ISSN: 2063-5346
Section A-Research paper

CATECHIN/AG/TITANIA (CAT NPS) NANOPARTICLES AS NOVEL ANTIFOULING COMPOUND AGAINST BIOFILM FORMING BACTERIA OF VISAKHAPATNAM COAST, ANDHRA PRADESH, INDIA

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Article History: Received: 12-03-2023 **Revised:** 14-05-2023 **Published:** 27-05-2023

Abstract
In the realm of nanotechnology, there is a need for the creation of novel experimental procedures for the production of nanoparticles. The sol-gel process was used to synthesize Catechin-Ag-Titania nanoparticles. Catechin-Ag-Titania NPs is proposed as a novel cost-effective and ecofriendly antifouling compound. Well-dispersed Catechin-Ag-Titania NPs designated as CAT NP has been achieved through catechin mediated reduction of silver and TiO_2 ions at ambient temperature. The CAT NP showed effective antifouling activity against the screened biofilm forming bacteria. SEM, XRD, FTIR and TEM showed the properties of CAT NP and antifouling properties of CAT NP will provide new opportunities to develop cost-effective and ecofriendly antifouling composition that is 10 times effective compared with control.
Keywords: CAT NP, Catechin, Ag, Titania, TEM.

DOI: 10.48047/ecb/2023.12.Si5.365

Introduction

Nanotechnology is an evolving technology that has contributed towards the development of great diversity of materials. It entails the production and utilization of extremely small particles about 1–100 nm in size, where the synthesis of these particles is controlled or modified at the molecular level (Badawy *et al.*, 2010). The extreme size and the bottom-up approach to synthesize new materials (Rolison, 2003) are the two special factors of nanoparticles, which are making them the building blocks of next generation. During the recent years, approximately 200 products which contain engineered nano material in them were introduced into the market (Breggin *et al*., 2007). Due to their desirable material characteristics and significant potential in a variety of applications, the synthesis of different nanostructures and titanium nanoparticles has attracted attention in recent years (Mills *et al*., 1993). Among the existing photo catalytic materials TiO_2 NPs are considered to be the best. The reasons attributing to this photo catalytic nature is strong oxidizing power, prolonged thermodynamic stability and relative non-toxicity (Krishna *et al*., 2006).

For the synthesis of nanoparticles, biological approaches are regarded as secure, economical, long-lasting, and environmentally benign processes (Schmid, 2011). Many bacteria, fungi, and

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plants have been used to successfully create silver nanoparticles, including Kalimuthu *et al.,* in 2008, Chaudhari *et al.,* in 2012, Saifuddin in 2009, Kathiresan *et al.,* in 2009, and Raut *et al.,* in 2010 and Masurkar *et al.,* in 2011. By adding coatings or capping agents and functionalizing their surface, NMs can also be manipulated and distinguished from one another. Consequently, two particles of the same substance that are varied in size and shape may have various physical and chemical characteristics and applications (Tiede *et al.,* 2009; Ju-Nam and Lead, 2008). Numerous commercial products contain titanium dioxide nanoparticles (TiO_2-NPs), which are currently produced in large quantities around the world. The formation of nanoparticles in different sizes and varied chemical compositions and regulated mono-dispersities is a fundamental topic in nanotechnology study. The need to create ecologically friendly nanoparticle manufacturing methods is currently increasing. The current study focuses on synthesis of novel nanocompound of catechin from plant extract, silver doped with Titania as antifouling agent.

Materials and methods

Preparation of Catechin-Silver-TiO_2Nanoparticles

In brief, 50 ml of pure ethanol was mixed vigorously with 20 mM tetra butyl orthotitanate to create a solution. After stirring for 20 minutes at room temperature, 3 mM concentrated HNO_3 was added. The reaction mixture was then supplemented with 1Mm of Catechin powder and 1Mm of $AgN0_3$ (the molar ratio of Catechin to Silver was 1:1) while being continuously stirred for 60 minutes until Silver Nitrate and Catechin Powder had dissolved. The aforementioned solution was then injected with 2mL of deionized water. The obtained mixture of the solution was then kept for 2hours at room temperature while being constantly stirred to create a gel, which was then left to set for 12 hours. The Catechin/Ag/TiO_2 sample is first dried at 80°C over the course of a night before being annealed at 400°C for three hours in order to produce a greyish material.

Determination of Minimum Inhibitory Concentrations (MICs) by agar dilution (slight modification)

Mueller-Hinton agar was prepared as per the conventional method. The sterilized agar was allowed to cool in water bath to 50ºC. Dilutions of antimicrobial agents were prepared in 25–30 ml vials. To each container, 19 ml of molten agar was poured, carefully mixed and then these contents were poured into sterile Petri plates with pre-printed labels on a flat surface. The plates were allowed to dry so that there were no moisture traces on the agar surface and then cooled to room temperature. Care should be taken that the plates are not over dried. The inoculum density was set to a uniform 104 colony-forming units (CFU) per agar spot. Nearly four or five pure culture colonies were used to prevent choosing an unusual variation. Either overnight colonies emulsification from an agar medium or by diluting a broth culture inoculum can be prepared. After inoculation, the plates were incubated for 18 hours at 35–37°C in air. Minimum Inhibitory Concentration is nothing but the lowest concentration of an agent that fully suppresses observable growth that could be determined by a naked eye. It is to be noted that neither a single colony nor a thin haze inside the region of an infected spot should ever be taken into account for determining it.

SEM analysis

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The treatment of the iron panels followed the process outlined by Novitsky and MacSween (1989). A mixture of 2.5 percent glutaraldehyde, 85 percent filter-sterilized seawater, and 15 percent distilled water was used to fix iron panels. After fixing, salt crystals were removed from the iron panels by periodically washing them in double-distilled, sterile water. The grains were dehydrated in two stages using HMDS (hydroxyl mexamethyl di silazane) and a graded ethanol series (90% for 5 min). The iron panels were sputter-coated with gold after drying, and double-sided conductive tape was used to link them to SEM stubs.

Transmission electron microscopy

The electron source for the TEM is a tungsten filament cathode fitted with an electron pistol that emits a high voltage electron stream. Electrostatic and electromagnetic lenses focus the electron beam's acceleration (from 40 to 100 kV) so that it is directed at the object under observation. In proportion to the density of the sample, some electrons in the electron beam will scatter when they hit the specimen. Unscattered electrons that pass through the specimen will strike a fluorescent viewing screen that is covered in either phosphor or zinc sulfide. The varying shades of blackness in the resulting image are related to the sample density and dispersed electrons.

X-Ray diffraction analysis

The technique known as X-ray diffraction analysis (XRD) is employed to examine size and structure of crystals. At the Advanced Analytical Laboratory at Andhra University, XRD analysis using a Siemens D5000 diffractometer provided further details about particle size and crystal structure.

Results and Discussion

Recently, *Nyctanthes arbor-tristis* extract and other natural products have been used to create green TiO_2 nanoparticles (Jha *et al*., 2009). Ag NPs can be produced on TiO_2 surfaces via photochemical, sol-gel techniques, aqueous reduction and liquid phase deposition (Cozzoli *et al.,* 2004; Guin *et al.,* 2007). Natural products such as *Eclipta prostrate* aqueous leaf extract, *Nyctanthes arbor-tristis* extract, *Catharanthu sroseus* aqueous leaf extract, and *Aspergillus flavus* aqueous extract have all been used in the synthesis of TiO_2 NPs (Sundrarajan and Gowri, 2011, Velayutham *et al*., 2012, Kirthi *et al*., 2011). According to Adams, *et al.,* (2006) and Long, *et al.,* (2006), TiO_2 is one of the best opacifiers and is used as a pigment in paints, inks, paper, sunscreens, cosmetics, and plastics. Additionally, several researchers have developed silver supported Titania materials as a photo catalytic bactericide using these fundamentals and the antibacterial properties of silver (Castro *et al*., 2002; Chao *et al*., 2003 and Akhavan, 2009). Using silver nitrate precursor and catechin, we created silver nanoparticles supported on Titania by sol-gel in the current study.

Data were compared using the XRD patterns for synthetic TiO_2 NPs and the control sample of pure TiO_2 (Figure 1). About 25.35, 38.08, 48.08, 54.19, 54.95, 62.63, 68.21, 70.39, 74.94, and 82.73 are the well-defined diffraction peaks with 2, which are ascribed to the (101), (004), (200), (105), (211), (204), (116), (220), (215), and (224) crystal planes, respectively. This XRD characteristic pattern is compatible with the typical JCPDS values of anatase TiO_2 (JCPDS Card No. 21-1272) (Maduda *et al.*, 2009 and Ge *et al.*, 2006), tetragonal structure, and did not occur in rutile and brookite form. Using Scherrer's formula, the average crystallite size of nanoparticle

samples was determined from FWHM of the anatase (101) reflection plane (Figure 1) (Dhage *et al.*, 2004). The Ag NPs produced by our environmentally friendly approach were nanocrystalline in nature, as shown by the occurrence of structural peaks in XRD patterns and average crystalline size of roughly 22 nm. Debye-Scherrer equation can be used to determine the average particle size of silver nanoparticles produced by the current green approach (Ahmad *et al.*, 2010; Vidhu *et al*., 2011).

$d = K\lambda/\beta\cos\theta$,

Here in the above equation, d represents crystallite size, K represents constant (=0.9, assuming the particles to be spherical), λ is wavelength of X-ray diffraction. β is the FWHM and the angle of diffraction is represented by θ. The samples that were synthesized possessed small crystallite sizes, with an average size of 19 nm, according to the predicted crystallite size, it was discovered. From this, it is clear that the catechin and silver additions somewhat reduced the size of the TiO_2 crystallites. Comparing (101) reflection plane of pure TiO_2 with Catechin, Silver, and TiO_2, it can be seen that the inclusion of Catechin and silver into TiO_2 has a broadening effect, which may account for the decrease in TiO_2 particle size. The predicted crystallite size decreased as the intensity was decreased and the width of the (101) reflection plane was increased. Additionally, the Catechin/Silver/TiO_2 samples' (101) reflection plane changed to a greater diffraction angle in comparison to pure TiO_2 (Figure1). The measured values can be used to calculate the lattice parameter (a) for the spacing of the (111) plane, respectively. The results of the lattice parameter and the inter planer spacing's measured in the XRD analysis, the lattice constriction was found as represented in Table 1.

In an attempt to determine the functional groups of catechin/Ag/TiO_2 nanoparticles and pure TiO_2 NPs, the FTIR spectra of the nanoparticles were taken. FTIR spectra of catechin and silver co-doped TiO_2 nanoparticles and un-doped TiO_2 are displayed in (Figure 2 and 3) respectively. Undoped TiO_2's spectra contained significant absorbance bands at 3387, 2925, 2853, 1627, 1383, 662, 626, 584, and 552 cm^{-1}. The O-H group's stretching vibrations in undoped TiO_2 could be responsible for the broad band that was seen at 3387, 2925, and 2853 cm^{-1}. The bands at 2853cm^{1} depict the asymmetric stretching, scissoring, twisting, and rocking vibrations of methylene groups, respectively. The distinctly asymmetrical stretch of the carboxylate group may be responsible for the stronger band at 1635 cm^{-1}. The band that is present at 1311 cm^{-1} is what causes the carboxylate group to extend in a symmetrical manner. According to Jung *et al*., (2000) and Ding *et al*., (2000), the ether C-O stretching vibration resulted in formation of peak at 1068cm^{-1} and that of alcoholic group at 1028 cm^{-1}, and 500 to 900 cm^{-1} Ti-O-Ti. Catechin/silver/TiO_2 nanoparticles' spectra, however, displayed distinctive absorbance bands at 3416, 2920, 2852, 2326, 2111, 1635, 1462, 1384, 1097, 1018, 663, and 606. The hydroxyl group's stretching vibration and the water molecules interlayer can be linked to the shift in absorbance peaks that was seen in the IR spectrum of nanoparticles (Liu *et al*., 2007). The major key contributors to this phenomenon were (a) Ti-O-H stretching vibration and (b) powder surface adsorption.

According to Li *et al*., (2008), for all samples, the band near 1635 cm^{-1} can be attributed to the bending vibration of the H-O-H bond on the titanium dioxide catalyst, and the band at 1384 cm^{-1}

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indicates the binding of Ag-O-Ti, respectively. Furthermore, the existence of transitional metal carbonyls at the peaks at 2326 cm and 2111 cm confirms that the oxidation of the hydroxyl and carbonyl groups is connected to reduction of the silver ions, indicating that the catechin is more thoroughly oxidized. It may be comprehended that the carbonyl and hydroxyl groups of catechin are actively involved in the creation of Ag NPs depending up on the band shift in these groups, there may be a loss of any existing carbonyl, or may be an emergence of a new carbonyl peak. The band at 1462cm^{-1} corresponds to asymmetric (Djaoued *et al.*, 2002) and that at 663 cm^{-1} denotes anatase peak of TiO_2.

The energy dispersive spectroscopy (EDS) analysis of the TiO_2 nanoparticles confirmed the presence of elemental metal signal (Figure 4).The shape of the titanium dioxide nanoparticles was seen in the scanning electron micrograph and was roughly spherical, indicating that they were aggregated. When viewed closely, it was clearly evident that the powder particles seemed to be slightly aggregated (Figure 5a & 5b).

Poly disperse nanoparticles with spherical forms could be seen in the TEM images (Figure 6a). The TEM micrograph of the TiO_2 NPs generated using silver and acacia extract clearly shows that each individual nanoparticle has a nearly spherical shape and measures 232 nm in size. Our experimental results confirm the effective manufacture of smaller anatase TiO_2 nanoparticles as compared to the literature report (Prasad *et al.,* 2007). Titanium ions have been transformed into metallic nanoparticles by catechin. According to Sasaki *et al.,* (2004), a distinct diffraction rings that corresponded to the anatase phase were displayed by the selected area electron diffraction SAED pattern (Figure 6b).

TiO_2 particles can be utilized in paints and coatings, sunscreens, energy storage devices, and other products (Wang *et al*., 2007; Vargas *et al*., 2006; Wildeson *et al*., 2008). Rutile, brookite, and anatase are the three crystalline phases of TiO_2 that can be produced. The latter are the materials for photocatalysis that have been investigated the most, and each one has unique features and consequently distinct applications (Boccaccini *et al.,* 2004). TiO_2 NPs are regarded as a superior band-gap semiconductor, much like ZnO NPs (Ju-Nam and Lead, 2008). TiO_2 nanoparticles, on the other hand, frequently agglomerate. Due to their potential oxidation strength, high photo stability, and lack of toxicity, TiO_2 nanoparticles outstand among the various other metal oxide nanoparticles in terms of their intense range of applications in air as well as water purification, DSSC, and other fields (Li *et al*., 2004, Al-Salim *et al*., 2010, and Ito *et al*., 1999). Therefore, in this study, we prepared unique nano compounds that can be employed as antifouling chemicals using TiO_2 and Ag.

The silver nanoparticles are effective (Kwok and Leung, 2005) and mercuric chloride (Wisley and Blick, 1967) and is ecofriendly chemical and less hazardous to the marine environment. The catechin from *Acacia catechu* showed highest inhibitory activity against the biofilms in our earlier studies, so catechin and silver along with Titania is used to prepare a hybrid compound CAT nanoparticle. Surprisingly it showed the best results compared to toxic chemicals which were used as controls in this experiment, The CAT nanoparticles are 10 times more effective when compared to the control copper sulphate and mercuric chloride (Figure 7) and is ecofriendly chemical and less hazardous to the marine environment.

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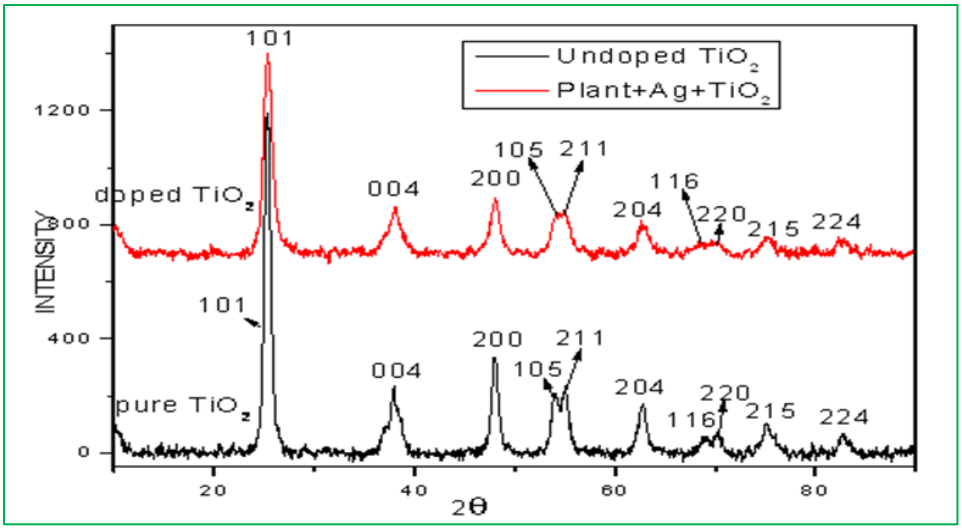

Figure 1: Powder X-ray Diffraction XRD patterns of C/Ag/TiO_2 Nanoparticles.

Table 1: Lattice parameters

	2θ	**d**	**FWHM of intense peak *β* (radians)**	**Size of the crystallite (D) nm**	**Lattice Parameters**	**hkl**
Pure TiO_2	25.28	3.520	0.0083	17	*a*=*b*=3.7852 *c*=9.5139	101
Catechin/Ag Doped TiO_2	25.35	3.511	0.0166	9	*a*=*b*=3.780 *c*=9.444	101

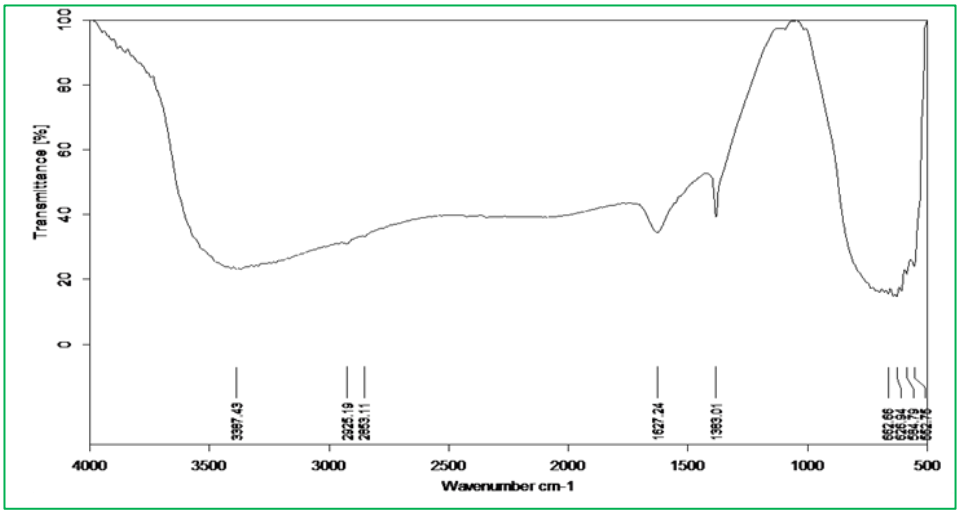

Figure 2: FT-IR of pure TiO_2

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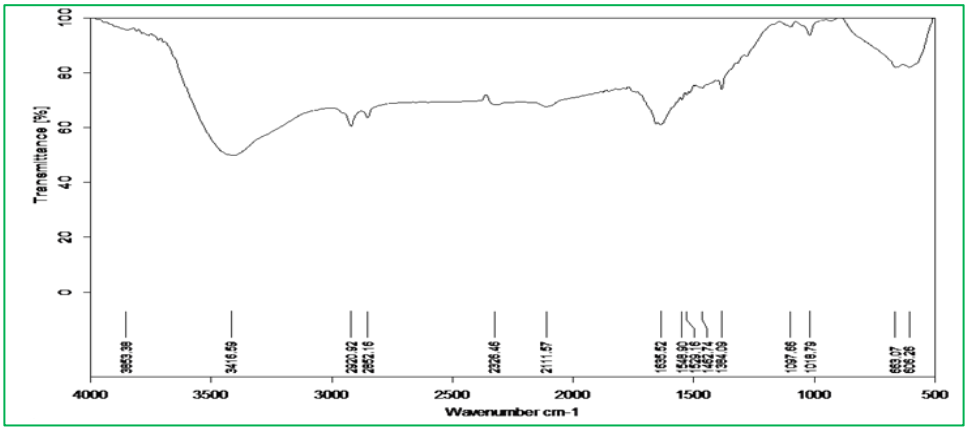


Figure 3: FT-IR of Catechi-Ag-TiO_2

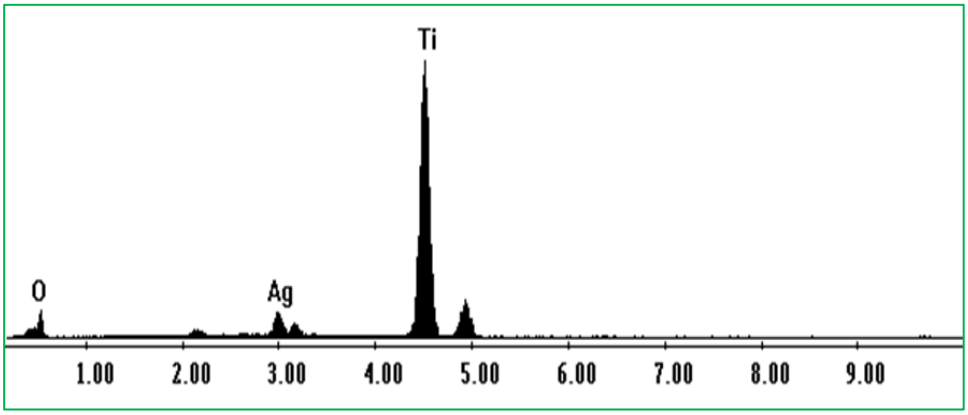
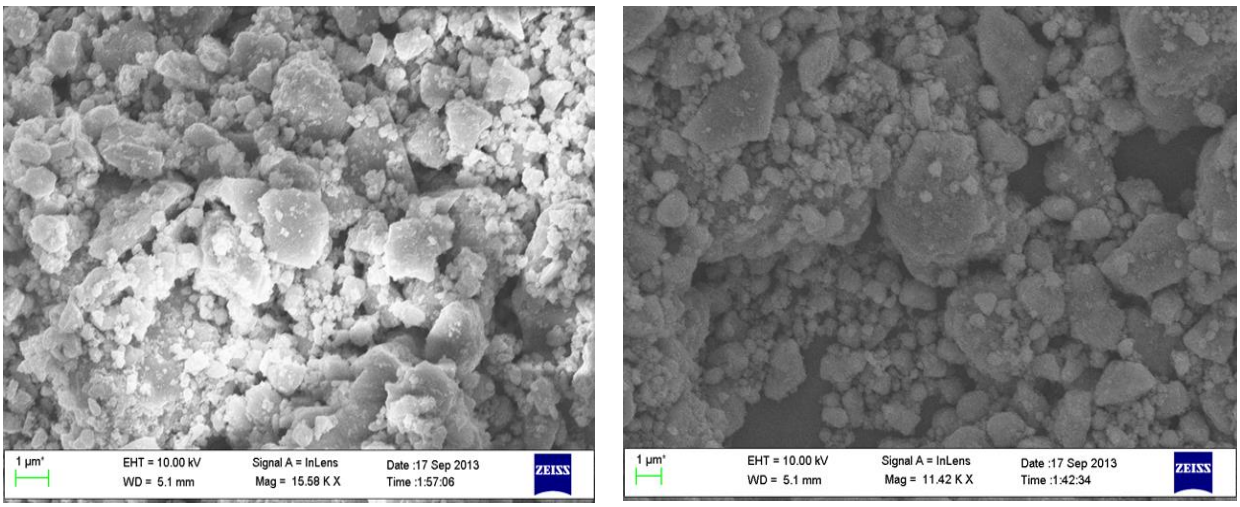


Figure 4: Energy Dispersive Spectrometry (EDS)



(a) **(b)**

Figure 5: (a) FE-SEM micrograph of Catechin/ Silver/ TiO_2 nanoparticles (b) FE-SEM micrograph of pure TiO_2.

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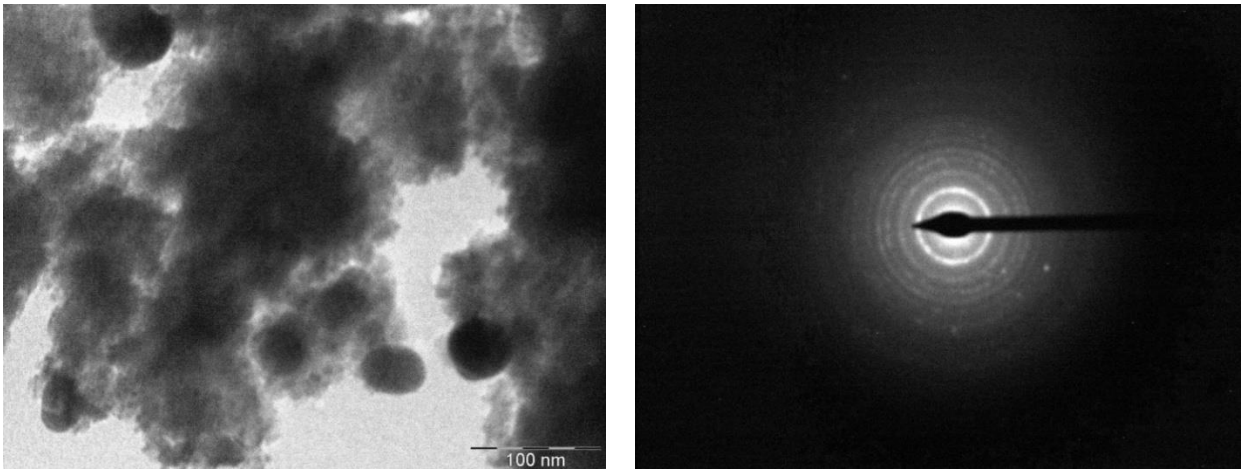
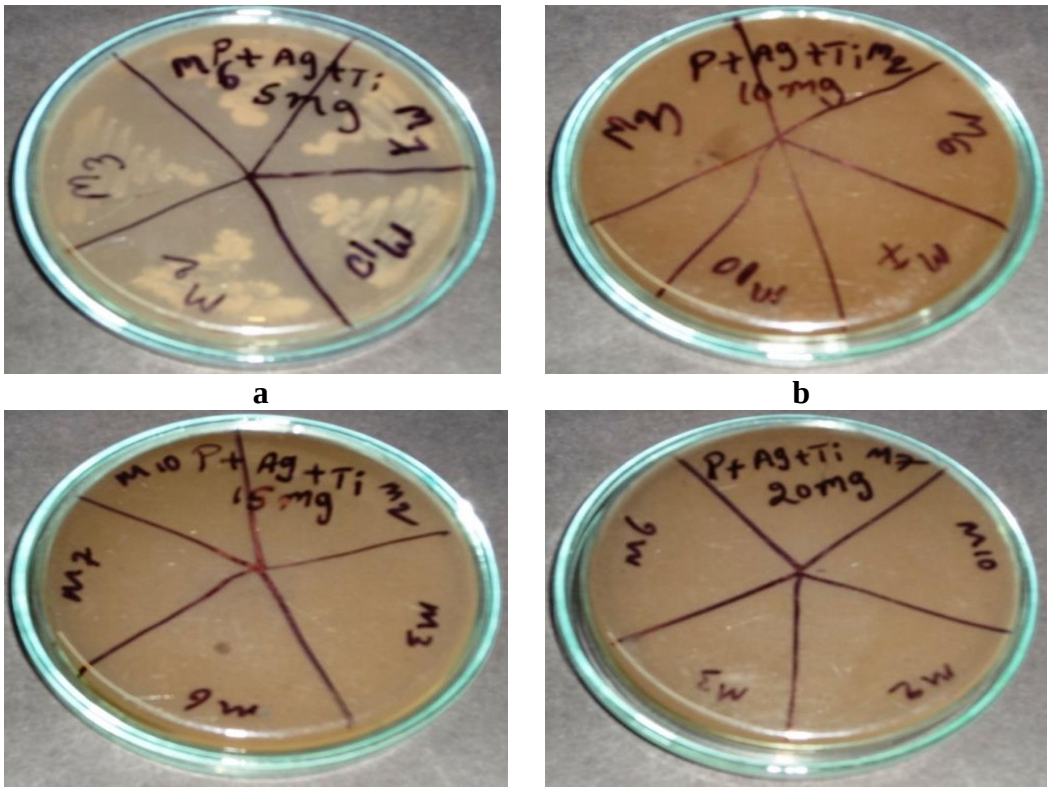


(a) **(b)**

Figure 6: (a) TEM micrograph of Catechin/Ag/TiO_2 and (b) the selected area electron diffraction pattern of Catechin/ Ag/TiO_2 Nanoparticles.



a **b**

c **d**

Figure 7: Determination of Minimum Inhibitory Concentrations (MICs) by agar dilution method: the concentration decreasing from 20 mg 10 ml^{-1} to 5 mg 10 ml^{-1} is tested, growth is observed up to 5 mg 10 ml^{-1} and the growth is inhibited at 10 mg 10 ml^{-1}, the final MIC is 1.0 mg ml^{-1}.

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Conclusion

All maritime industrial equipment has a significant operational problem when it comes to controlling marine microfouling and its detrimental impact on submerged surfaces. From this finding, it may be inferred that Catechin-silver-Titania nanoparticles may contribute to biofilm prevention. This work also demonstrates the synergistic effect of CAT nanoparticles against biofilm forming bacteria. In comparison to the test compounds, the synthesized new CAT NPs demonstrated the best results. CAT NPs may be coated on marine industrial surfaces in order to take use of its potential antifouling activity.

Acknowledgement

Authors greatly acknowledge the UGC, for the financial support under RGNF scheme, and thankful to the institution for providing the laboratory facilities.

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