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Antibacterial, Antifeedant and Photocatalytic Potential of the Silver Oxide-Bismuth Bimetallic Nanoparticles Biogenically Synthesized with *Curcuma longa* Ethanolic Extract

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Abstract

The recent rise in the drug resistance in microorganisms and in agriculturally important pests has created havoc in the society costing severe economic losses. Continuous uses of synthetic pesticides are threatening the agricultural field and soil fertility. This has forced mankind to look for an innovative solution to combat pollution and drug resistance in harmful microorganisms as well as agriculturally important pests. Particularly, *Spodoptera litura* is an economically important polyphagous pest which has the propensity to damage a number of economically important crops and other food crops. Plant extracts and nanoparticles are playing important role in control of insect pest and the biogenic synthesis of metallic and the dual- metallic nanoparticles are increasingly being studied in the recent times owing to their environmental friendliness. The present study has reported the synthesis of a combination of Silver Oxide and Bismuth bimetallic nanoparticles for the first time using biogenic techniques with the help of an ethanolic extract of rhizome (*Curcuma longa*). The nanoparticles were tested against antibacterial, antifeedant and catalytic properties for the degradation of the dye methylene blue. The X- Ray Diffractometry has been used to characterize and calculate the size of the nanomaterials. The results revealed that 5000µg and 1000µg concentration of nanoparticles had shown maximum zones of inhibition of 13mm and 12mm, respectively, against *S. aureus* and *E. coli*.

Keywords: Antibacterial, Antifeedant, Photocatalytic, Potential, *Spodoptera litura*.

INTRODUCTION

The nano materials are of promising use and interesting potential due to their physical properties and significance in several fields of applications. The properties of nanomaterial are affected not only by their chemical composition, but also by their structure, shape and size [1]. It is well known that the reduction in the particle size leads to new and novel properties (NPs) [2,3] In the rapidly emerging field of nano biotechnology, metal nanoparticles (MNPs) are extensively used in drug delivery [4] biosensors, [5] bio-imaging [6] antimicrobial activities [7] and food preservation [8], by exploiting their unique physical chemical and biological properties. Their nano scale size, three-dimensional structure, large surface area and negligible side effects make them highly effective for biomedical applications such as molecular imaging [9] and cancer therapy [10]. Several studies report that nanoparticles exhibit antibacterial activity independent of their size, concentration and oxidation state [11]. Antimicrobial activity of the nanoparticles is known to be a function of the surface area in contact with the microorganisms. The ions released by the nanoparticles may attach to the negatively charged bacterial cell wall and rupture it, thereby leading to protein denaturation and cell [12]. Recent attention has been turned to the development of synthetic procedures that are environmentally friendly in order to minimize chemical waste as well as potential safety issues associated. On the other hand, the attention is focused to fully understand the interaction mechanisms between nanoparticles and living systems. *C. longa* is a well- known medicinal plant, which belongs to *Zingiberaceae* (ginger) family. The rhizome of *C. longa* is traditionally used throughout the Asian countries. Mainly it is grown in Asian countries such as China, India, and other tropical countries [13]. *Curcuma longa* has been reported by Lee et al [14] for the ethanolic extract ability to reduce obesity in high fat-induced diet mice by activating the AMPK Signaling pathway.

Shanmughanathan et al have reported the green synthesis of cobalt oxide nanoparticles using *Curcuma longa* water extract. These nanoparticles were evaluated successfully for the antimicrobial, antioxidant, anticancer and dye degradation properties. [15]

The present study is aimed to biogenically synthesize nanoparticles using combination of silver oxide and Bismuth bimetallic nanoparticles for the first time from the ethanolic extract of rhizome (*Curcuma longa*). To test against antibacterial, antifeedant and photocatalytic degradation activity.

MATERIALS AND METHODS

Extract Preparation

Five grams of freshly processed ''viralimanjal'' variety of powdered *Curcuma longa* rhizomes were dissolved in 75 mL of ethanol. The extract was then obtained by evaporating in rotary vacuum evaporator until an orangish brown liquid was obtained.

Phytochemical Analysis

The Phytochemical analysis was carried out for detecting the qualitative presence of alkaloids, phenolics, flavonoids, tannins and proteins as per a modified protocol of Shaikh and Patil [16].

Detection of alkaloids

To the extract, 3-4 drops of picric acid solution was added, dark orangish brown precipitate indicates the formation of alkaloids.

Detection of flavonoids

To the extract, 1 mg/ mL of lead acetate solution was added and formation of greenish-yellow solution indicated formation of flavonoids.

Detection of phenolics

To the extract, 1 mg/ mL of ferric chloride solution was added. Formation of dark green colour indicated the formation of phenolics.

Detection of tannins

To the extract, 1 mg/ mL of Ferric chloride solution along with a few drops of water were added simultaneously. The formation of dark green color indicated the presence of tannins.

Detection of proteins

To the extract, a few drops of concentrated nitric acid were added. Formation of a yellow color indicated the presence of proteins.

Green synthesized Silver Oxide- Bismuth nanoparticles procedure

Silver Oxide –Bismuth bimetallic nano particles green synthesized with *Curcuma longa.* 1 mL of 60.6 g/L *C. longa* ethanolic extract (rhizome) was taken and dissolved in 99 mL of dd water. To this admixture, 0.1 M of silver nitrate and bismuth nitrate pentahydrate solutions each were added to the solution. The color change was observed. Heating was done at 42- 45 degrees Celsius for 30 minutes. The supernatant was slowly and carefully decanted in such a way that the deposited residue contained the nanoparticles.

Stock Solution Preparation

50 mg of green synthesized Silver Oxide –Bismuth bimetallic nano particles was added to 500µL DMSO and mixed thoroughly. In this 100 µL contains 10mg (10000 µg); 10 µL contains 1mg (1000 µg) and 1 µL contains 0.1mg (100 µg) of the test compound

Test Bacteria

Two Gram Positive bacteria such as *S aureus, B. subtilis* and two Gram Negative bacteria *E. Coli*, and *P. aeruginosa* were tested by well diffusion method at 125µg to 5000µg concentrations.

Well Diffusion Assay

Antibacterial activity by well Diffusion Method

Antimicrobial activities of the green synthesized Silver Oxide –Bismuth bimetallic nano particles were performed against both Gram-negative (*E. coli, P aeruginosa*), Gram-positive (*B. subtilis* and *S. aureus*) bacteria. The antibacterial activity was carried out by well diffusion method (15). In brief the pure cultures of organisms were sub cultured in Müller-Hinton broth at 35°C ± 2°C on a rotary shaker at 160 rpm. For bacterial growth, a lawn of culture was prepared by spreading the 100 µL fresh culture having 10-6 colony-forming units (CFU)/mL of each test organism on Muller Hinton Agar (MHA) plates with the help of a sterile glass-rod spreader or sterile cotton swab. Plates were left standing for 10 minutes to let the culture get absorbed. Then 6 mm wells were punched into the MHA plates using a sterile cork-borer aseptically for testing nanomaterial for antimicrobial activity. Wells were sealed with one drop of molten agar (0.8% agar) to prevent leakage of nano materials from the bottom of the wells. Using a micropipette, 50µL (5000µg, 1000µg, 500µg, 250µg and 125µg) of the sample of green synthesized Silver Oxide– Bismuth bimetallic nano particle suspension was poured onto each of five wells on all plates. Streptomycin (10 µg), was used as Standard reference drug (positive control). After overnight incubation at 37°C ± 0.5°C, the different levels of zone of inhibition were measured.

Minimum Inhibitory Concentration (MIC) activity by Broth Micro Dilution Method:

Minimum Inhibitory concentration (MIC) for green synthesized Silver Oxide –Bismuth bimetallic nano particles was determined by the broth-dilution method in 96 well round bottom micro titer plates was performed against selected bacteria. Streptomycin at 10 µg was used as Positive control standard drug. A 100µL of Muller Hinton broth medium amended with biogenically synthesized Silver oxide–Bismuth bimetallic nano particles (2500µg/mL) was prepared separately. A 100 µL Muller Hinton broth was added in all the wells A1 to A8. 100 µL of 2500 µg biogenically synthesized Silver oxide –Bismuth bimetallic nano particles suspension was added in well A1 and mixed well by pipetting aspiration, from this 100 µL was transferred to well A2 by doubling dilution and similarly up to A8, and 100 µL from A8 was discarded (concentration in A1 to A8 ranges from 1250µg to 10µg). Each set was inoculated aseptically with 10µL of respective bacterial suspension (10-5CFU/mL). Standard reference drug control, Positive control, and Negative controls were maintained. The inoculated 96 well plates were incubated at 37°C ± 0.5°C for 24 hours. By drop plating method the viable bacterial colonies corresponding to the each well for the biogenically Ag_2O-Bi nanoparticles concentration were counted and recorded by the naked eye to determine the lowest concentration that arrested bacteria growth, defining this as the MIC (bacteriostatic) and the complete growth inhibition (Bactericidal) was defined as MBC (Minimum Bactericidal Concentration). Streptomycin at 10 µg was used as Standard reference drug [17].

Catalytic activity of Ag_2O- Bi bimetallic NPs for photolytic dye degradation of Methylene Blue (MB):

The catalytic activity of Ag2O-Bi bimetallic NPs for photolytic dye degradation of MB was evaluated by Dr. A. Manikandan at Karpagam Academy of Higher Education (Deemed to be University), Coimbatore, TN, India. Concentration of 1 % (w/v) methylene blue was taken. A suitable concentration of the catalyst (Ag_2O- Bi) bimetallic NPs was chosen for the study. The dark condition was chosen as negative control and the light condition without the NPs was the positive control. The test phase with the nanoparticles was observed for photodegradation of dyes [18].

Antifeedant Assay

The antifeedant activity was determined by a modified protocol of Napal et al [19], 1000 ppm of stock concentration of green synthesized bimetallic (Silver Oxide- Bismuth) nanoparticles were prepared in acetone as solvent. Then they were serially diluted two-fold to reach a concentration of 125 ppm. Filter paper discs were inserted into Petri plates. 6 cm castor leaf discs were immersed 1000, 500, 250, 125 ppm of discs. The leaf discs were inserted into the petri plates in such a way that the leaf discs were pressed firmly against the filter paper disc. Each larva of *Spodoptera litura* was inserted into the Petri plate. This process was repeated five times for all the four concentrations totally. Acetone was used as control.

Characterization

The Silver Oxide- Bismuth bimetallic nanoparticles were characterized using XRD and the size was calculated using Debye-Scherrer equation with the help of InstaNANO software. Bruker D8 Advance XRD instrument was used for recording the XRD Spectrum.

RESULTS

Phytochemical Analysis

The phytochemical analyses indicated the presence of alkaloids, phenolics, tannins, flavonoids and proteins.

Characterization

XRD

The size of the nanoparticles was found to be 38.81 nm on average. Silver Oxide (Crystallography Open Database Card Number: [-00-101—0604] was found at Experimental 2- Theta position 26. 236 degrees. And Bismuth [Crystallography Open Database Card Number: -00-500-0215] was found to be at 27.30 degrees 2- Theta position. Moreover, during the biogenic synthesis, it was noted that impurities i.e., the phytochemicals have been chelated along with Silver Oxide and Bismuth. The results were recorded and the peaks were smoothened with the help of Qual-X software.

Antimicrobial Activity

Antibacterial activity results revealed that green synthesized Silver Oxide–Bismuth bimetallic nano particles exhibited antibacterial activity against both Gram-positive and Gram-negative bacteria but when compared to standard antibiotic Streptomycin (10 µg), the activity was very less. Silver –Bismuth bimetallic nano particles did not show any activity against the pathogenic *P. aeruginosa* at tested concentrations, Table 1 and Figure 1 shows the zone of inhibition by green synthesized Silver Oxide –Bismuth bimetallic nano particles against both the pathogenic Gram-positive and Gram-negative bacteria. Green synthesized Silver Oxide –Bismuth bimetallic nanoparticles exhibited maximum (14mm) bacterial growth inhibition against gas forming pathogen *B. subtilis* at highest tested concentration. In contrast, at the same concentrations the pathogen *P. aeruginosa* did not show any activity at any tested concentrations. But whereas among the tested concentrations 5000µg and 1000µg had shown maximum zones of inhibition of 13mm and 12mm, respectively, against the pathogenic bacterial organisms namely, *S. aureus* and *E. coli.* In the case of *E. coli* the maximum growth, inhibition zones were found to be the following; 12mm, 10mm, 8mm, and no activity was shown at 250µg and 125µg whereas at 250µg *S. aureus* had shown 8mm zone of inhibition. (Figure 1). There was no activity showed at 125µg against all the tested bacteria.

Table 1: Antibacterial activity of green synthesized CL- Ag_2O Bi nanoparticles by well diffusion method

S. No	**Test Sample**	**Conc (µg)**	**Zone of Inhibition (in mm)**			
			S. aureus	***B. subtilis***	***E. coli***	***P. aeruginosa***
1	CL- Ag Bi np	5000	*13*	*14*	*12*	-
		1000	*11*	*11*	*09*	-
		500	10	08	08	-
		250	8	-	-	-
		125	-	-	-	-
2	Streptomycin	10	15	18	12	10

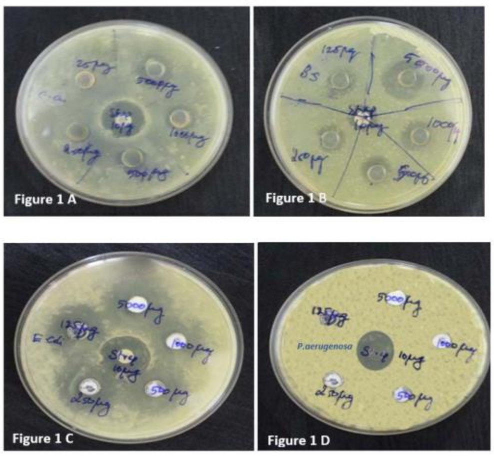

Figure 1: Figure 1A to 1D Muller Hinton Agar plates showing zones of inhibition by well diffusion method against selected bacteria

Figure 1 A- Antibacterial activity of CL- Ag_2O- Bi bimetallic NPs against *Staphylococcus aureus*
Figure 1 B- Antibacterial activity of CL- Ag_2O- Bi Bimetallic NPs against *Bacillus subtilis*
Figure 1 C- Antibacterial activity of CL- Ag_2O- Bi Bimetallic NPs against *Escherichia coli*
Figure 1 D- Antibacterial activity of CL- Ag_2O- Bi Bimetallic NPs against *P. aeruginosa*

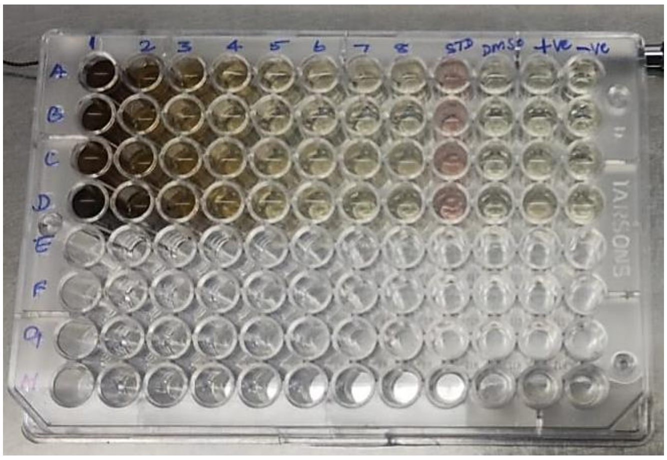

Figure 2: Minimum Inhibitory Concentration (MIC) of green synthesized CL- Ag Bi nanoparticles against selected bacteria by Broth Micro Dilution Method

i. A1-A8 – *S. aureus Vs* Test control (1250 µg; 625µg, 312µg, 156µg, 78µg, 39 µg, 19.5µg, and 10µg); A9 – STD control Streptomycin (10µg); A10 - Vehicle control; A11- Positive control; A12- Negative control- Blank.
ii. B1-B8 – *B. subtilisVs.*Test control (1250 µg; 625µg, 312µg, 156µg, 78µg, 39 µg, 19.5µg, and 10µg); B9 – STD control - Streptomycin (10µg); B10 - Vehicle control; B11- Positive control; B12- Negative control- Blank.
iii. C1-C8 – *E.coliVs.*Test control (1250 µg;625µg, 312µg, 156µg, 78µg, 39 µg, 19.5µg, and 10µg); C9 – STD control Streptomycin (10µg); C10 - Vehicle control; C11- Positive control;C12- Negative control- Blank.
iv. D1-D8 – *P. aerugenosa Vs.* Test control (1250 µg;625µg, 312µg, 156µg, 78µg, 39 µg, 19.5µg, and 10µg); D9 – STD control Streptomycin (10µg); D10 - Vehicle control; D11- Positive control; D12- Negative control- Blank.

Table 2: Minimum Inhibitory Concentration (MIC) of green synthesized CL- Ag Bi nanoparticles against selected bacteria by Broth Micro Dilution Method

S. No	**Microorganisms**	**MIC (µg)**	**MBC (µg)**	**Streptomycin (10 µg)**
1	*Staphylococcus aureus*	156	>312	MBC
2	*Bacillus subtilis*	156	312	MBC
3	*E. coli*	312	625	MIC
4	*P. aerugenosa*	312	625	MIC

Determination of Minimum Inhibitory Concentration (MIC)

The results of broth micro dilution to determine the Minimum Inhibitory concentration (MIC) exhibited the antibacterial activity against all the tested dental pathogenic bacteria. The Results obtained in the well diffusion method were having similarity in terms of bacteriostatic concentrations and bactericidal concentrations. The bacteriostatic activity was observed from the lowest concentrations namely from 78µg, and 156µg, and the bactericidal activity was observed 312µg and 625µg concentrations for the tested bacteria. The results observed were tabulated with the corresponding MIC and MBC Values. Among the tested pathogenic bacteria, *Bacillus subtilis, Staph aureus, E. coli* and *P. aeruginosa* the MIC value was 156µg and the MBC value was 312µg or more, among the gram-positive bacteria and for gram negative bacteria the MIC values were 312µg and subsequently the Minimal Bactericidal Concentration (MBC) values were greater than 625µg which is much higher concentration than the gram-positive bacteria (Table 2).

Antifeedant activity

Antifeedant activity was found to be the highest in 1000 ppm concentration followed by 500 ppm concentration of the bimetallic nanoparticles (Table 3).

Photocatalytic Activity

From the photocatalytic activity studies and the graph, it is inferred that the incomplete dye degradation has taken place in the light condition in the presence of nanoparticles in two hours. Kinetic mode of degradation has been employed in the degradation of the dye in the presence of light wherein the nanomaterials acted as catalysts (Figure 3).

Table 3: Antifeedant Activity

1000 ppm		
Diseased %	Primary Area (cm^2)	Diseased Area (cm^2)
0.852	12.6	0.108
0.0274	11.68	0.0032
2.105	15.6771	0.234
0	13.04	0
0	11.81	0
500 ppm		
Diseased %	Primary Area (cm^2)	Diseased Area (cm^2)
62.1061	8.77	26.32
3.712	17.67	3.43
47.31	10.003	8.16
45.84	8.3	6.92

44.26	9.62	9.62
250 ppm		
Diseased %	Primary Area (cm^2)	Diseased Area (cm^2)
41.81	8.14	5.1
40.8	7.26	4.26
0	17.36	0
35.41	14.26	4.82
33.77	84.7	2.97
125 ppm		
Diseased %	Primary Area (cm^2)	Diseased Area (cm^2)
2.66	13.92	3.809
0	11.36	0
5.849	13.83	0.93
12.28	10.94	1.26
11.96	9.95	1.12

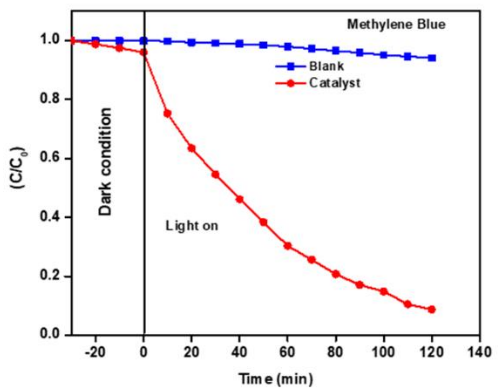

Figure 3: Photocatalytic Study

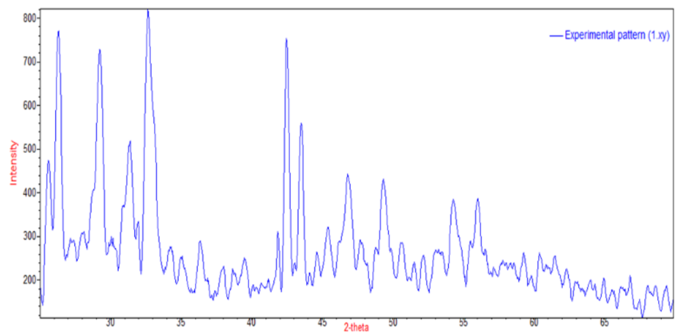

Figure 4: Smoothened XRD Peak of Ag_2O- Bi bimetallic NPs

DISCUSSION

The antimicrobial activity observations have been made for green synthesized Silver Oxide –Bismuth bimetallic nano particles with *Curcuma longa* ethanolic extract. However, it should also be noticed that Gram-negative, Gram-positive bacterial strains such as *E. coli*, *B. subtilis* and *S. aureus* exhibited inhibition-zones that were more or less had shown similar activity in a dose dependent manner at tested concentrations other than *P. aeruginosa*.

Ranga Reddy et al have fabricated Ag- Au bimetallic NPs and have evaluated their antimicrobial activity as against *Bacillus sp*. These results are greater in terms of magnitude against three out of four pathogens tested. The application of the study was in the development of hydrogels [20]. Sharma et al have unveiled the antimicrobial activities of Ag and Au NPs synthesized using Shimla, Mandi and Bilaspur varieties of *Curcuma longa* rhizome extracts. A high antimicrobial and anticancerous activity were observed in this report [21].

Velammal et al have synthesized Ag and Au NPs using the bark extract of *Plumbago zeylanica* [22]. Kirby Bauer Disc diffusion method was employed in the antimicrobial assay. The characterization techniques involved in the study include XRD analyses. A significant antimicrobial activity was obtained for the synthesized monometallic nanoparticles. Ahmad et al [23] have synthesized Mn- Cu bimetallic nanoparticles using the *Vinca rosea* extract and have evaluated the antioxidant, antibacterial and catalytic activities. The tested bacteria included *E. coli, S. typhi, S. aureus* and *K. pneumoniae*. The phytochemicals serve as capping ligands in reducing agents for green synthesis of nanoparticles. The photocatalytic degradation of eriochrome black and methyl orange was achieved successfully. The photo-degradation activity was found to be higher due to the catalytic activity of the bimetallic NPs. Antifeedant assay was successfully determined in the biogenically synthesized silver oxide- bismuth nanoparticles as against *S. litura* also known as tobacco cutworm, which is a polyphagous pest of many important food and cash crops. In comparison with previous reports, the antifeedant activity was found to be greater in magnitude [31, 32, 33, 34, 35].

From the XRD diffractogram above it is inferred that the structure of Ag2O- Bi NPs is bimetallic and 57% amorphous and 43% crystalline. During the preparation and purification of the nanoparticles, it has been heated at 45 degrees Celsius for 30 minutes. This structure has closest resemblance with silver bismuthate (Crystallography Open database: entry number 1509761). The diffraction pattern of the biogenically synthesized silver oxide- bismuth nanoparticles was closest in resemblance to Oberndorfer et al in terms of appearance of peaks. The silver and bismuth have been oxidized and hence there might have been a possibility of formation of a binary compound of silver and/or bismuth. This study is the first visible study in green synthesis of Ag2O- Bi bimetallic NPs. [24]

With regards to antifeedant activity, a significant antifeedant activity was observed as against *Spodoptera litura*. *S. litura* is a deadly polyphagous pest of cash crops such as tobacco, chillies, sunflowers, groundnuts, pulses [27]. The bimetallic NPs were found to show efficacy as against the third instar larvae of *S. litura*. Arivoli and Tennyson have determined the antifeedant activity as against *S. litura* using various medicinal plants. The highest mortality rate observed was by the medicinal plant *Zanthoxylum limonella* at 56 %. This study has reported the antifeedant activity at 1000 ppm [28].

With respect to the Ag_2O- Bi dual metallic combination, our combination corroborates with the study carried out by Ruiz- Ruiz et al. The difference carried out in the current study and the report of Ruiz- Ruiz et al, mechanochemical methods have been employed to synthesize Ag- Bi bimetallic nanoparticles in the method of Ruiz- Ruiz et al. [30, 31, 36]. Whereas in this study, biogenic synthesis has been carried out. Our study is the first visible report on such a combination. The XRD shows a dual set of peaks indicating closer interplanar distances in both the lattices. Further studies need to be carried out to ascertain the extent of the formation of the nano-admixture between Ag_2O and Bi.

From the above studies, it is inferred that our study had shown comparable antimicrobial and antifeedant activity in terms of magnitude as against the three pathogens namely *E. coli*, *S. aureus* and *B. subtilis* which is comparable with the previous literature.

CONCLUSION

It is inferred that a protocol for green synthesis for bimetallic nanoparticle (Ag_2O- Bi) has been developed and the particles were found to possess antimicrobial, antifeedant and catalytic properties. The nanoparticles were synthesized by biogenic methods and when compared with previous literature, were synthesized without the need of toxic chemicals as reducing agents. The size of the nanoparticles was found to be 38.81 nm and the antimicrobial activity was comparable with magnitude when compared to the test standard streptomycin. The antifeedant activity was greater in magnitude when on comparison with previous literature. The nanoparticles can be further explored for *in vivo studies* in animal models for the determination of toxicity of nanoparticles in future. Further studies using ICP- MS need to be carried out to ascertain the formation of the nano-admixture between Ag_2O and Bi.

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Authors' Contributions

Adithya C.G. performed the extraction of crude extract, phytochemical analysis and preparation of the bimetallic NPs. Dr. Joseph Devadass B. performed the antimicrobial assays and performed the serial dilutions of the bimetallic nanoparticles. Dr. M. Muthupandi and Adithya C.G performed the antifeedant assays. Dr. A. Manikandan performed the dye degradation assay. Dr. M.F. Valan, Dr. V. Duraipandiyan helped to design the work and helped in overall editing of the manuscript.

Conflict of interest

There is no conflict of interest.

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