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Green Synthesis of Bismuth Oxide Nanoparticles and Its *In Vitro* Antimicrobial Study Using Red River Gum (*Eucalyptus camaldulensis)* Leaf

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Abstract

Nanotechnology has been stated as research and development at the atomic, molecular or macromolecular scales. Nanoparticles are considered to be the building blocks for nanotechnology, and are referred to particles with at least 1 to 100 nm dimension. The green synthesis of Bi_2O_3 nanoparticles was demonstrated in this work by employing an extract from Red River Gum (*Eucalyptus camaldulensis*) leaf as a reducing agent. The existence of bioactive chemicals, which were essential to the formation of Bi_2O_3 nanoparticles, was discovered by phytochemical analysis of the leaf extract. FTIR confirms the presence of Bi-O bond at 1043.65459 cm^{-1}, SEM revealed the morphological characteristics of the synthesized Bi_2O_3 nanoparticles, displaying the presence of some crystalline substances within an amorphous, XRD shows that, the synthesized Bi_2O_3 nanoparticles is in an amorphous state, and UV-Vis spectroscopy the excitation of electrons of Bi_2O_3 nanoparticles from the valence band to the conduction band was responsible for the absorption peaks at 480 nm and 580 nm, with a band gap energy of 2.58eV and 2.13eV respectively were used to characterized the synthesized Bi_2O_3 nanoparticles, and confirmed their synthesis. Bi_2O_3 nanoparticles and red river gum leaf extract showed antimicrobial activity against gram positives staphylococcus epidermis and staphylococcus aureus and gram negatives pseudomonas aeruginosa, and escherichia coli bacteria, Bi_2O_3 nanoparticles were more effective, and both exhibited increased activity with higher concentration. This study demonstrates the potential of extract's from red river gum (*Eucalyptus camaldulensis*) leaf assistance in green synthesis of Bi_2O_3 nanoparticles.

Keywords: Greensynthesis, nanoparticles, *Eucalyptus camaldulensis*, Phytochemical, Antimicrobial.

Introduction

Over time, nanotechnology has attracted a lot of attention. The nanoparticles are the basic building block of nanotechnology. Particles of carbon, metal, metal oxides, or organic substances that range in size from 1 to 100 nanometers are known as nanoparticles [1]. In contrast to their counterparts at larger scales, the nanoparticles display distinct physical, chemical, and biological characteristics at the nanoscale. Increased mechanical strength, more reactivity or stability in a chemical reaction, a comparatively bigger surface area to volume, etc. are the causes of this phenomenon. Because of these characteristics, nanoparticles are used in many different applications. Aside from their composition, the nanoparticles vary in size, shape, and dimensions [2].

There are four types of nanoparticles: zero-dimensional, which has length, width, and height fixed at one point (like nano dots); one-dimensional, which can have only one parameter (like graphene); two-dimensional, which has length and width (like carbon nanotubes); and three-dimensional, which has all of the parameters (like gold nanoparticles). The size, shape, and structure of the nanoparticles vary. They range in size from 1 to 100 nm and can be spherical, cylindrical, tubular, conical, hollow core, spiral, flat, etc., or irregular. Depending on surface differences, the surface may be uniform or uneven. There are crystalline and amorphous nanoparticles that contain loose or agglomerated single or multiple crystal solids. The secret to a sustainable and clean future lies in nanotechnology [3].

Nanoparticles are generally classified into three classes: the organic, inorganic and carbon based.

The organic nanoparticles include: Ferritin, liposomes, dendrimers, and micelles are examples of organic nanoparticles or polymers [4]. The inorganic nanoparticles include: Metal and metal oxide based nanoparticles are generally categorized as inorganic nanoparticles. On the other hand, Carbon-based nanoparticles are particles that are entirely composed of carbon [5]. Fullerenes, graphene, carbon nanotubes (CNT), carbon nanofibers, carbon black, and occasionally activated carbon in nanoscale are among their classifications [6].

Nanoparticles are characterized by AAS, UV visible, XRD, TEM, IR, SEM etc.

Some of the significant applications of nanoparticles include: Cosmetics [7], electronics [8], catalysis [9], medicine [10], food [11], renewable energy.

Red River Gum (also known as *Eucalyptus camaldulensis*) is one of the most prominent species in the genus of eucalyptus trees found in Australia [12].

Figure 1. Red River Gum (*Eucalyptus camaldulensis*) tree

Figure 2. River Red Gum (*Eucalyptus camaldulensis*) leaves

Therfore, the aim of this study is to biologically synthesize bismuth oxide nanoparticle using Red River Gum (*Eucalyptus camaldulensis)* leaf and study its *In vitro* antimicrobial activity.

Materials and Methods

All the chemicals used were of analytical grade and were used without any further purification

Method

The methodologies adopted in this study include extraction, phytochemical screening, nanoparticles synthesis and antimicrobial analysis:

Collection of *Eucalyptus camaldulensis extract*

Fresh *Eucalyptus camaldulensis* leaf samples were collected from the *Eucalyptus camaldulensis* tree growing in the Umaru Musa Yar'adua University Katsina, Katsina State, Nigeria in 2024, beside the Central Mosque and was taken to Biology laboratory of the University for identification. The leaves samples were transported to the Chemistry laboratory of the Umaru Musa Yar'adua University for extraction.

Extraction method

The leaf samples collected were placed into the mortar to be grounded into powder. A 40 g amount of the leaves powder was added in 1040ml of boiling distilled water. The mixture was poured into a 500 ml of conical flask wrapped in aluminum foil to avoid evaporation and exposure to light for 3 days at room temperature. The ericnmeyer flask was placed on a platform shaker at 70 rpm for 24hrs. The mixture was filtered using a filter and stirred using magnetic stirrer [13].

Phytochemical Analysis of River Red Gum (*Eucalyptus camaldulensis)* leaves extract

Chemical test for the identification of bioactive chemical constituents in the red river gum *(Eucalyptus camaldulensis)* was carried out as shown in the following procedure below:

Test for Phenols and Tannins

The 2 ml of the extract was placed in a test tube and mixed with 3ml of 2% solution of $FeCl_3$ the blue color will be indicated [14].

Test for Terpenoids (Salkowski's Test)

The 2ml of the extract was mixed with 3ml of chloroform. Then 3ml concentrated sulfuric acid was added carefully and shaken gently. A reddish brown coloration formed at the interphase showed positive result for the presence of terpenoid [15].

Test for Glycosides

The 2 ml of the extract was mixed with 3ml of glacial acetic acid containing 3 drops of 2% $FeCl_3$. To another test tube containing 3ml of concentrated sulfuric acid, the mixed was poured into it. A brown ring at the interphase indicated the presence of glycosides [16].

Test for Flavonoids (Shinoda Test)

The 2 ml of the extract was mixed with 5 pieces of magnesium ribbons and concentrated hydrochloric acid was added drop while noting the color change. Pink or orange coloration indicated the presence of flavonoids [17].

Test for Saponins

The 4 ml of the solvent extract was placed in a test tube and shaken vigorously noting down the observations. The formation of stable form indicated the presence of saponins [18].

Synthesis of Bi_2O_3 Nanoparticles

Ten gram of powdered *Eucalyptus* leaves sample was weighed in to a beaker. 100ml of distilled water was added in to the beaker containing *Eucalyptus* leaves sample and maintained at 60°C for 10 min. After 10 min, the eucalyptus extract was filtered using filter paper. For the synthesis of Bismuth oxide (Bi_2O_3) nanoparticles, 12 ml of eucalyptus extract was added slowly in to 100ml of 1.0M Bismuth nitrate ($Bi(NO_3)_3$) in conical flask at room temperature by continuous stirring with magnetic stirrer. In order to maintain the pH of 12, 1.0M solution of sodium hydroxide (NaOH) was used. After reaching the desired pH the mixture was allowed in the stirrer for 2hours until the formation of precipitate was observed. The color change of the solution was observed. The mixture was centrifuged. The pellets were washed using de-ionized water and dried at 50°C in hot air overnight. The synthesized Bismuth oxide nanoparticles was characterized by UV-vis spectroscopy, X-ray diffraction (XRD), Fourier transform infrared (FTIR) spectroscopy, and scanning electron microscopy (SEM) [19].

Results and Discussion

Phytochemical Analysis of *(Eucalyptus camaldulensis)* leaf extract

Table 1: Phytochemical analysis results of river red gum leaves extract *(Eucalyptus camaldulensis).*

S/N	Test	Observation
1	Glycosides	+
2	Terpenoids	+
3	Flavonoids	+
4	Saponins	+
5	Tannins	+
6	Phenols	+

From table 1, the leaves extract shows the presence of glycosides, terpenoids, flavonoids, saponins, tannins, phenols denoted by the symbol '+'. The secondary metabolites present will be the natural capping agents that will be supplied by the *eucalyptus camaldulensis* leave extract to influence the formation of the Bi_2O_3 nanoparticles.

Green Synthesized Bi_2O_3 Nanoparticles

Figure 3. Synthesized Bi_2O_3 nanoparticles

A number of benefits over conventional synthesis techniques are provided by the environmentally friendly synthesis of Bi_2O_3 nanoparticles utilizing an extract from *Eucalyptus camaldulensis* leaves. By using plant extract, hazardous chemicals are not required, which lessens the synthesis process's negative environmental effects. The process also uses less energy because it doesn't call for high temperatures or pressures. Because of their super shape and crystalline structure, the resultant Bi_2O_3 nanoparticles can be used in a variety of fields, such as optoelectronics, sensing and catalysis.

Fourier Transformation Infrared Radiation (FT-IR) Analysis of Green Synthesized Bi_2O_3 Nanoparticles

Table 2: FT-IR spectral data obtained

Peak number	Wave number (cm^{-1})	Intensity
1	834.923680	77.36436
2	1043.65459	67.47026
3	1252.38551	76.58994
4	1371.66032	74.33699
5	1580.39124	73.40883
6	2922.23286	86.25352
7	3242.78392	83.03083

The absorption peak at 1043.65459 cm^{-1} confirms the presence of Bi-O bond. The higher wave number range was due to the presence of surface impurities (i.e graphite, silicone oxide, urea and orthoclase) in the sample.

The FTIR analysis also suggests that the Bi_2O_3 nanoparticles may have a surface modification or capping with organic compounds from the red river gum (*Eucalyptus camaldulensis)* leaves extract. This surface modification may influence the optical, electrical, and catalytic properties of the Bi_2O_3 nanoparticles.

UV Analysis of Green Synthesized Bi_2O_3 Nanoparticles

Table 3: UV analysis of Bi_2O_3 nanoparticle at 480 nm

Solvent absorbance = 0.735

S/N	Concentration	Wave length	Absorbance
1	1ppm	480nm	1.589
2	2ppm	480nm	2.219
3	3ppm	480nm	2.893
4	4ppm	480nm	3.582
5	5ppm	480nm	4.046

Table 4. UV analysis of Bi_2O_3 nanoparticle at 580nm

Solvent absorbance = 0.735

S/N	Concentration	Wave length	Absorbance
1	1ppm	580nm	0.973
2	2ppm	580nm	1.125
3	3ppm	580nm	1.478
4	4ppm	580nm	1.88
5	5ppm	580nm	2.494

The excitation of electrons of the synthesized Bi_2O_3 nanoparticles from the valence band to the conduction band was responsible for the absorption peak at 480nm and 580nm, with a band gap energy of 2.58eV and 2.13eV respectively.

Scanning Electron Microscope (SEM) Analysis of Green Synthesized Bi_2O_3 Nanoparticles

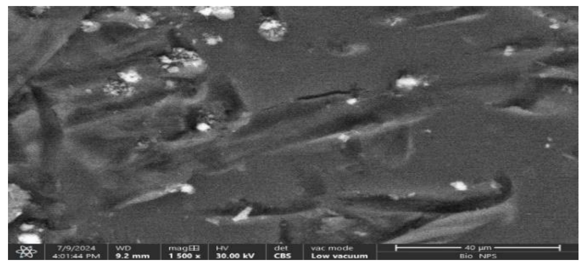

Figure 4: (a) SEM Analysis

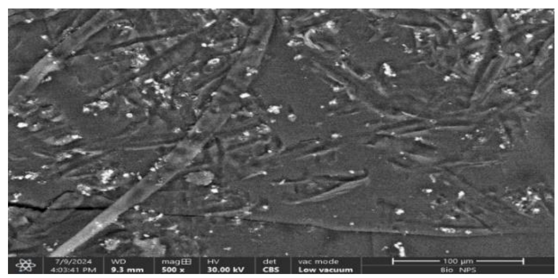

Figure 5: (b) SEM Analysis

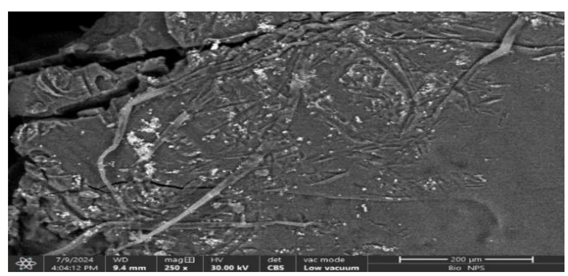

Figure 6: (c) SEM Analysis

The scanning electron microscope revealed the morphological characteristics of the synthesized Bi_2O_3 nanoparticles, displaying the presence of some crystalline substance within an amorphous. This suggested the x-ray diffraction (XRD) analysis to confirm and identify the crystalline and amorphous substance present.

X-Ray Diffraction (XRD) Analysis of Green Synthesized Bi_2O_3 Nanoparticles

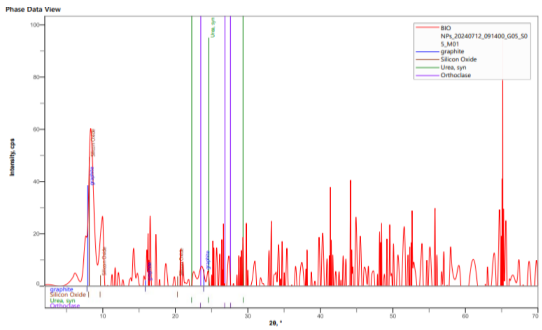

Figure 7. Phase data view

The X-ray Diffraction (XRD) analysis confirms the present of surface impurities in a crystalline state. This is because, all the sharp peaks may be attributed to the surface impurities. The absence of sharp peak corresponding to the Bi_2O_3 also confirms that, the synthesized Bi_2O_3 nanoparticles is in amorphous state. The presence of surface impurities is due to the application of crude extract in the synthesis process.

***In Vitro* Antimicrobial Activity of The Green Synthesized Bi_2O_3 Nanoparticles and *Eucalyptus camaldulensis* Leaves Extract**

Table 5: Bi_2O_3 nanoparticles antimicrobial activity results

S/N	Organisms	200mg/ml	100mg/ml	50mg/ml	25mg/ml
1	Staphylococcus epidermis	11.63	8.90	7.70	ND
2	Pseudomonas aeruginosa	10.0	8.35	7.30	ND
3	Staphylococcus aureus	12.10	8.90	7.40	ND
4	Escherichia coli	9.60	7.20	ND	ND

Table 6: Red river gum (E*ucalyptus camaldulensis)* leaves extract antimicrobial activity results

S/N	Organisms	200mg/ml	100mg/ml	50mg/ml	25mg/ml
1	Staphylococcus epidermis	27.0	17.5	14.0	11.5
2	Pseudomonas aeruginosa	13.0	12.5	ND	ND
3	Staphylococcus aureus	10.5	7.5	ND	ND
4	Escherichia coli	12.0	ND	ND	ND

Table 7: Minimum inhibition concentration (MIC) and Minimum bacterial concentration (MBC) of Bi_2O_3 nanoparticles.

S/N	Organism	Minimum inhibitory concentration (MIC)	Minimum bactericidal concentration (MBC)
1	Staphylococcus epidermis	50mg/ml	Not detected
2	Pseudomonas aeruginosa	50mg/ml	200mg/ml
3	Staphylococcus aureus	50mg/ml	Not detected
4	Escherichia coli	100mg/ml	100mg/ml

Table 8: Minimum inhibition concentration (MIC) and Minimum bacterial concentration (MBC) of red river gum (*Eucalyptus camaldulensis)* leaves extract.

S/N	Organism	Minimum inhibitory concentration (MIC)	Minimum bactericidal concentration (MBC)
1	Staphylococcus epidermis	200mg/ml	200mg/ml
2	Pseudomonas aeruginosa	100mg/ml	100mg/ml
3	Staphylococcus aureus	100mg/ml	100mg/ml
4	Escherichia coli	200mg/ml	200mg/ml

The antimicrobial activity of the green synthesized Bi_2O_3 nanoparticles using red river gum (*Eucalyptus camaldulensis)* leaves extract and the leaves extract itself revealed a significant finding.

For the synthesized Bi_2O_3 nanoparticles, the growth of *Staphylococcus epidermis, Pseudomonas aeruginosa and Staphylococcus aureus* started at 50mg/ml while that of *Escherichia coli* stopped at 100mg/ml (referred to minimum inhibitory concentration). Also the *Pseudomonas aeruginosa* and *Escherichia coli* were completely killed at 200mg/ml and 100mg/ml respectively (referred to minimum bactericidal concentration).

For the Red river gum *(Eucalyptus camaldulensis)* leaves extract, the growth of the *Pseudomonas aeruginosa and Staphylococcus aureus* started at 100mg/ml, while that of *Staphylococcus epidermis and Escherichia coli* stopped at 200mg/ml (referred to minimum inhibitory concentration). Also, *Pseudomonas aeruginosa and Staphylococcus aureus* were completely killed at 100mg/ml, while *Staphylococcus epidermis and Escherichia coli* were completely killed at 200mg/ml (referred to minimum bactericidal concentration).

In conclusion, based on the minimum inhibitory concentration and minimum bactericidal concentration, the synthesized Bi_2O_3 nanoparticles is more effective than the extract from the red river gum *(Eucalyptus camaldulensis),* and also the activity of both, increases with the increase in concentration.

Conclusion

To sum up, this study shows that, red river gum *(Eucalyptus camaldulensis)* leaves extract was used successfully to synthesized bismuth oxide nanoparticles in an environmentally friendly manner. The bioactive substances included in the leaves extract, as determined by phytochemical analysis, and confirmed by IR were essential for the stability and reduction of the bismuth oxide nanoparticles.

FTIR, UV-Vis spectroscopy, SEM, and XRD, analysis, were used to characterize the synthesized Bi_2O_3 nanoparticles, and confirmed their synthesis. While the FTIR study, demonstrated the existence of functional groups that might be in charge of the nanoparticles' antibacterial action and revealed the Bi-O at 1043.65459 cm^{-1}. UV shows that, the excitation of electrons of the synthesized Bi_2O_3 nanoparticles from the valence band to the conduction band was responsible for the absorption peaks at 480nm and 580nm, with a band gap energy of 2.58eV and 2.13eV respectively. SEM revealed the morphological characteristics of the synthesized Bi_2O_3 nanoparticles, displaying the presence of some crystalline substances within an amorphous. XRD confirms the present of surface impurities in a crystalline state and Bi_2O_3 nanoparticles in an amorphous state.

According to the antimicrobial study, the minimum inhibitory concentration and minimum bactericidal concentration revealed that, the synthesized Bi_2O_3 nanoparticles is more effective than the extract from the red river gum *(eucalyptus camaldulensis),* and also the activity of both, increases with the increase in concentration.

Recommendation

Further characterization should be carry out on particle size to confirm the actual size of the synthesized nanoparticles.

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