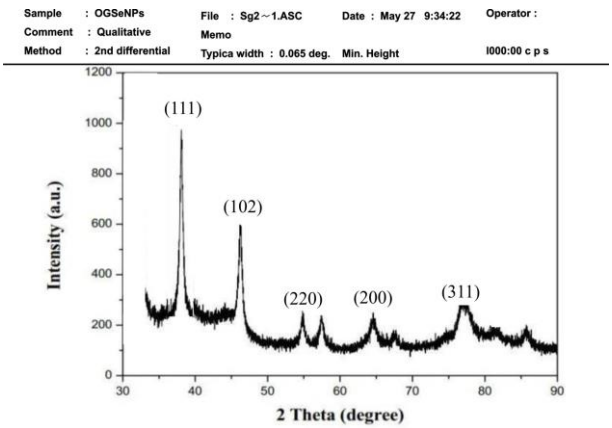

Figure 3. XRD analysis of synthesized SeNPs using *O. gratissimum*

FTIR was used to determine the functional groups present in the sample. The FTIR spectrum of *O. gratissimum* extract is shown in Figure 4a. Peaks were present at 3778.31, 3376.00, 2918.00, 2853.01, 2360.17, 1595.00, 1369.00, 1032.00 and 367.33 cm^{-1}. The peaks at 3376.00 cm^{-1} and 3778.31 cm^{-1} were observed to have the strongest and broadest absorption peak indicating a stretching vibration of hydroxyl group (OH). The characteristic peaks appearing at 2918.00 cm^{-1} corresponds to C – H asymmetrical stretching of alkanes. The peak at 2853.01 cm^{-1} indicated the O-H stretching of carboxylic acid. The peak at 1595.00 cm-1 corresponds to C=C stretching of cyclic alkene. The peak at 1369.00 cm^{-1} attributed to O-H bending of alcohol. The peak observed at 1032.53 cm^{-1} and 1032.00 cm^{-1} was due to C-N stretching of amine. These characteristics absorption peak indicated that hydroxyl, aldehyde, amine and ester may be responsible for the ability of *O. gratissimum* extracts to adhere to the surface of nanoparticles as a form of stabilization.

The FTIR spectrum of biosynthesized SeNPs is shown in Figure 4b. Peaks appeared at 3458.00, 2929.45, 2355.16, 1737.50, 1637.33, 1449.00, 1387.40, 1111.33, 1032.53, 540.00 and 458.73 cm^{-1}. The peak at 3458.00 cm^{-1} was observed to have the strongest and broadest absorption peak indicating a stretching vibration of hydroxyl group (OH). The characteristic peaks appearing at 2929.45 cm^{-1} corresponds to C – H asymmetrical stretching of alkanes. The peak at 1737.50 cm^{-1} attributed to C=O stretching of esters. The peak at 1595.00 cm-1 corresponds to C=C stretching of cyclic alkene. The peak at 1387.40 cm^{-1} was assigned to C-H bending of aldehyde. The peak at 1111.33 cm^{-1} corresponds to C-O stretch of secondary alcohol and aliphatic ether. The peak observed at 1032.53 cm^{-1} was due to C-N stretching of amine while the peak at 540.00 cm^{-1} represents the C-I and C-Br stretching of halo compounds. These characteristics absorption peak indicated that hydroxyl, aldehyde, amine and ester may be responsible for the efficient stabilization and bioreduction of Sodium selenite (Na_2SeO_3) into Selenium nanoparticles (SeNPs). These functional groups are present as a result of the presence of biomolecules in the extract which was involved in the synthesis process. This is in accordance with the work of Alagesan and Venugopa, (2019) who reported that the presence of various functional groups as biomolecules may be responsible for both reduction and stabilization of the SeNPs. These functional groups are present as a result of the presence of biomolecules in the extract which was involved in the biosynthesis process. Previous reports have also suggested the role of phytochemicals as a stabilizing agent for the synthesis of metal nanoparticles (Coccia et al., 2012).

The effect of physicochemical parameters on the synthesis of SeNPs

Temperature is one of the most important physical parameter on the synthesis of SeNPs. Effect of temperature on the synthesis of SeNPs was evaluated. The UV-Visible absorption spectrum of the effect of temperature on the synthesis of SeNPs is shown in Figure 5a. Strong SPR peak was observed at 300 nm for all the temperatures and broad bands ranging from 200 nm – 400 nm. The result showed that the nanoparticles biosynthesis increased by enhancing temperature to 37°C and no appreciable production of nanoparticles was attained at 45°C. The ability of 37°C to be the best temperature for the synthesis of SeNPs may be due to the fact that the biomolecules or proteins responsible for the reduction of sodium selenite are only active around 30°C and at 37°C. So, 37°C was considered as the optimum temperature. Also, at higher temperature denaturation of proteins capping may occur and this leads to higher rate of agglomeration. This result is in agreement with the work of Lortie et al. (1992) who reported that there was no synthesis of SeNPs at higher temperature. Correa-Llanten et al. (2014), reported that synthesis of SeNPs by *Citrus reticulata* peel extract was found to be efficient at 40°C

The effect of incubation time on the biosynthesis of SeNPs using *O. gratissimum* extract was studied and the UV- Visible spectrum was recorded after time intervals of 24 hrs, 48 hrs and 72 hrs. Figure 5b shows the UV-Visible absorption spectrum of the effect of incubation time on the biosynthesis of SeNPs. SPR peak was observed at 300 nm and a broad band ranging from 200 nm – 400 nm for all the incubation time. Maximum absorbance was recorded at 72 hrs while the least absorbance was recorded at 24 hrs for the biosynthesis of SeNPs. The ability of the biosynthesized SeNPs to increase in absorbance with time may be as a result of continued reduction of the selenium ions and the collision frequency between the particles. This is in accordance with the work of Sani-e-Zahra et al. (2022) who reported that after 96 hr maximum absorbance was observed. Sarkar et al. (2022), who reported that the synthesis of SeNPs depends on time.

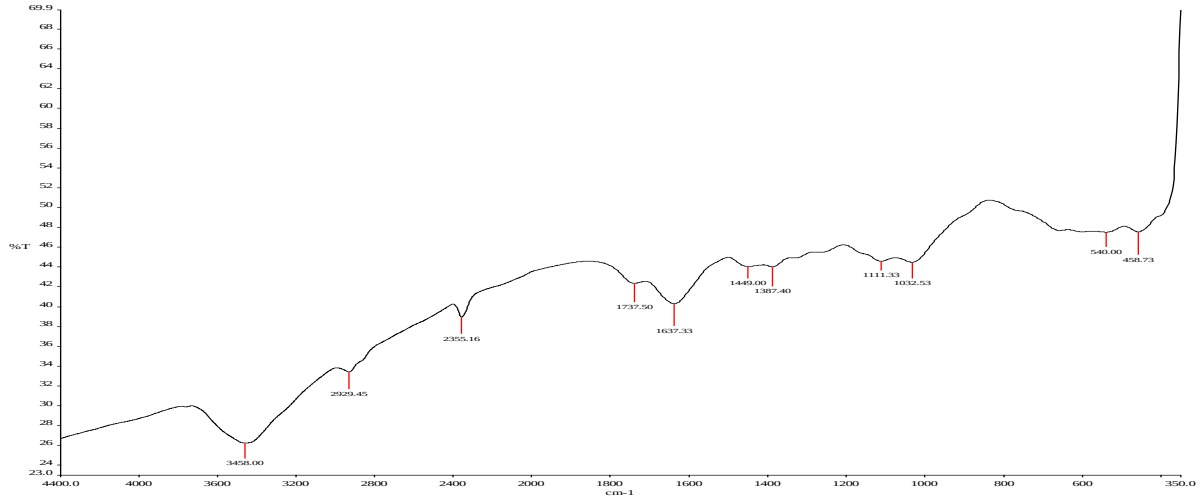

Figure 4a. FTIR spectrum of *O. gratissimum* extract

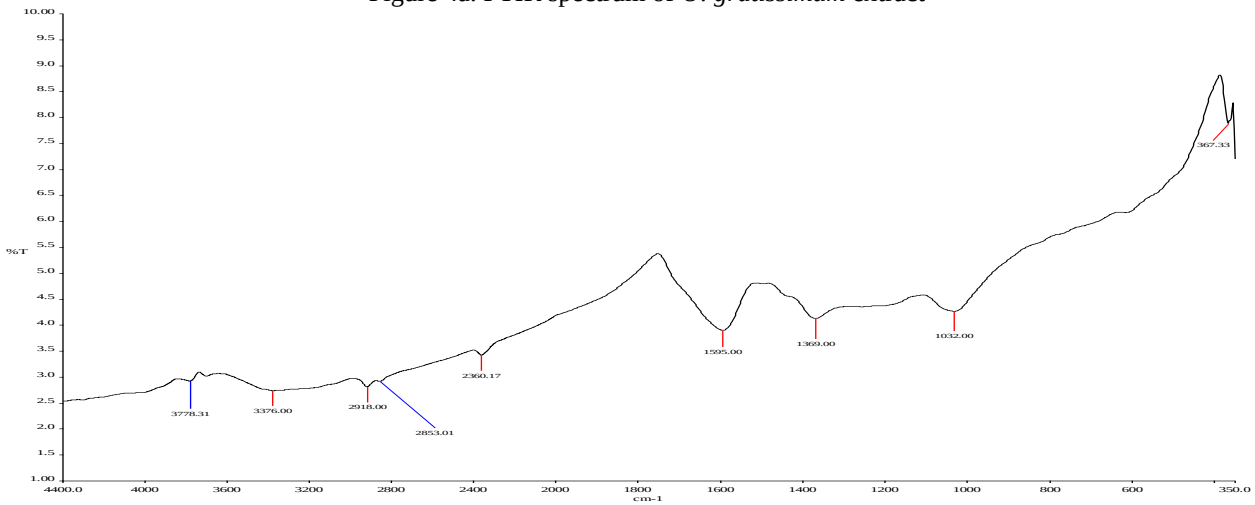

Figure 4b. FTIR spectrum of synthesized SeNPs using *O. gratissimum* extract

Table 2. Antimicrobial activity of greenly SeNPs using *O. gratissimum* (OGSeNPs) on some pathogens

	Zone of inhibition (mm)			
Test organisms	Na_2SeO_3	OGE	OGSeNPs	CPR
S. pneumoniae	-	18	26	28
E. coli	-	19	28	26
K. pneumoniae	-	15	27	24
P. aeruginosa	-	-	22	24
S. aureus	-	20	27	28
C. albicans	-	14	24	24
P. alvei	-	16	20	24

Key: Na_2SeO_3: Sodium selenite, OGE: *O. gratissimum* extract, OGSeNPs: Biosynthesized selenium nanoparticles using *O. gratissimum;* CPR: Ciprofloxacin

The effect of different concentration (2 mM, 4 mM, 6 mM, 8 mM) of Na_2SeO_3 on the biosynthesis of SeNPs was evaluated and is shown in Figure 5c. The SeNPs showed strong SPR peak at 300 nm with broad band at 200 nm - 400 nm for all concentrations. The highest absorbance was recorded at 8 mM while the least absorbance was recorded at 2 mM. It was observed that the best concentration for SeNPs synthesis was 8 mM. The ability of 8 mM Na_2SeO_3 concentration to produce the highest SeNPs biosynthesis may be due to the fast rate of bio-reduction with increase in concentration of the salt. This result is in agreement with the work of Sani-e-Zahra et al. (2022) who reported maximum amount of SeNPs as the concentration of sodium selenite increased.

The effect of pH on the biosynthesis of SeNPs using *O. gratissimum* extract was evaluated at different range (2, 5, 7, 9 and 11) and monitored with the UV-Visible spectrophotometer. The UV-Visible absorption spectrum of the effect of different pH levels on the biosynthesis of SeNPs is shown in Figure 5d. SPR peak was observed at 300 nm and broad bands ranging from 200 nm – 400 nm for the pH levels with pH 9 and 11. The highest absorbance was recorded against pH 11 indicating the increased synthesis of selenium nanoparticles under alkaline conditions which may be as a result of more availability of bio-reductions while the lowest absorbance was recorded at pH 2. The decreased reduction rate in acidic medium could be ascribed to the denaturation and /or degradation of bioactive compounds present in the *O. gratissiumum*.

This report is in agreement with the work of Sani-e-Zahra et al. (2022) who reported that a maximum absorption was observed at pH 7 and no reaction occurred at pH 5. Similarly, Roopan et al. (2011) also reported the formation of highly dispersed nanoparticles at pH 11 whereas, no reaction occurred at pH 2.

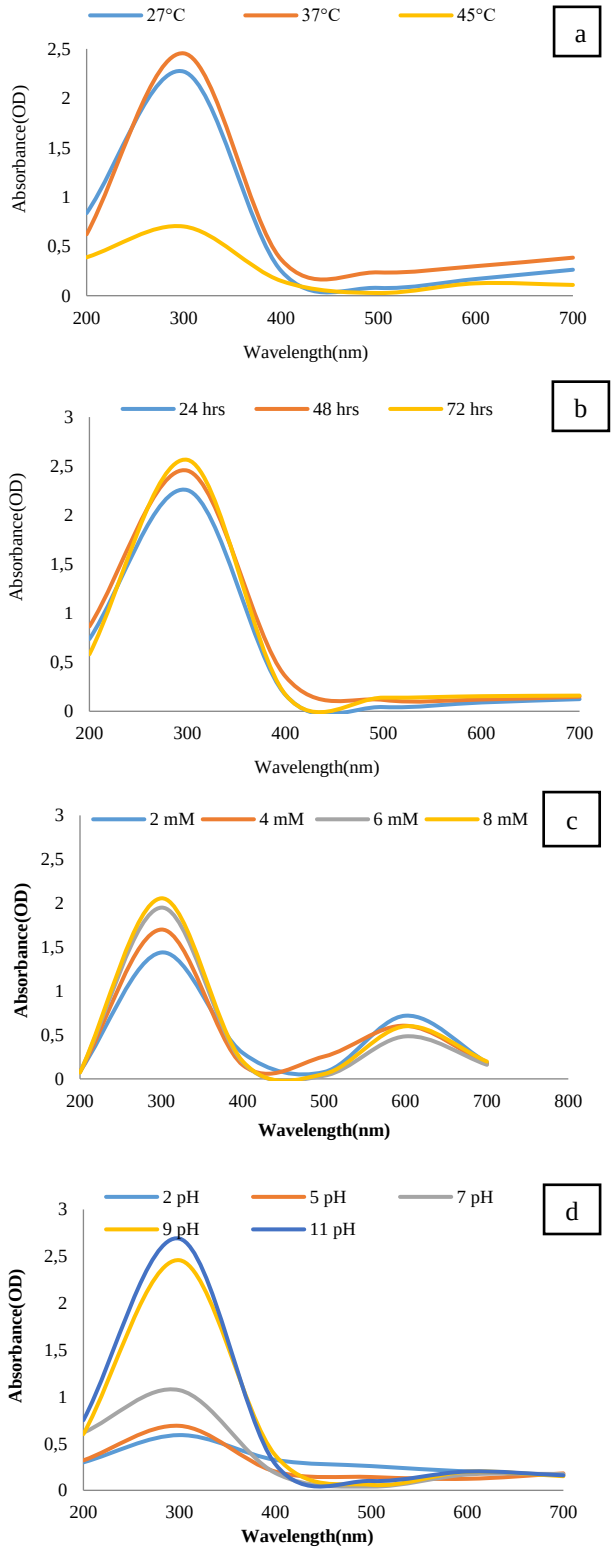

Figure 5. UV-visible absorption spectra of synthesized SeNPs

(a) at different incubation temperature; (b) at different incubation time; (c) at different pH of reaction mixture; (d); at different Na_2SeO_3 concentration

Antimicrobial activity of greenly SeNPs using *O. gratissimum* (OGSeNPs)

One of the most remarkable and peculiar properties of selenium nanoparticles is their antimicrobial activities. The ability of the biosynthesized SeNPs to inhibit the growth of selected pathogens was investigated and the biosynthesized SeNPs exhibited an excellent antimicrobial activity against the selected pathogens. They were also found to have a broad spectrum of activity against the pathogens. The gram negative bacteria were more susceptible to the SeNPs than the gram positive bacteria which may be due to the composition of bacterial cell wall. Gram negative bacteria are significantly protected with a cytoplasmic membrane and outer cell membrane, containing a thin layer of the peptidoglycan between them with periplasmic compartment, while all gram positive bacteria are bound by only a single unit lipid membrane and thick layer of peptidoglycan. The result is similar to the work of Hidayat et al. (2020), who reported that the inhibitory effects of SeNPs were more pronounced on gram negative compared to gram positive bacteria due to the thickness of the cell wall and cell components. Eswarapriya and Jegatheesan (2015) reported that there was an antifungal activity of biosynthesized SeNPs against *Candida albicans* and *Aspergillus niger*. Also, Zonaro et al. (2015) reported that SeNPs exhibit more inhibitory actions against gram positive bacteria species as a result of the lesser surface charges of nanoparticles that effectively enable them to bind to the bacterial cell membrane. This study is in accordance with the work of Hernández-Díaz et al. (2021) which reported that selenium nanoparticles had a wide range of activities against *B. subtilis*, *E. coli*, and *S. aureus*.

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