Biogenic Synthesis of Selenium Nanoparticles Using *Beta vulgaris* Fruit Extract and Its Antimicrobial Potential

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Abstract
Generally biological synthesis of nanoparticles has a large number of biomedical applications because it is safe and eco-friendly in nature. *Beta vulgaris* (Beetroot) is a fruit, rich in vitamins, minerals, and nutrients. This study demonstrated the phytochemical analysis, synthesis of selenium nanoparticles and the antimicrobial activity of aqueous fruit extract of *B. vulgaris*. Phytochemical studies were carried out using standard procedures. Biosynthesized SeNPs by *B. vulgaris* fruit extract were confirmed by visual detection, UV–Visible spectrophotometer and characterized by Scanning Electron Microscopy, Transmission Electron Microscopy, Energy dispersive spectroscopy, Fourier Transform Infra-red spectroscopy and X-ray diffractor was used to confirm the formation, nature, morphology, size and shape of the nanoparticles. Agar well diffusion method was used for the antimicrobial activity. Phytochemical analyses of *B. vulgaris* fruit extract revealed the presence of saponins, tannins, flavonoids, terpenoids, alkaloids, and phenol which act as both reducing and potential stabilizing agents in the synthesis of SeNPs. UV-visible spectrophotometer revealed the surface plasmon resonance peak at 350 nm within a broad band range of 300 – 400 nm. The SeNPs are crystallographic, spherical and having size range from 20–100 nm. Esters, amino acids, hydroxyl, aldehyde, and carboxylic acid were present and responsible for the synthesis of SeNPs. The biosynthesized SeNPs showed activity against the test pathogens and the zone of inhibition ranged between 9 - 25 mm. In conclusion, *B. vulgaris* fruit extract can be used for the synthesis of SeNPs with potent antimicrobial activity on fungal and bacteria.

Keywords: *Beta vulgaris*, Selenium nanoparticles, Biosynthesis, Antimicrobial activities, Phytochemical analysis

1. Introduction

Nanoparticles represent the leading edge of rapidly growing field of Nanotechnology. They are particles of matter within the range of 1 and 100 nanometer (nm) in diameter [1]. Nano-scale technology involves the use of materials on the atomic or molecular scale to produce objects which are not more than a few nanometres in diameter [2, 3]. Nanoparticles possess high specific surface area than micro-particles which enhances their functional capabilities. Furthermore, nanoparticles bridge the gap between bulk materials and atomic or molecular structures which gives it a unique interest [4]. However, nanoparticles are not simple molecules but are composed of three layers. The surface layer, which may be functionalized with a variety of small molecules, metal ions, surfactants and polymers-the shell layer, which is a chemically different material from the core in all aspects and the core, which is essentially the central portion of the nanoparticles and usually referred to as the nanoparticle itself [5]. In addition, nanoparticles have found their usage in drug delivery, chemical, and biological sensing as well as -gas sensing [6], carbon dioxide capturing, and other related applications [7].

Selenium is a trace mineral needed in the body only in small quantities [8]. It is found naturally in foods and used as a supplement. Selenium is an essential component of various enzymes and proteins, called selenoproteins, which helps the Deoxyribonucleic acid and protects against cell damage and infections. These proteins are also involved in reproduction and the metabolism of thyroid hormones as well as induces and modulates the expression of stress-responsive proteins and genes [8]. Selenium nanoparticles are metallic nanoparticles that have attracted many researchers due to it biocompatibility, bioavailability, and low toxicity [9]. In addition, selenium nanoparticles possess antimicrobial activity which enables them to inhibit the growth of microbes such as bacteria, fungi and viruses. Selenium is an essential trace element in the diet, required for maintenance of health and one of the

interesting compounds to integrate with antibacterial agents. Previously, selenium nanoparticles have been used in the management and treatment of several diseases, such as diabetes, Alzheimer's disease (AD) inflammation-related diseases, and rheumatoid arthritis [9].

Nanoparticles can be synthesized using physical, chemical, and biological approaches. Biological synthesis approach emphasizes the use of natural organisms, microorganism, microalgae, enzyme, plant, and plant extracts which offers a reliable, simple, non-toxic, low-cost approach, stable nature and its eco-friendly [10]. Plants provide a better platform for nanoparticles synthesis as they are free from toxic chemicals, provide natural capping agents and easier to scale-up for large scale production due to their abundance and availability in nature [11]. The use of plant extracts also reduces the cost of microorganisms' isolation and culture media enhancing cost competitive feasibility over the use of microorganisms for nanoparticles synthesis [12].

Medicinal plants contain essential bioactive compounds that are mostly referred to as phytochemicals which plays a bactericidal role in most pathogenic organisms. Generally, phytochemicals perform intermediary metabolic activities and function as primary metabolites such as fats and sugars found in plants, as well as secondary metabolites found in some range of plants and provide specialized functional compounds with therapeutic properties [13]. Plants such as guava (*Psidium guajava*), beetroot (*Beta vulgaris*), ginger (*Zingiber officinale*), neem (*Azadirachta indica*), and moringa (*Moringa oleifera*) have been found to exhibit broad range of antimicrobial activities [14]. Many plants used as herbs and spices, have been used as medicines. However, there are evidence-based studies to verify the efficacy of medicinal plants, and some of these shreds of evidence have provided insights into plant-based synthesis [17].

Beta vulgaris (Beetroot) is a root fruit vegetable with medicinal properties and is commonly known as red beet, table beet, garden beet, or beet. *B. vulgaris* is enriched with essential nutrients, fiber, folate (vitamin B9), manganese, potassium, iron and vitamin C [16]. Its fruit is alkaline in nature (pH range of 7.5 to 8.0) and has been acclaimed for its health benefits, particularly for its antioxidant potential [17]. *B. vulgaris* fruit can be roasted, boiled, or steamed and eaten as a condiment or salad. Nutritionally, beetroot contains 88% water, 10% carbohydrate, 2% protein, and less than 1% fat [17] and a good source of minerals, including manganese (good for bone health), magnesium, potassium, sodium, phosphorus, iron, zinc, copper, boron, silica, and selenium [18]

This study aimed to synthesise, characterize and evaluate the antimicrobial activity of *B. vulgaris* fruit extract-selenium nanoparticles.

2. Materials and Methods

2.1 Sample Collection

Beta vulgaris fruits were purchased from Shoprite supermarket in Ibadan, Oyo State, South-West, Nigeria. They were identified and authenticated at the Herbarium unit Department of Botany, University of Ibadan, Nigeria. The fruits were labelled and transported to the laboratory for immediate analysis.

2.2 Preparation of *Beta vulgaris* Fruit Extract

The fresh *Beta vulgaris* fruits were washed thoroughly with tap water and subsequently with sterile distilled water to remove all the unwanted visible particles. The fruits (20 g) were cut into fine pieces, grinded, sieved and filtered using Whatman filter paper No 1. The fruit extract obtained was used for further analysis [19].

2.3 Phytochemical Analysis

Standard qualitative and quantitative phyto-chemical tests were carried out on *B. vulgaris* fruit extract for the determination of phytochemical constituents [20, 21]

2.4 Biogenic Synthesis of Selenium Nanoparticles

In a typical reaction procedure, 10 mL fruit extract of *B. vulgaris* was added to 90 mL of 5 mM sodium selenite solution and heated up to 90°C for 5 minutes, ascorbic acid was added as an initiator of reduction reaction. The gradual colour change of the resulting mixture signified the formation of selenium nanoparticles. The reacting solution was stirred at room temperature for 72 hrs and was repeatedly centrifuge at 10000 rpm for 15 minutes. The obtained precipitate was used for further analysis.

2.5 Characterization of Selenium Nanoparticles

2.5.1 Visual Detection and UV-Visible Spectroscopy Analysis

Visual observation of the synthesized SeNPs was carried out by visually observing the gradual change in colour of the fruit extract and the resulting sodium selenite mixture.

The formation of SeNPs was confirmed by determining the absorbance wavelength of the formulation using UV-Visible spectrophotometer. 2 mL of the reaction mixture was scanned in UV-Visible spectra (Lambda 752 UV-Vis spectrophotometer) between the wavelengths of 250 - 750 nm with a resolution of 1 nm [22].

2.5.2 Fourier Transform Infrared Spectroscopy (FT-IR)

The BTSeNPs were dried and diluted with potassium bromide (FT-IR grade) in the ratio of 1:100. The FT-IR spectrum of samples was recorded on an FT-IR spectrophotometer (Jasco FT/IR-6300) equipped with JASCO IRT-7000 Intron Infrared Microscope. The spectrum was recorded with the wavelength ranging from 350 - 4,400 cm^{-1} at a resolution of 4 cm^{-1} [23].

2.5.3 Scanning Electron Microscopy and Transmission Electron Microscopy Analysis

The morphology of the biogenic synthesized SeNPs was determined using Scanning Electron Microscopic (SEM) machine. The samples were freeze dried and mounted on a cover slip, coated with gold using a coater (JEOL-JSM-7600F, US). The images of the SeNPs were obtained, details regarding applied voltage, magnification used, and size of the contents of the image were also on the images [24].

A small amount of the sample was deposited on a carbon-coated copper grid, a thin film of the sample was created. BTSeNPs was centrifuged for 10 min at 13,000 rpm. The pellet was rinsed in de-ionized water before centrifugation at 13,000 rpm for 10 min to eliminate any possible organic contamination. The pellet was frozen dry using a lyophilizer. Transmission electron micrographs were obtained using TEM-ARM200F-G Verios 460L, USA [15].

2.5.4 Energy Dispersive X-Ray (EDX)

The elemental analysis of the biosynthesized SeNPs using *B. vulgaris* fruit extract (BTSeNPs) was determined using EDX spectroscopy. The pellet from the centrifuged selenium nanoparticles samples was dried. 100 nm thick section of the pellet was placed on the grid and analyzed using EDX machine at a voltage of 40.0 Kv and current of 350 µA (JEOL - JSM - 7600F, US). Pure selenium standard was used for calibration, and then a working curve was selected [25].

2.5.5 X-Ray Diffraction (XRD)

The crystallographic nature of the biogenic synthesized BTSeNPs was determined using XRD. Slurry of the dried BTSeNPs samples was placed in a quartz disk. The disk was placed on an XRD slit and analyzed using an X-ray diffractometer (Rigaku D/ Max111c, PW1800, China) in the region of 2^{θ} operating at a voltage of 45 kV and a current of 30 mA at room temperature and using Cu K α radiation wavelength $\lambda = 1.541Å$ [22].

2.6 Screening for Antimicrobial Activities of Synthesized SELENIUM Nanoparticles Using *B. vulgaris* Fruit

The antimicrobial activity of the biogenic synthesized BTSeNPs against some selected test pathogens were assessed using the agar well diffusion method. 18 hrs old culture of *Staphylococcus aureus*, *Escherichia coli*, *Bacillus alvei*, *Streptococcus pneumonia*, *Candida albicans*, *Shigella* spp, *Pseudomonas aeruginosa and Klebsiella pneumoniae* grown on nutrient agar at 37ºC was suspended in saline. A lawn of the indicator strain was made by spreading the cell suspension over the surface of Mueller Hinton agar plates with a sterile cotton wool swab and the plates were allowed to dry. A cork borer of 5 mm diameter was used to bore uniform wells in the agar. BTSeNPs solution was loaded in the wells. Bacteria plates were then incubated at 37ºC for 24 h, after which the zones of inhibition were measured [1].

2.7 Statistical Analysis

Statistical analysis of antimicrobial activities of synthesized SeNPs was done using SPSS software (version 24, SPSS lnc, Chicago, IL, USA). ANOVA was used to compare the results. $P < 0.05$ was considered significant.

3.0 Results

Phytochemical Screening of the Aqueous Fruit extract of *B. vulgaris*

The qualitative and quantitative results of *B. vulgaris* fruit extract is shown in Table 1. The qualitative analysis revealed the presence of saponins, tannins, flavonoids, terpenoids, alkaloids and phenol while cardiac glycosides, anthraquinones and steroids were absent. Quantitatively, tannins have the highest quantity (389 mg/100g) while saponin has the least quantity (65 mg/100g).

Table 1: Qualitative and Quantitative Phytochemical Constituents of *B. vulgaris* Fruit Extract

S/N	Phytochemical Parameters	Qualitative	Quantitative (mg / 100g)
1.	Saponins	+ve	65
2.	Tannins	+ve	389
3.	Flavonoids	+ve	316
4.	Cardiac glycosides	-ve	-
5.	Anthraquinones	-ve	-
6.	Steroids	-ve	-
7.	Terpenoids	+ve	100
8.	Alkaloids	+ve	360
9.	Phenol	+ve	69

Key: +ve: present, -ve: absent

Biogenic Synthesis and Characterization of Selenium Nanoparticles

The synthesis of the SeNPs was observed from the colour change that occurred after the interaction of the sodium selenite with the aqueous extract of *B. vulgaris* fruit. There was a colour change from red to ruby red indicating the formation of SeNPs as shown in Plate 1.

Plate 1: Visual Characterization of Biogenic Synthesized SeNPs using *B. vulgaris* Fruit

Key: Beetroot extract (*B. vulgaris*); Na_2SeO_3: Sodium Selenite solution; Beetroot+ Na_2SeO_3 = BTSeNPs

UV- Visible spectroscopy

The synthesized SeNPs were characterized with UV- Visible spectrophotometer and the spectra obtained are shown in Figure 1. Surface plasma resonance peak (SRP) was at 350 nm for BTSeNPs and the broad band between 300 – 400 nm, indicating the formation of SeNPs. The Surface plasma resonance peak was at 450 nm for BT extract, having a broad band of 300 - 550 nm.

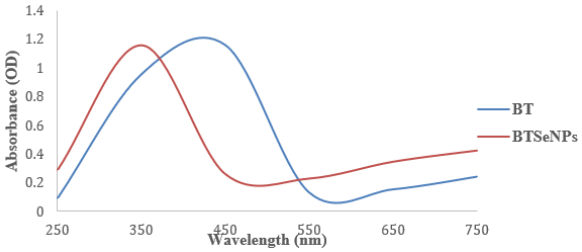

Figure 1: UV-Visible absorption spectra of SeNPs synthesized using *B. vulgaris* fruit.

Key: BT – *Beta vulgaris* fruit extract; BTSeNPs – Synthesized selenium nanoparticles by *B. vulgaris* fruit extract

Scanning Electron Microscopy (SEM)

SEM revealed the morphology of the synthesized SeNPs. The SEM micrograph of the synthesized SeNPs by *B. vulgaris* is shown in Plate 2. The SeNPs synthesized by the *B. vulgaris* fruit extracts revealed spherical shapes with uniform particle size distribution.

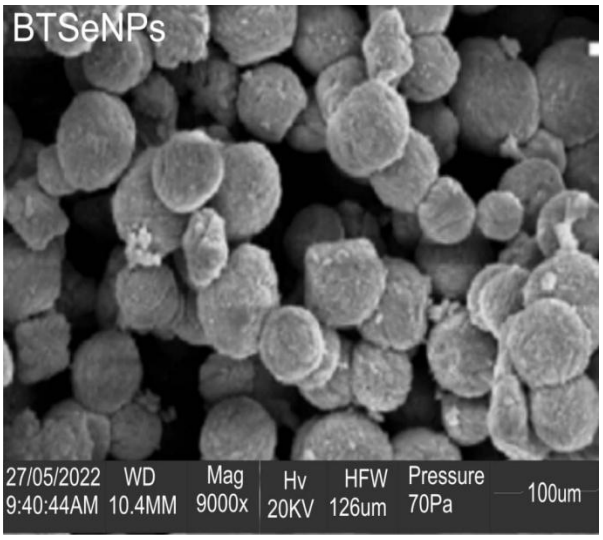

Plate 2: Scanning electron micrograph of the synthesized SeNPs using *B. vulgaris* fruit

Transmission Electron Microscopy (TEM)

TEM was performed to determine the size of the biosynthesized selenium nanoparticle. The TEM micrograph of the biosynthesized SeNPs by *B. vulgaris* fruit is shown in Plate 3. The particles size ranged from 20 -100 nm.

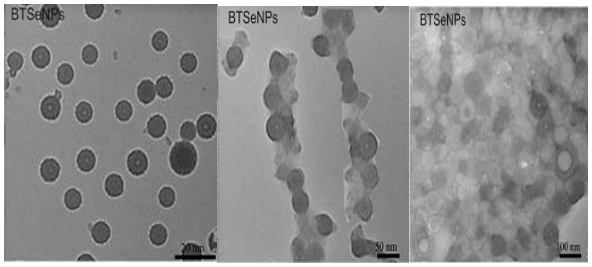

Plate 3: Transmission electron micrographs of the synthesized SeNPs using *B. vulgaris* fruit.

Energy Dispersive X-Ray (EDX)

The EDX analysis of the synthesized SeNPs by *B. vulgaris* fruit extract is shown in Figure 2. The EDX spectrum exhibited the presence of strong signals corresponding to elemental selenium which was 72%. The other peaks being observed was Oxygen (20%), Carbon (24%) and Silicon (3%). No signal for other elements and impurities was observed. The occurrence of elemental selenium confirmed the bio-reduction of the selenium ions to elemental form.

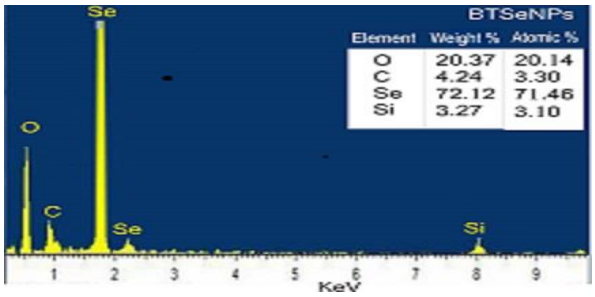

Figure 2: EDX analysis of the synthesized SeNPs by B. vulgaris fruit extract.

FTIR analysis

The FTIR spectrum of biosynthesized SeNPs using *B. vulgaris* fruit extract is shown in Figure 3a. Ten bands were present at 3759.57, 3413.00, 2927.86, 2289.00, 1574.66, 1390.06, 1266.53, 1030.54, 541.21 and 370.23 cm^{-1} as shown in Figure 3.5a. The peak at 3759.57 cm^{-1} was observed to have a medium and sharp absorption peak indicating a stretching of hydroxyl group (O-H). The peak at 3413.00 cm^{-1} indicates the presence of O-H stretching alcohol while the peak at 2927.86 cm^{-1} indicates the presence of C-H stretching alkenes. The absorption peak at 2289.00 cm^{-1} indicates the presence of a O=C=O strong stretching carbon dioxide group and 1574.66 cm^{-1}peak shows the presence N-H bending of amine. The peak at 1390.06 cm^{-1} indicates C-H in plane bend of alkanes. The peak at 1266.53 cm^{-1} showed the presence of C-O stretch of alkyl aryl ether while 1030.54 cm^{-1} indicates the presence of S=O stretching of sulfoxide. The peaks at 541.21 cm^{-1} and 370.23 cm^{-1} showed the presence of C-Br halo compound stretch. From all indications with the observed functional groups, hydroxyl, ether, alcohol and proteins may be responsible for the stability and bio-reduction of sodium selenite to SeNPs.

The FTIR spectrum of *B. vulgaris* fruit extract showed the presence of 15 bands at 3762.00, 3438.00, 2926.29, 2369.33, 1728.45, 1637.57, 1514.38, 1427.00, 1381.79, 1329.00, 1256.29, 1099.45, 605.00, 495.76 and 360.52 cm^{-1} in Figure 3b. The peaks at 3762.00 cm^{-1} and 3438.00 cm^{-1} has been identified as O-H stretch of alcohol. The peak at 2926.29 cm^{-1} indicated the N-H stretching of amine salt. The absorption peak at 2369.33 cm^{-1} indicates the presence of a N=C=O strong

stretching isocyanate group. The peak at 1728.45 cm^{-1} indicated the C=O symmetrical stretching of aldehydes. The absorption peak at 1637.57 cm^{-1} indicates the presence of C=C stretch of alkene. The peak at 1514.38 cm^{-1} showed the presence of N-O nitro compound stretch. The absorption peak at 1427.00 cm^{-1} shows the presence O-H bending of carboxylic acid and 1381.79 cm^{-1} peak shows the presence of S=O stretch of sulfonyl chloride. The peak at 1329.00 cm^{-1} indicates the presence of S=O strong stretch of sulfone group. The peaks at 1256.29 cm^{-1} and 1099.45 cm^{-1} indicated the presence of C-O stretch of aromatic ester and C-O aliphatic ether stretch. The absorption peaks at 605.00 cm^{-1}, 495.76 cm^{-1} and 360.52 cm^{-1} indicated the presence of C-l of halo compound stretch. The functional groups observed generally indicated the presence of aldehyde, carboxylic acid, ester and alkenes.

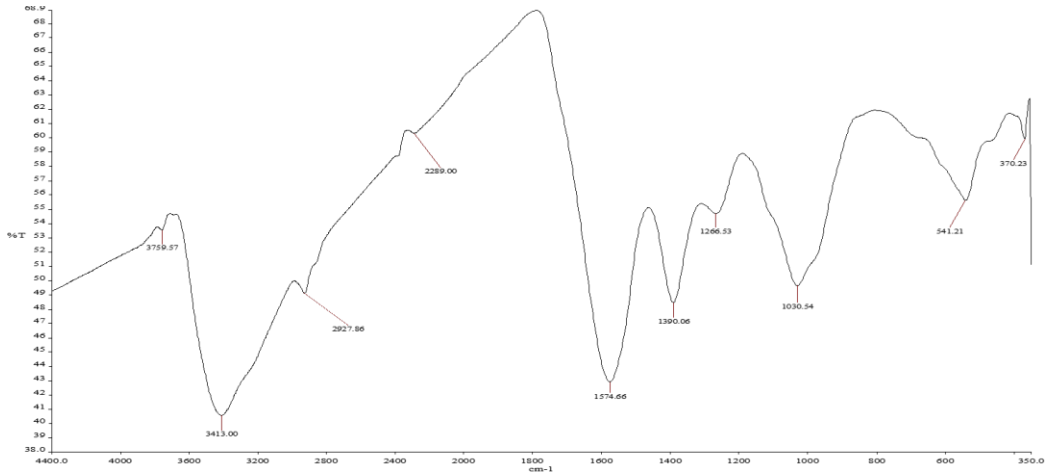

Figure 3a: FTIR spectrum of the synthesized SeNPs using *B. vulgaris* fruit.

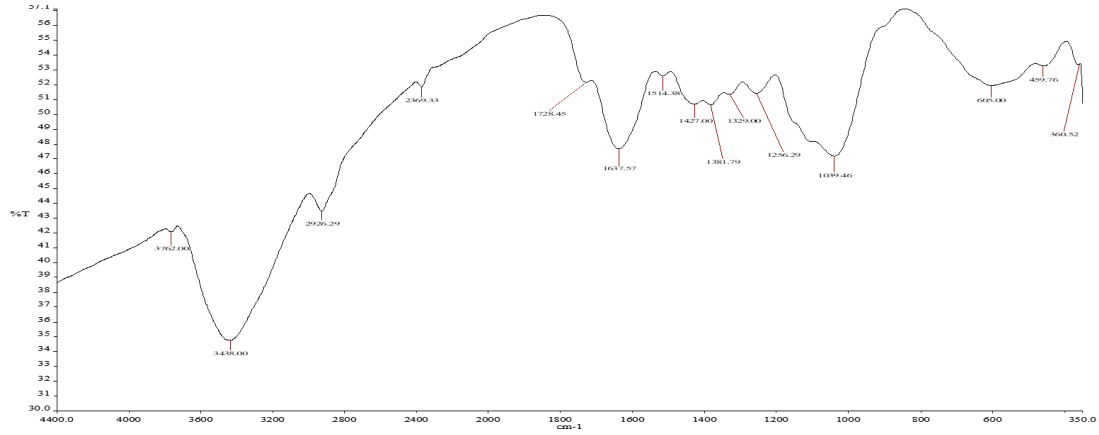

Figure 3b: FTIR spectrum for *B. vulgaris* fruit extract.

Characterization of the SeNPs using X-Ray Diffraction (XRD)

The synthesized SeNPs using *B. vulgaris* fruit extract was characterized using XRD which showed the information about the arrangement of atoms within a crystalline material. XRD pattern of the SeNPs synthesized by *B. vulgaris* is shown in Figure 4. The diffraction for BTSeNP peaks were observed at 2θ angles of 24.16^0, 30.24^0, 40.11^0, 46.21^0, 57.32^0, 67.47^0, 71.24^0 and 87.10^0 corresponding to lattice planes 012, 104, 111, 200, 116, 220, 300 and 311 respectively in line with face-centered cubic (FCC).

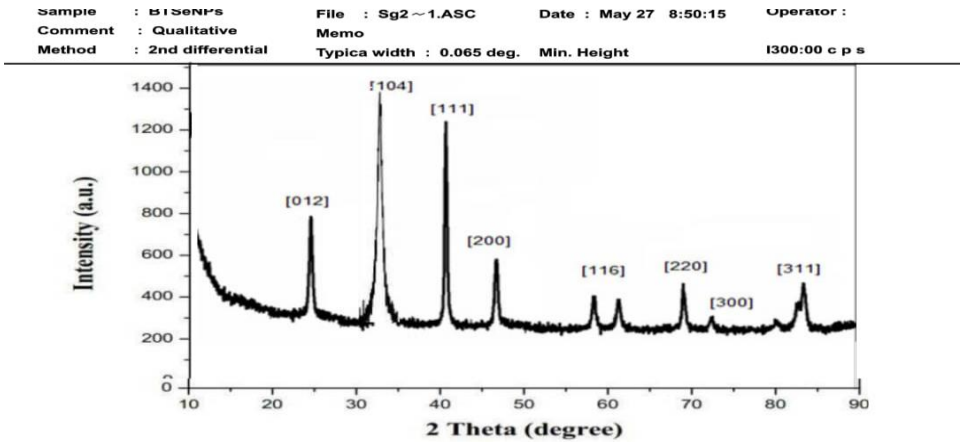

Figure 4: X-ray diffraction pattern of the synthesized SeNPs using *B. vulgaris* fruit.

Antimicrobial Activity of SeNPs

The antimicrobial activity of SeNPs, Sodium selenite (Na_2SeO_3), *B. vulgaris* fruit extract and Ciprofloxacin against some selected test organisms is shown in Table 2. BTSeNPs antimicrobial activity ranged from 13 mm - 24 mm. The highest susceptibility was recorded against *K. pneumonia* while *C. albican* had the least susceptibility. Na_2SeO_3 antibacterial activity ranged from 11 mm - 18 mm. *S. aureus* had the highest susceptibility while the least susceptibility was recorded against *K. pneumonia*. The antibacterial activity for *B. vulgaris* (BT) fruit extract ranged from 9 mm – 15 mm, *Shigella* had the highest susceptibility while *E. coli* had the least susceptibility. Ciprofloxacin had the highest antibacterial activities followed by the biosynthesized SeNPs by *B. vulgaris* fruit (BTSeNPs) and the least activity was observed by *B. vulgaris* fruit extract. The antimicrobial activity of Ciprofloxacin ranged from 18 mm - 25 mm. The highest susceptibility was recorded against *S. pneumoniae* (25 mm), followed by *S. aureus* (23 mm), and the least susceptibility was recorded against *C. albican* (18 mm).

Table 2: Antimicrobial activity of *B. vulgaris* Fruit Extract, Sodium Selenite, BTSeNPs and Ciprofloxacin against Some Test Organisms

Test Organisms	Zone of inhibition (mm)			
	Na_2SeO_3	BTSeNPs	BT	Ciprofloxacin
S. pneumoniae	12± 0.28	19± 0.33	-	25± 0.49
E. coli	-	17± 0.51	9± 0.38	20± 0.25
K. pneumonia	11± 0.40	23± 0.27	-	22± 0.31
***Shigella* spp**	13± 0.25	20± 0.42	15± 0.23	21± 0.46
P. aeruginosa	12 ± 0.37	18± 0.25	-	20± 0.53
S. aureus	18± 0.53	24± 0.34	10± 0.41	23± 0.22
C. albicans	-	13± 0.62	-	18± 0.41
B. alvei	-	16± 0.51	12± 0.20	21± 0.33

The mean values reported with different superscript in each row indicated significant difference ($P < 0.05$)
Key; Na_2SeO_3: Sodium selenite; BTSeNPs: SeNPs synthesized from *B. vulgaris* fruit extract
BT: *B. vulgaris* fruit extract

4. Discussion

The roles of biogenic synthesis in nano-science technology are significant in the synthesis of diverse nanomaterials. Biological approach syntheses are of great importance due to it non-toxic, eco-friendly nature and cost effectiveness in large scale production [10]. In this study, *B. vulgaris* fruit extract was evaluated for the biogenic synthesis of selenium nanoparticles (SeNPs). Qualitative and quantitative

phytochemical analysis conducted on *B. vulgaris* fruit extract revealed the presence of saponins, tannins, flavonoids, terpenoids, alkaloids and phenol while cardiac glycosides, anthraquinones and steroids were absent. Tannins had the highest quantity (389 mg/100g) while saponin had the least quantity (65 mg/100g) This is in line with the work of Dambalkar, [16] who reported that *B. vulgaris* have an important content of B-vitamins (B1 - thiamine, B2 -riboflavin, B3 -niacin, B5 - pantothenic acid, B6 -pyridoxine, B9 -folates and B12-cyancobalamin), as well as folic acid and antioxidants, such as triterpenes, terpenoids, carotenoids, coumarins, flavonoids (tiliroside, astragalin, rhamnocitrin, rhamnetin, kaempferol), betalains and phenolic compounds.

Biosynthesized SeNPs using *B. vulgaris* fruit extract (BTSeNPs) was observed visually based on the colour changes after incubation. There was a change in colour to ruby red after 72 h of incubation. This colour change is attributed to higher reduction of sodium selenite and changes in the atomic size of the metal to nanosized. This result correlates with the work of Lalitha *et al*. [26] who reported that colour change is an important factor for the synthesis of SeNPs. *B. vulgaris* extracts reduce the salt i.e. Na_2SeO_3 and result in the production of SeNPs. This report is in line with the work of Cui *et al*. [10], who observed that colour changes and the synthesis of SeNPs depend on time.

UV-Visible spectrophotometer is a very useful technique for the characterization analysis of nanoparticles. The surface plasmon resonance (SPR) peak obtained for *B. vulgaris* SeNPs fruit extract was at 350 nm and having a broad band between 300 - 400 nm. SeNPs exhibits size and shape dependent on SPR bands and also, the peaks obtained confirmed the synthesis of SeNPs. This correlates with the work of Cui *et al.* [10], who reported that the UV-Visible spectra of biosynthesized SeNPs of Hawthorn extract were validated by the progressive rise in optical phenomenon within the characteristic peak between 200 - 400 nm. This result is like the work of Cheon *et al*. [27], who reported that SeNPs synthesized by plant extract showed maximum absorbance peaks between 300 - 600 nm with a broad band and size. Ramamurthy *et al*. [28] reported a maximum peak at 390 nm and a broad band between 200 and 400 nm during the biosynthesis of SeNPs by Fenugreek extract.

Scanning Electron Microscope was used to elucidate the morphology, size, and shape of the biogenic synthesized SeNPs by *B. vulgaris* fruit. The shape and size of the nanoparticles are the most important factor to decide their biological activity [27]. The SeNPs in this study were spherical. This work is in agreement with the work of Cheon *et al.* [27] who reported that the nanoparticles are spherical in the biosynthesis of selenium nanoparticles by *Vitis vinifera*. Ojeda *et al*. [29] also obtained spherical shaped particles with the synthesis of selenium using *Juglans regia*.

TEM is the most suitable method for providing detailed information about the size and very fine structures in nanoparticles. The size of the nanoparticles synthesized by *B. vulgaris* fruit ranged from 20 nm – 100 nm. This report is also in accordance with Shalaby *et al*. [30] who reported that the TEM imaging of SeNPs synthesized from spice extracts revealed spherical shapes. Also, with the work of Alagesan and Venugopa [22] who reported that the nanoparticles produced were spherical particles within the diameter range of 45 – 90 nm.

Energy Dispersive X-ray (EDX) analysis was used to determine the elemental composition of nanoparticle as selenium. Strong signals from the selenium atom in the nanoparticles were observed while there were weaker signals from Carbon, Oxygen and Silicon atoms. The presence of a strong peak of the selenium atom is as a result of the Surface Plasmon Resonance. The Carbon, Oxygen, and Silicon signals may be due to the X-ray emission from proteins. The sharp principal absorption signal of about 1.78 keV for SeNPs was obtained which is in accordance with the results of Cui *et al*. [10] who reported that the peaks clearly indicate the presence of elemental selenium. Kora *et al*. [31] reported the emission peak of 1.37keV and 11.22keV in the presence of selenium.

FTIR spectra analyses are used to identify the potential functional groups of biomolecules responsible for reducing and capping the bio-reduced SeNPs. The FTIR spectra of biosynthesized SeNPs by *B. vulgaris* showed that different functional groups were present in the BTSeNPs compared to *B. vulgaris* fruit extract. Hydroxyl group, amine, aldehyde, and carboxylic acids, among others are responsible for the synthesis of SeNPs. These functional groups are present because of the phytochemical constituents which support the formation of the biomolecules.

Carbonyl groups from the amino acid residues and peptides of protein has strong ability to bind selenium. This protein forms a coat covering the metal nanoparticles and aids its stabilization. The presence of C-H, C=C, and C-O functional groups is like the report of Ananth *et al*. [32]. Similarly, Hidayat *et al*. [33] reported the presence of proteins and amino acids which are stabilising agents for SeNPs.

XRD analysis is used to determine the crystallographic nature of the nanoparticles. XRD confirmed the formation of selenium nanoparticles in the form of nanocrystals. All diffraction peaks corresponded to the characteristic crystalline face-centered cubic (FCC). Two peaks at 2^{θ} with values of (31.36, 40.03), corresponding to (104), (111) planes of selenium were observed for the biosynthesized SeNPs by *B. vulgaris.* Cittrarasu *et al*. [34], clearly revealed the crystalline nature of greenly synthesised selenium nanoparticles mediated by *Ceropegia bulbosa Roxb* extract. Similarly, El-Deeba *et al.* [35] who reported the pure crystalline formation of selenium nanoparticles.

The synthesized SeNPs from *B. vulgaris* exhibited varied antimicrobial activities against the selected test organisms. BTSeNPs exhibited excellent antimicrobial activity by inhibiting both the Gram-positive and Gram-negative organisms which was indicated by the zones of inhibition. The Gram-positive bacteria were more susceptible to the SeNPs than the Gram-negative bacteria. Most SeNPs obtained from medicinal plants have been credited with high antimicrobial properties, especially against drug-resistant human pathogenic and food spoilage microbes [36]. The result is similar to the work of Hidayat *et al*. [33] 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. Also, Zonaro *et al*. [37] reported that SeNPs exhibited more inhibitory actions against Gram positive bacteria species because of the lesser surface charges of nanoparticles that effectively enable them to bind to the bacterial cell membrane.

Conclusion

Selenium nanoparticles were synthesized using *B. vulgaris* fruit extract. The biosynthesized SeNPs had SPR peak at 350 nm, spherical shapes with uniform particle size distribution, had strong signals corresponding to elemental selenium and presence of different functional groups. There were significant zones of inhibition. Biologically synthesized selenium nanoparticles using *B. vulgaris* can be used for the development of newer and more potent antimicrobial agents.

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