

Biogenesis, Characterization and Bioefficacy of Tin Oxide Nanoparticles from Averrhoa Bilimbi Fruit Extract



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Abstract: A brisk and one-step green method of stannic oxide (Tin oxide) nanoparticles synthesis from the fruit essence of Averrhoa bilimbi has been described in this paper. Nanoparticle was synthesized using the method of co-precipitation and reduction with plant extract. The synthesized tin oxide nanoparticles have been characterized using different analytical techniques. Characterization was performed using UV-Vis Diffuse Reflectance Spectroscopy, Fourier-Transform Infrared Spectroscopy, Scanning Electron Microscopy, High-resolution Transmission Electron Microscopy, Energy Dispersive Spectroscopy, and X-ray Powder Diffraction. UV-Vis spectrum showed an absorbance in the range of 280 nm to 290 nm. The scanning electron microscopy analysis revealed the spherical morphology of the green synthesized nanoparticles and the energy-dispersive spectroscopy spectrum sights the intense peaks of Sn and O which validate the constitution of tin oxide nanoparticle. Further, X-ray diffraction analysis confirmed the formation of SnO₂ nanoparticles in tetragonal crystal structure and the crystalline size of the nanoparticles estimated falls in the range of 2.6 nm. The nanoparticles size is determined to be at close range of 3.08 nm from the transmission electron microscopy studies. The exertion of the SnO₂ nanoparticles was found against Klebsiella aerogenes and Staphylococcus aureus. Biosynthesized SnO₂ nanoparticles showed antioxidant activity too. These findings support the use of tin oxide nanoparticles in distinct applications remarkably in medical field. SnO₂ nanoparticles synthesis using the Averrhoa bilimbi fruit extract is being reported for the first time in this study to the best of our knowledge.

Keywords: Antibacterial, antioxidant, Averrhoa bilimbi, green synthesis, Tin oxide nanoparticle

I. INTRODUCTION

Nanostructures of metal oxide have emerged as an innovative disquisition due to its relevance in applied science. Metal oxide nanoparticles have diverse application in the areas of electronics, medicine and many other industries. As the nanoparticles acts with different decorum from bulk materials, it is a major domain of interest for researchers [1]. Stannic oxide (SnO₂) is an important metal oxide on account of its strong physical and chemical interactions, low operating temperature, and tough thermal stability up to 500 °C [2], [3].

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Tin oxide nanoparticles have an expanded spectrum of application in diverse field. The photocatalytic activity of tin oxide nanoparticles from the leaf extracts of *Calotropis gigantea* has been demonstrated with the degradation of methylene orange [4].

Photocatalytic counterattack on methylene blue, Congo red and eosin Y through green synthesized SnO₂ nanoparticles has been studied by Diallo et al. from *Aspalathus linearis* [5].

Rhodamine B degradation was studied using tin oxide nanoparticles from *Plectranthus amboinicus* [6]. Krishnakumar et al. [7] successfully synthesized SnO₂ nanoparticles by chemical digestion method and proved the humidity sensing a property of tin oxide nanoparticles and also there is a study on gas like carbon monoxide sensing properties of SnO₂ nanoparticles by Celine et al [8]. Antimicrobial activity of

SnO₂ nanoparticles was proved against a wide range of fungi, gram negative and gram-positive bacteria and also it was found that SnO₂ has the ability to reduce body weight. Henceforth it can make use in a good deal with the medical field [9]–[11]. Different methods have been established for the synthesis of tin oxide nanoparticles in a chemical way and physical way of approach. The sol-gel method, co-precipitation method, hydrothermal, mechanochemical, spray pyrolysis, combustion route, micro-emulsion technique, electrochemical deposition, laser ablations are few of them [12], [13]. SnO₂ is an *n*-type semiconductor with band gap falling in the range of 3.1-3.8 eV at 300K [14], [15].

Dealing with the toxicity and cost-effectiveness of the synthesis of nanoparticles, the concept of green chemistry or green synthesis of metal nanoparticles has been accounted into the research. Biosynthesis or green synthesis of the nanoparticles is a remunerative process and also an eco-friendly alternative for the physical and chemical synthesis methods. Extracts of plants naturally act as reducing agents and represent a major source for green synthesis approach. The green synthesis of approximately 20 nm sized SnO₂ nanoparticles was demonstrated employing nontoxic chemicals and green tea, *Camellia sinensis*[16]. Muthugana et al. [17] has reported the reducing property and the effect of green plant extract as capping agent in the process of synthesizing metal oxide nanoparticles. In this study, the ethanolic extract of *Steviare baundiana* leaves extract was used as the reducing agent. In another work, a methanolic extract of *Cleistanthum collinus* leaves extract was used for the preparation of 49.26 nm sized nanoparticles and further explained its antimicrobial and antioxidant effect [15].



The first time use of amino acid arginine for SnO₂ nanoparticles synthesis with an average of 4-5 nm diameter size was reported by Architha et al. [18]. It has been reported that the bio-waste material, the eggshell membrane was used for the synthesis of SnO₂ nanoparticles. The collagen proteins present inside the egg shell membrane acts as the reducing agent for the synthesis[19]. Toxicity of green synthesized nanoparticles may depend on the experimental techniques, their size, and presence of capping agents [20]. Accumulating research evidences confirms that SnO₂ nanoparticles shows little toxicity. A study on daphnia mortality investigates the toxicity of tin oxide nanoparticles on the basic food chain of nature as the food for daphnias, fishes and finally human. It was explained that the tin oxide nanoparticles have less toxicity on *Daphnia magna* [21]. Less toxicity of SnO₂ nanoparticles on zebra fish when compared to other metal oxide nanoparticles like TiO₂, CeO₂, and ZnO₂ was reported by Leah et al.[22].

Averrhoa bilimbi fruit is consumable and it belongs to Oxalidaceae family. The fruits of bilimbi are cylindrical in shape and it will acquire its maximum weight and size at the ripened stage. The color of the fruit will change to yellow from green at its fully blown phase. The fruits and leaves of the plant are endowed with many biological and clinical properties like anti-inflammatory, antimicrobial activities, antihypertensive property and many other pharmacological properties [23]. In India, the extracts of bilimbi fruits were used to treat diarrhea, hepatitis, fever, etc and the mature fruits were used to make dishes like jams, jellies, pickle etc. The fruit extract contains a high amount of oxalic acid also [24], [25]. It's already been reported the synthesis of different nanoparticles from the *A. bilimbi* plant part extract. Iron oxide nanoparticles was synthesized from the fruit, leaf, and bark aqueous extract of bilimbi and it exhibited considerable antioxidant activity [26]. Vijay et al. [27] have synthesized silver nanoparticles from *A. bilimbi* fruits within the size range 10 nm to 50 nm. It was described that the phytoconstituents present in the fruits may influence the stability of the silver nanoparticles. There is a report of superior antioxidant activity of naturally synthesized zinc oxide nanoparticles from *A. bilimbi* extract using co-precipitation method for the first time by Rajita et al.[28]. An instant synthesis of gold and silver nanoparticles from *A. bilimbi* extract was described by Rimal et al. and it was with diameter in the range 50-150 nm. It was shown that they possess a hexagonal or rhomboidal shape [29].

In the present work, a precise, low cost, and green method of SnO₂ nanoparticles from the fruit essence of *A. bilimbi* is demonstrated. Synthesized nanoparticles were characterized by various methods, such as UV-Vis diffuse reflectance spectroscopy (UV-Vis DRS), X-ray diffraction (XRD), high-resolution transmission electron microscopy (HR-TEM), and scanning electron microscopy (SEM). The elemental composition was obtained by energy-dispersive X-ray (EDS) and the functional group analysis is done with Fourier-transform infrared spectroscopy (FTIR). The antioxidant activity with 1, 1-diphenyl-2-picryl hydrazyl (DPPH) assay was got done. The effectiveness of the SnO₂ nanoparticles in opposition to the growth of bacteria was also determined.

II. MATERIALS AND METHODS

A. Plant Details

The plant *Averrhoa bilimbi* belongs to the kingdom Plantae. It comes under the family Oxalidaceae and genus *Averrhoa* and species bilimbi (Fig. 1)



Fig. 1 *Averrhoa bilimbi* fruit bearing plant

B. Plant Extract

Fresh fruits of *Averrhoa bilimbi* at full bloom stage were bagged from Alappuzha district Kerala and transferred to the lab in a fresh sterilized bag. The fruits were washed several times in tap water and further in distilled water to clear the external junks. Further, the fruits were cut into small pieces and retained for extraction (50g in 100ml distilled water) in the water bath. The extract was then refined using a clean muslin cloth and further through Whatmann filter paper and stored for further use [30].

C. Phytochemical analysis of *Averrhoa bilimbi* fruit extract

Phytochemical integrant of fruit essence of *A. bilimbi* were detected by qualitative assay. The more well-prepared aqueous fruit essence was qualitatively proven for the existence of tannin, saponin, flavonoids, terpenoids, alkaloids, cardiac glycosides and anthraquinones using the standard protocol reported formerly [31].

D. Synthesis of Tin Oxide (SnO₂) nanoparticles:

10 mL extract of *A. bilimbi* was diluted with 90 ml of distilled water. 0.1 M of tin chloride (Sigma Aldrich) was mixed with an equal volume of *A. bilimbi* extract using a magnetic stirrer for 4h. After 4h of reaction, the mixture was left static to settle down and then it was centrifuged at 5000 rpm for 15m. It was then washed with ethanol followed by three times washing with distilled water. The precipitate was acquired and dehydrated in an oven for 1h at 80°C. Further, the powder was calcinated for 2h at 400°C.

E. Characterization of SnO₂ nanoparticles:

The crystal structure and crystalline sizes of calcinated SnO₂ nanoparticles from *A. bilimbi* were determined from the XRD patterns. The sample was browsed over the 2θ range from 20° to 80°.

The microstructure configuration of the nanoparticles was analyzed by using HR-TEM. The optical reflectance was exemplified using UV-DRS in the wavelength domain from 200 to 800 nm.

The surface morphology was reviewed using SEM. The nanoparticle's elemental composition was obtained by EDS analysis overlooked with a spectrometer which is supported to the scanning electron microscope. The FTIR spectrum of the *A. bilimbi* SnO₂ nanoparticles was studied to analyze the functional group present in the nanoparticles.

F. Assessment of Antibacterial activity:

The antibacterial vibrancy of calcinated SnO₂ in contra with both gram-negative *Klebsiella* and gram-positive *Staphylococcus aureus* were attained on the basis of standard agar well diffusion method [32]. Standard antibiotic streptomycin was used as positive control. 100 µg and 200 µg of calcinated SnO₂ were dissolved in dimethyl sulfoxide (DMSO) by ultrasonication. The liability of the microbes towards the samples was carried out in nutrient agar medium. The samples were spilled into the well which has been made in the nutrient agar plate in which the respected bacterium has inoculated. The plates were maintained for 24 h at 37 °C to carry out the bioactive reaction. Later, the plates were checked after 24 h to monitor the zone of inhibition.

G. Antioxidant activity (DPPH assay):

The antioxidant activity was assessed by using DPPH assay. Varied concentrations of the synthesized nanoparticles sample were made up to the required volume of the working solution and 0.1 mM of DPPH was added and kept for incubation in dark. After half hour incubation, the absorbance of the mixture was read at 517 nm. The decline in the absorption of the DPPH solution with the addition of tin oxide nanoparticles has been monitored. Ascorbic acid was used as the standard reference.

DPPH scavenging activity (%) = (Abs Blank - Abs Sample / Abs Blank) x 100

Where, Abs Blank = Optical density of Control.

Abs Sample = Optical density of sample.

Statistical analysis:

All the experimental were performed in triplicate and the results were expressed as mean ± Standard Deviation. Origin software was used for preparing the graphs. Statistical significance between the groups was determined by one-way ANOVA followed by a test for trial trend.

The overall methodology which has been done in this piece of work is illustrated in Fig. 2

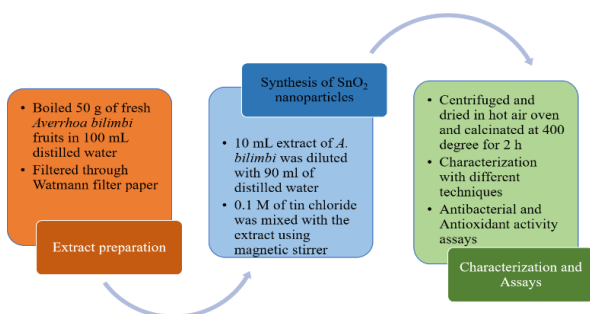


Fig. 2 The overall methodology of SnO₂ nanoparticle synthesis

III. RESULTS

A. Phytochemical assessment:

Freshly made raw aqueous fruit extract of *A. bilimbi* was qualitatively validated for the existence of diverse secondary metabolites and the result is given in Table 1.

Table 1 Phytoconstituents of Averrhoa bilimbi extract

S. No	Phytochemical Analysis	Results
1	Alkaloids	present
2	Flavonoids	present
3	Tannin	present
4	Cardiac glycosides	present
5	Saponin	absent
6	Anthraquinones	absent
7	Terpenoids	present

B. Characterization of nanoparticles:

X-ray diffraction technique:

The crystal structure of biosynthesized tin oxide nanoparticles was recorded using X-ray diffraction technique and is given in Fig. 3. The crystalline size of the SnO₂ nanoparticles was verified using Scherrer's formula $D = k\lambda/\beta\cos\theta$.

The crystalline size is estimated as 2.6 nm from the equation.

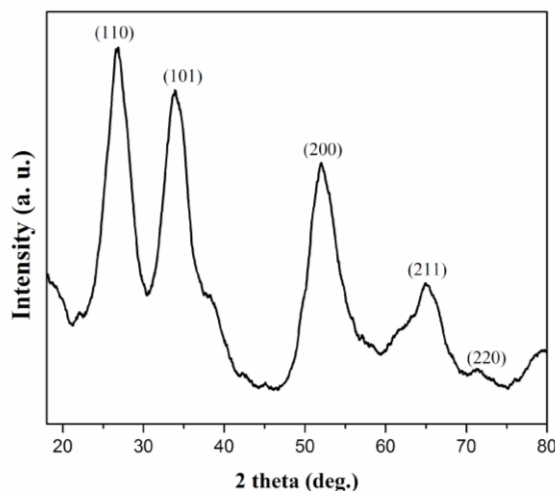


Fig. 3 XRD pattern of SnO₂ nanoparticle from Averrhoa bilimbi

UV-Visible spectroscopy:

The UV-Vis DRS of SnO₂ nanoparticles synthesized from *A. bilimbi* aqueous fruit extract is presented in Fig. 4. The absorption peak observed at ~ 280nm again validates the genesis of SnO₂ nanoparticles. The band gap of the synthesized nanoparticles was estimated from UV-DRS data using Tauc's' relation,

$$F(R) = (\alpha \cdot hv)^2 / hv$$

Where F(R) is the Kubelka-Munk function, hv is the incident photon energy, α is the absorbance and the Tauc's plot is given in Fig. 5 and is found to be 3.36 eV.

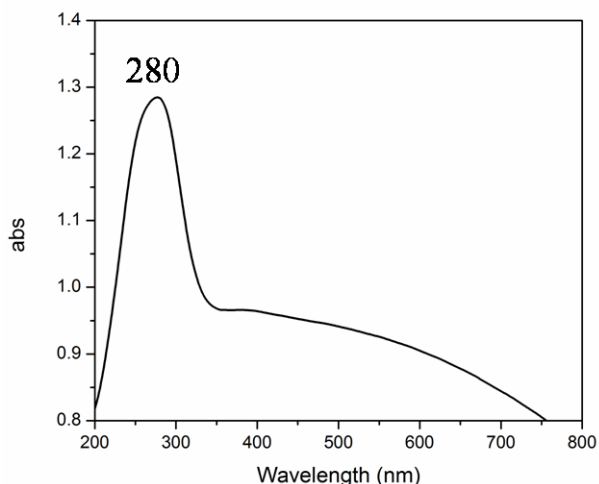


Fig. 4 UV-VIS DRS of SnO₂ nanoparticle from *Averrhoa bilimbi*

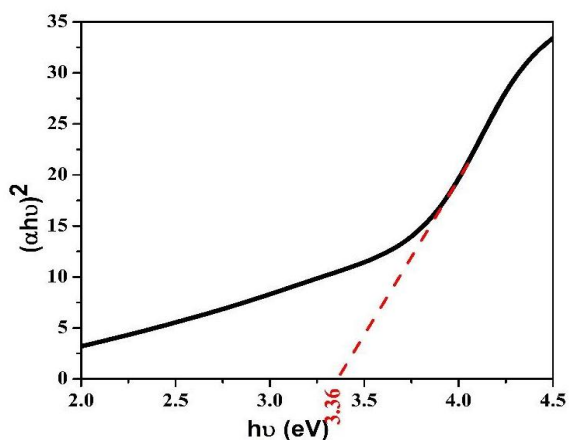


Fig. 5 Band gap of SnO₂ nanoparticle from *Averrhoa bilimbi*

Fourier-transform infrared spectroscopy:

The FTIR spectrum of SnO₂ nanoparticles from *A. bilimbi* was in a range of 400-4500 cm⁻¹. As shown in Fig. 6 the spectrum has bands at 3627 cm⁻¹, 2914 cm⁻¹, 2857 cm⁻¹, 1723 cm⁻¹, 1640 cm⁻¹, 1526 cm⁻¹, 1386 cm⁻¹, 1066 cm⁻¹, 787 cm⁻¹, 491 cm⁻¹ which demonstrate the functional groups.

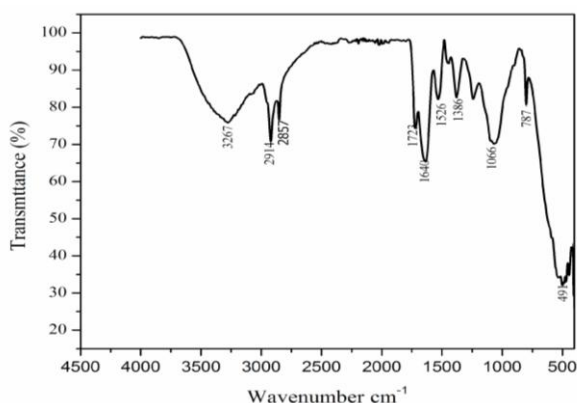


Fig. 6 FTIR spectra of SnO₂ nanoparticle from *Averrhoa bilimbi*

Scanning Electron Microscopy:

The morphology of biosynthesized SnO₂ NPs was analyzed using scanning electron microscopy and is given in Fig. 7. Based on the SEM analysis the morphology of SnO₂

nanoparticles is found to be spherical in shape and the EDS spectrum of SnO₂ nanoparticles, which symbolizes the potent peaks that confirm the composition of Sn and O (Table II).

Table II Composition table of SEM-EDS of SnO₂ nanoparticle from *Averrhoa bilimbi*

Element	Weight%	Atomic%
C K	6.27	17.03
O K	32.17	65.54
Cl K	0.82	0.75
Sn L	60.74	16.68
Totals	100.00	

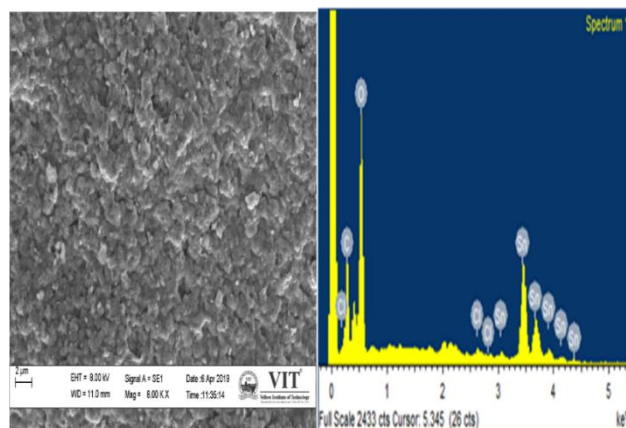


Fig. 7 SEM and EDS image of SnO₂ nanoparticle from *Averrhoa bilimbi*

Transmission Electron Microscopy:

The TEM image of the synthesized nanoparticles with higher magnification, recorded with high resolution microscopy is detailed in Fig.8. The size of the SnO₂ nanoparticles is determined to be 3.08 nm which is in an acceptance with the crystalline size of the same from the XRD data.

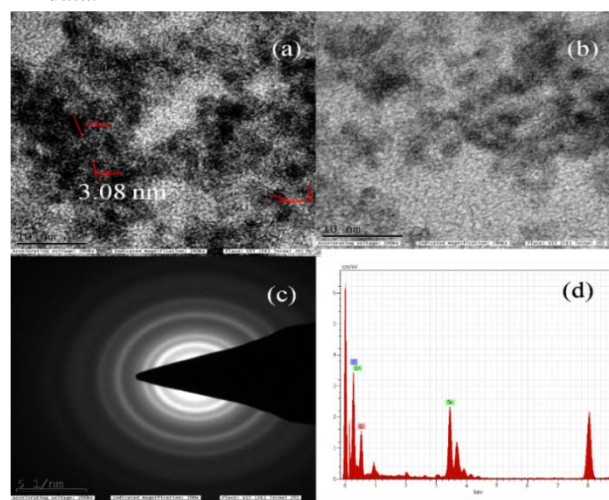


Fig. 8 Transmission Electron Microscopy of SnO₂

nanoparticle from *Averrhoa bilimbi*: (a) image which indicate the size of nanoparticle, (b) 10 nm size magnified image, (c) SAED pattern of SnO₂ nanoparticle and, (e) EDS image

C. Antibacterial activity:

The antibacterial study of the green synthesized SnO₂ nanoparticles from *A. bilimbi* showed a satisfying inhibitory activity against gram-positive *S. aureus* and gram-negative *K. aerogenes*. The hindering effects of the nanoparticles in higher concentration against the microbes were calculated by measuring the zone of inhibition around the diffused well in the plate culture. The zone of inhibition was measured in mm and was found to be 35 mm for calcinated SnO₂ nanoparticles against *Klebsiella* and 18mm for the same against *S. aureus* (Table III). The lower concentration of nanoparticles didn't give much promising result against the microbes [33], [34].

Table III: Zone of inhibition of antibacterial activity of SnO₂ nanoparticles against pathogen

Name of the Bacteria	Antibacterial activity Zone of inhibition (mm)		
	Standard	100µg	200µg
<i>Staphylococcus aureus</i>	40	5	35
<i>Klebsiella aerogenes</i>	22	8	18

D. Antioxidant activity:

The antioxidant activity of the green synthesized SnO₂ was assessed by considering the free radical scavenging activity using 1, 1-diphenyl-2-picryl hydrazyl (DPPH). The percentage of scavenging activity concerning with the concentration of sample and standard ascorbic acid is represented in Fig. 9. As the intensity at the band of 517nm decreased, the scavenging activity increased. The decreasing rate in the band intensity is directly proportional to the concentration of the SnO₂ nanoparticles. The highest concentration at 1000µg/ml showed the maximum antioxidant activity.

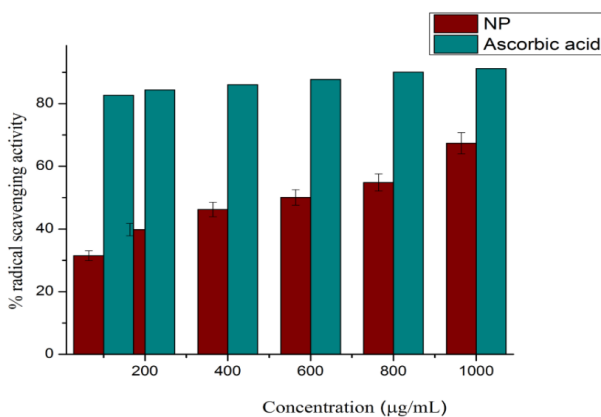


Fig. 9 Antioxidant activity (DPPH) of SnO₂ from *Averrhoa bilimbi*

IV. DISCUSSION

The green synthesis of SnO₂ nanoparticle from *A. bilimbi* fruit extract was carried out successfully. The aqueous extract showed the presence of alkaloid, flavonoids, tannin, glycosides, and terpenoids. Meanwhile, the test results turn out in the absence of saponin and anthraquinones [35], [36]. Studies have proved that the phytochemicals act as good reducing agent in the formation of nanoparticles from plant extract. Jaison et al interpreted that the flavonoids have the potentiality to chelate and reduce the metal ions into nanoparticles. As the *A. bilimbi* fruit holds the flavonoids in it and it has reduced the tin chloride into tin oxide nanoparticle [37]. The characterization using XRD pattern analysis revealed the size of the crystalline structure and it reflects the previously mentioned nanoparticle's size [38]. The 2θ value of the SnO₂ nanoparticles were 26.7°, 33.9°, 52.04°, 65.2°, 71.6° that complements the crystallographic plane, (110), (101), (200), (211), (220) which denotes the formation of SnO₂ nanoparticles in tetragonal structure according to the JCPDS card number (41-1445) [39], [40]. From the UV-Vis DRS of SnO₂ nanoparticles, it can be seen that the material has good absorption capacity in the visible region in the range of 200–800 nm. The estimated band gap of synthesized SnO₂ nanoparticles is 3.36 eV and is in agreement with the previously reported band gap value of SnO₂ nanoparticles [13], [41]. From the FTIR analysis the different band peaks at different points indicated the presence of different functional groups. The band at 3267 cm⁻¹ is accounted for the strong broad O-H stretching of the intermolecular bond. The peak ranging from 2914 cm⁻¹ - 2857 cm⁻¹ indicates the O-H stretching of the intramolecular bond and the C=O stretching gives a strong peak at 1723cm⁻¹. A C=C stretching of alkenes was found at 1640 cm⁻¹. The bands at 1526 cm⁻¹, 1386 cm⁻¹, and 1066 cm⁻¹ give an idea on the N-O stretching of nitro compounds, O-H bending of phenols and C-N stretching of ethers respectively. The bands obtained at the range of 500 to 400 are the specific one which pinpoints the Sn-O-Sn assignment[42], [43]. From the SEM analysis the morphology was found and the agglomeration of nanoparticles was observed which may be due to the small size and nature of the particle. Few weak peaks of Cl that appeared in the EDS spectrum points out the biometabolites present in the plant extracts [4]. The EDS image of the nanoparticles clearly indicates the purity of the sample with the presence of Sn and O. The extra peaks present is due to the elements which components the grid of the microscope. The SAED pattern reveals the structure related to the XRD. The above data guarantees with the previous research which has been conducted with the SnO₂ nanoparticles studies [38].

Nanoparticles exerts its anti-microbial activity by breaking the cell wall membrane and thereby causing disturbance in the cell functioning [44]. Previously, studies have reported that tin oxide nanoparticles possess significant anti-microbial activity [18]. In accordance with these studies, biosynthesized SnO₂ also showed remarkable anti-microbial activity.

However, they showed a different depth of anti-bacterial activity, possibly because of the difference in cell wall complexity of the test organisms. The DPPH assay reveals the antioxidant capacity of the SnO₂ nanoparticles. Studies have reported the antioxidant activity of SnO₂ nanoparticles through DPPH assay. The increase in concentration of nanoparticles, the increased was the result [45].

V. CONCLUSION

Here in this piece of investigation, we elucidated a low-cost and ecologically sound method of synthesizing tin oxide nanoparticles from the aqueous extract of the *A. bilimbi* fruit. The synthesized nanoparticles were characterized using the analytical techniques, UV-VIS DRS, XRD, FTIR, SEM, and TEM. The band gap of the SnO₂ nanoparticles is found to be 3.36 eV and from the SEM analysis the morphology of the nanoparticles is revealed as spherical in shape. The crystalline size of SnO₂ was found to be 2.6 nm from the XRD studies. The TEM data gives an idea about the average size of the nanoparticles and it is determined as 3.08 nm. The biological activity of the synthesized nanoparticles was found effective against two microbes, *Staphylococcus aureus* and, *Klebsiella aerogenes* and this study may help to find a new way into the medical research field to use the SnO₂ nanoparticles as an emerging medicine against microbes. The synthesized SnO₂ nanoparticles provides with a satisfying antioxidant activity with DPPH radical scavenging assay. All these investigation in this study will help to come across the possibilities in the green chemistry to explore the nanoparticle synthesis from the natural sources and its conscientiousness in different field of research and industry for the future development of mankind.

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