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# Green Synthesis And Characterisation of Encapsulated Barnacle with Copper Nanoparticles And Their Potential Biological Applications

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***ABSTRACT:*** *Marine biofilms formed due to adhesion of bacteria and other microorganisms on submerged surfaces are generally considered to be a major form of microfouling. Subsequent attachment of larvae of higherorganisms like barnacles, mussels, and so forth, on marine biofilms, causes macrofouling. Several approaches have been used to prevent micro and macrofouling. Copper and its oxide nanoparticles (CuONPs) are known to exhibit strong inhibitory and antimicrobial activity. Biological synthesis of CuONPs is rapidly gaining importance due to its growing success. Hence, the present study is focused on the biosynthesis of CuONPs using barnacle extract and APTMS protected nanoparticles was characterization through UV-Vis spectrophotometer, X-ray diffractometer (XRD), Fourier transform infrared spectroscopy (FTIR), and Antimicrobial Activity. The antimicrofouling effect of the Green synthesized CuoNPs was tested against marine bioassay forming bacteria and the results suggested that it could effectively inhibit biofilm formation. This preliminary study has proved that CuONPs may be used as antimicrofouling agent for the prevention of biofouling in the early stages.*

***Keywords:*** *Green synthesis, Copper, X-ray diffraction, Infrared spectroscopy, Escherichia coli.*

## I. INTRODUCTION

The emergence of nanoscience and nanotechnology in the last decade presents opportunities for exploring the bactericidal effect of metal nanoparticles. The bactericidal effect of metal nanoparticles has been attributed to their small size and high surface to volume ratio, which allows them to interact closely with microbial membranes and is not merely due to the release of metal ions in solution. A cell wall is present around the outside of the bacterial cell membrane and it is essential to the survival of bacteria. It is made from polysaccharides and peptides named peptidoglycon. There are broadly speaking two different types of cell wall in bacteria, called gram-positive and gram negative. The names originate from the reaction of cells to the gram stain, a test long-employed for the classification of bacterial species. Gram-positive bacteria possess a thick cell wall containing many layers of peptidoglycan. In contrast, gram negative bacteria have a relatively thin cell wall consisting of a few layers of peptidoglycan. Surfaces of copper nanoparticles affect / interact directly with the bacterial outer membrane, causing the membrane to rupture and killing bacteria. Antibacterial activity of copper nanoparticles synthesized by electrolysis was evaluated by using standard Zone of Inhibition (ZOI) microbiology assay [1]. Marine biofouling is one of the major problems encountered on the man-made objects in the marine environment. Biofouling has been defined as the undesirable accumulation of microorganisms, plants, and animals on artificial surfaces immersed in a common matrix [2]. The establishment of fouling community takes place in several stages. Initially, any submerged surface gets coated by a conditioning film consisting of organic and inorganic molecules. The subsequent onset of macrofouling may be preceded by the formation of bacterial biofilms (bacterial fouling) and such a biofilm may have a deleterious effect on the ability of the surface to remain free from larger fouling organisms. Use of chemical antifouling agents is one of the common and easy approaches to control fouling caused by micro- and macrofoulers [3]. However, many antimicrobial materials are less effective on microorganisms in biofilms compared to their planktonic counterparts [4]. Therefore, high concentrations of chemical antifoulants are required for the effective control of fouling resulting in harmful secondary effects. Future research on the antifouling strategies may target the formation of the conditioning layer as a way to prevent subsequent colonization of the surface [5].

### 1.1 Anti-Fouling Agent

Anti-fouling is the process of removing or preventing these accumulations from forming. In industrial processes, biodispersants can be used to control biofouling. In less controlled environments, organisms are killed or repelled with coatings using biocides encapsulated with the copper nanoparticle. Nontoxic mechanical strategies that prevent organisms from attaching include choosing a material or coating with a slippery surface, creation of an ultra low fouling surface with the use of epoxy resins as adhesive, or creation of nanoscale surface topologies[6-10].

### 1.2 Barnacle as a Coating Agent

A barnacle is a type of arthropod constituting the infraclass Cirripedia in thesubphylum Crustacea, and is hence related to crabs and lobsters. Barnacles are exclusively marine, and tend to live in shallow and tidal waters, typically in erosive settings. Barnacles are encrusters, attaching themselves permanently to a hard substrate. The most common, "acorn barnacles" (Sessilia), are sessile, growing their shells directly onto the substrate. The order Pedunculata ("goose barnacles" and others) attach themselves by means of a stalk [11-15]. An encapsulated copper and its oxide nanoparticles can be defined as a community of attached micro organisms connected by an extracellular biocide coating. Barnacle undergoes multiple developmental stages from planktonic to attached cells. The coating is both an adhesive and protective layer that modulates the diffusion of molecules in the biofilm. Consequently, Barnacle cells in coating materials are more resistant to antibiotics and antibacterial agents. The use of encapsulated copper nanoparticles based antifoulings provides clear advantages to the protection of the environment. Aside from the advantages of copper antifoulings over organotin antifoulings on marine life, the ability to provide continuously fouling free surfaces means substantial fuel savings and consequently reduced emissions of green house gases. Because of copper's biocidal properties at a boat's surface, copper and copper compounds have been used for centuries for the protection of the underwater hulls of numerous types of marine craft.

Over the ages three different purposes have been served in encapsulated copper nanoparticles barnacle based marine hull protection. In early wooden vessels, a sheathing of metallic copper was used to protect the timbers from being invaded by ship worms (worms that bore into wood and weaken the structure). Simultaneously, copper sheathed boats protected from the growth of barnacles and other marine fouling species which, if present, increased the frictional resistance of the water against the hull and slowed the speed of the boat. The major thing is the barnacle encapsulated copper nanoparticles kills other fouling agents such as algae's, moulds etc. They are semiparaitic in nature because they cause drag, skin irritations, and also be annoying. Copper, in various chemical compositions, has always been one of the primary active substances used in antifouling paints to control hard fouling on the boat's surface on either commercial shipping or pleasure craft. With the development of Self Polishing Copolymer coatings, as another major active ingredient in anti fouling paints we make the encapsulated copper nano with binding agent is standard for antifoulings paints used on ship underwater hulls, as well as pilings, levee and dock structures [16-20].

In this process, chemical reaction is induced by mechanical energy. The chemical forerunners are mostly a mixture of chlorides, oxides and/or metals that react during milling or subsequent heat treatment to produce a composite powder in which ultra fine particles in a stable salt matrix are dispersed. These ultra fine particles are recovered by washing with suitable solvent from selective removal of the matrix. The present investigation reports, the novel synthesis of Copper and Copper oxide nanoparticles using Chemical reduction method. The present work to synthesize the Copper(II) salacilate, Copper(II) phthalate, Copper (II) benzoate, Copper(II) nitrobenzoate and Copper(II) cinnamate precursors from their corresponding aromatic acids and Sodium Hydroxide. To synthesize the Copper nanoparticles by reducing Copper(II) salacilate, Copper(II) phthalate, Copper(II) benzoate, Copper(II) nitro benzoate and Copper(II) cinnamate precursors using hydrazine by mechano chemical reduction method. To characterize the Copper nanoparticles with the help of analytical Instruments such as UV- VISIBLE, FT- IR, XRD analysis. To analysis the anti bacterial activity of the copper nanoparticles which is encapsulated with barnacle with both gram positive and gram negative bacteria and fugal also. To investigate the effect of encapsulated copper nanoparticles with Barnacle as a antifouling agent [21]. The green chemistry approach used in the present work for the synthesis of nanoparticles is simple, cost effective and the resultant nanoparticles are highly stable and reproducible. This method was developed to synthesize nanoparticles by reducing Copper precursor with hydrazine without using any solvent.

## II. MATERIALS AND METHOD

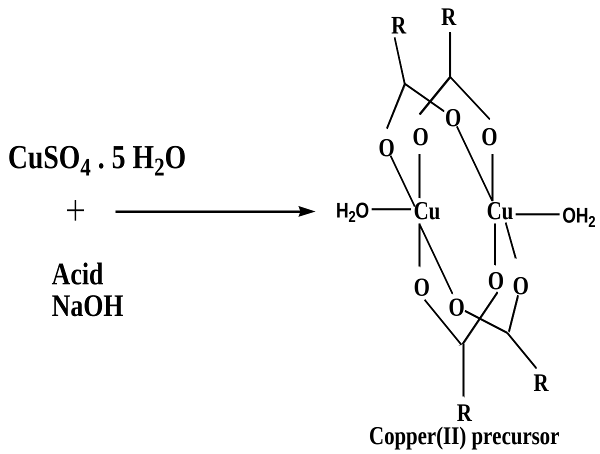
Copper Sulphate, Sodium Hydroxide, Cinnamic Acid, Pthalaic Acid, Benzoic Acid, Nitro Benzoic Acid, Salicylic Acid, Hydrazine, Barnacle (3-Aminipropyl) trimethoxy silane (APTMS), Ethanol in sigma aldrich.

**2.1 Mechanochemical Synthesis / Green Chemical Reduction Method**

In this process, chemical reaction is induced by mechanical energy. The chemical precursors such as Copper(II) Salacilate, Phthalate, Benzoate, Nitro Benzoate and Cinnamate were produce as a composite powder in which they act as an ultra fine particle. The present investigation reports, the novel synthesis of encapsulated barnacle with Copper and Copper oxide nanoparticles using Chemical reduction method [22].

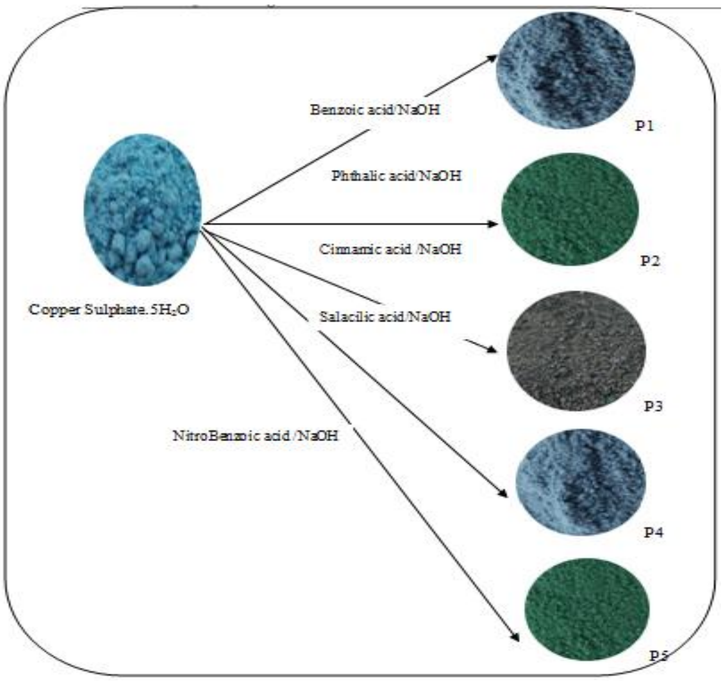
**2.2 Synthesis of Copper(II) benzoate Precursor**

6 gms of Benzoic acid (20mmol) and 2 gms of Sodium Hydroxide (20mmol) is mixed in a mortar pestle and grinded it for fifteen minutes and the mixture is colorless. Then add Copper Sulphate Penta Hydrate $CuSO_4.5H_2O$ (8 gms, 20mmol) which is blue in color in to the mixture and grind it for 65 minutes. The dark blue color mixture was changed into pale blue, this mixture is washed with the help of ethanol and filtered and dried. The Same Procedure follows other organic acids [23].




**Scheme 1**: Schematic diagram of the formation of Copper(II) Precursors.

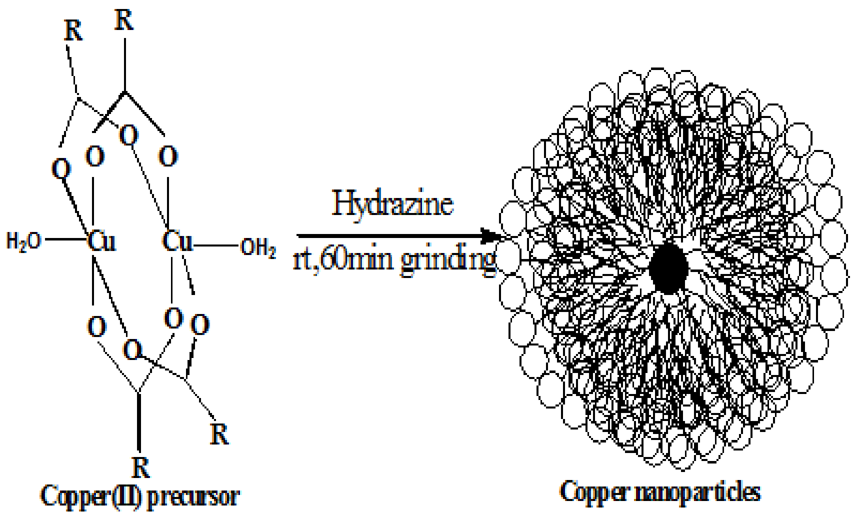
R – Benzoic acid, Phthalic acid, Cinnamic acid, Succinic acid and Nitro benzoic acid
P1 - Copper(II) benzoate, P2 - Copper(II) phthalate, P3 - Copper(II) cinnamate, P4 – Copper(II) Salacilate, P5 – Copper(II) nitro benzoate.




**Scheme 2:** Photographic picture of the formation of Copper(II) Precursors and encapsulated barnacle with Copper & its oxide Nanoparticles.

**2.4. Synthesis of encapsulated barnacle with Copper Nanoparticles from Copper (II) benzoate Precursor**

The blue color of Copper(II) benzoate is taken and it is reduced with the help of Hydrazine by grinding it in a mortal pestle for an hour. The pale blue color was gradually changed in to a black color powder and then 2 gram of finely grinded barnacle was mixed along with the 4 ml of (3-Aminipropyl) trimethoxy silane (APTMS) which act as a binding agent. The mixture was grinded on the constant speed for forty five minutes in the ambient temperature. The resultant mixture obtained was barnacle encapsulated Copper and its oxide Nanoparticles.




R – Benzoic acid, Phthalic acid, Cinnamic acid, Salicylic acid and Nitro benzoic acid

**Scheme 3:** Schematic diagram of the formation of encapsulated barnacle with Copper and its oxide Nanoparticles.

**2.5 Synthesis of encapsulated barnacle with Copper Nanoparticles from Copper(II) phthalate Precursor**

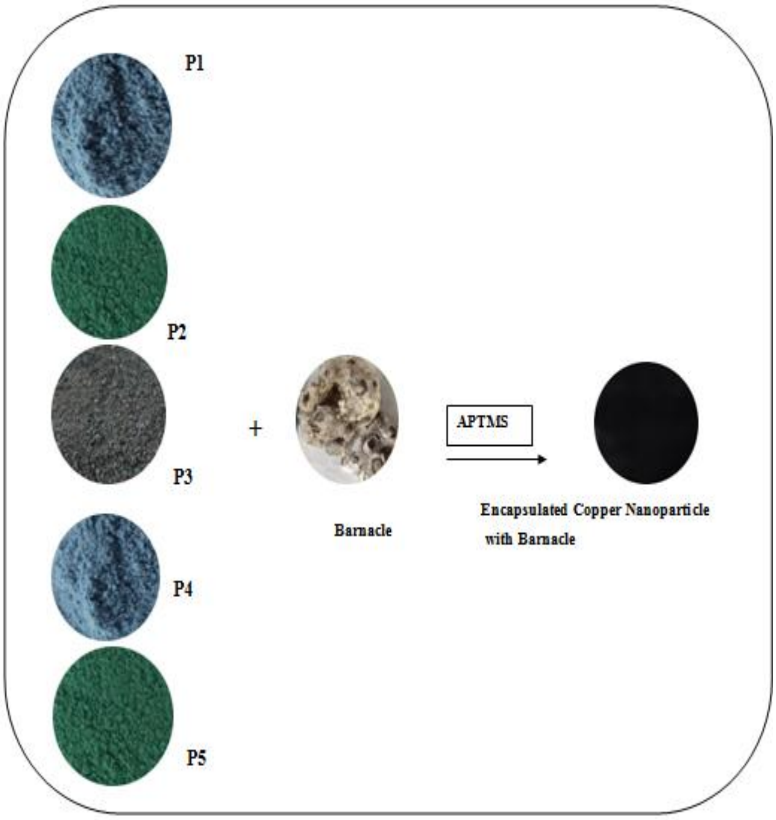
The dark green color of Copper(II) phthalate is taken and it is reduced with the help of Hydrazine by grinding it in a mortal pestle for an hour. The dark green color was gradually changed in to a black color powder and then 1 gram of finely grinded barnacle was mixed along with the 2 ml of (3-Aminipropyl) trimethoxy silane (APTMS) which act as a binding agent. The mixture was grinded on the constant speed for forty five minutes in the ambient temperature. The resultant mixture obtained was barnacle encapsulated Copper and its oxide Nanoparticles.

**2.6 Synthesis of encapsulated barnacle with Copper and its oxide Nanoparticles from Copper(II) salicylate Precursor**

The dark gray color of Copper(II) Salicylate is taken and it is reduced with the help of Hydrazine by grinding it in a mortal pestle for an hour. The dark gray color was gradually changed in to a black color powder and then 1 gram of finely grinded barnacle was mixed along with the 2 ml of (3-Aminipropyl) trimethoxy silane (APTMS) which act as a binding agent. The mixture was grinded on the constant speed for forty five minutes in the ambient temperature. The resultant mixture obtained was barnacle encapsulated Copper and its oxide Nanoparticles.

**2.7 Synthesis of encapsulated barnacle with Copper and its oxide Nanoparticles from Copper(II) nitro benzoate Precursor**

The pale green color of Copper(II) nitro benzoate is taken and it is reduced with the help of Hydrazine by grinding it in a mortal pestle for an hour. The pale green color was gradually changed in to a black color powder and then 1 gram of finely grinded barnacle was mixed along with the 2 ml of (3-Aminipropyl) trimethoxy silane (APTMS) which act as a binding agent. The mixture was grinded on the constant speed for forty five minutes in the ambient temperature. The resultant mixture obtained was barnacle encapsulated copper nanoparticles.




## III. RESULT AND DISCUSSION

### 3.1 UV – Visible Spectroscopy Analysis

Figure 1 shows a) & (b) UV visible spectra of Copper(II) Precursors and (c) CuONPs UV – Visible absorption spectroscopy is a useful technique to monitor the size dependent optical properties of the nanomaterials, due to the quantum confinement of the photo generated electron – hole carriers in the particles. The below figure shows the UV- visible spectra of various precursors were recorded in Methanol solution in the wavelength range 200-800nm. The given table 1shows the values.

**Table -1** Shows UV visible spectrum of Copper(II) Precursors and Copper and its oxide nanoparticles.

| Compound | Charge transfer |
|---|---|
| P1 | 235nm,735 nm |
| P2 | 225nm,780nm |
| P3 | 720 nm |
| P4 | 385 nm ,710 nm |
| P5 | 225 nm,730 nm |

| Compound | Nanoparticles |
|---|---|
| N1 | 430nm |
| N2 | 590nm |
| N3 | 489nm |
| N4 | 510nm |
| N5 | 595nm |

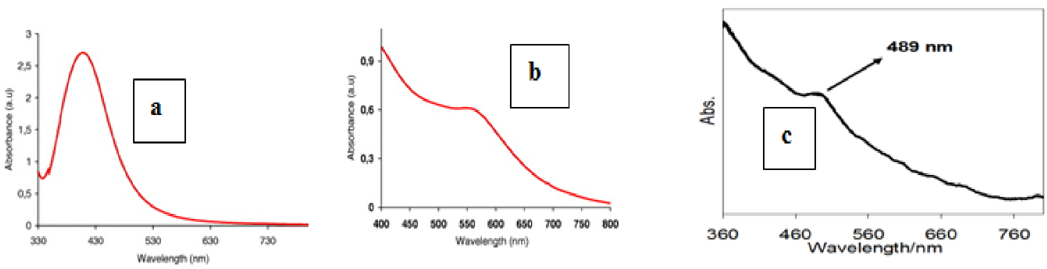



**Figure 1. (a) & (b)** UV visible spectra of Copper(II) Precursors and (c) CuONPs.

### 3.2 IR –Spectra Analysis

Figure 2 shows FT IR spectroscopy is a useful tool to understand the functional group of any organic molecule. The effect of digestion of particles in aqueous dispersion of reducing agent on particle size is described in Table 3 which shows that more concentration of reducing agent reduces the size of the barnacle encapsulated CuO nanoparticles [24].

**Table -2** shows FT IR spectrum of Copper(II) Precursors and CuONPs.

| Compound | O-H | C-H | C=0 | C-H | $C_6H_6$ | C-O | Cu-O |
|---|---|---|---|---|---|---|---|
| P1 | 3446 | 2900 | 1640 | 1447 | 3061.53 | 1140 | 540,692 |
| P2 | 3464 | 3066 | 1612 | 1443 | 3066.78 | 1144 | 638,575,719 |
| P3 | 3400 | 3000 | 1668 | 1450 | 3100.10 | 1100 | 540,582,640 |
| P4 | 3359 | 2928 | 1639 | 1450 | 3083.02 | 1089 | 585,528 |
| P5 | 3394 | 2934 | 1607 | 1406 | 3135.11 | 1119 | 532,617 |
| N1 | - | - | - | - | 2956.68 | - | 669,518 |
| N2 | - | - | - | - | 2924.23 | - | 586,527 |
| N3 | - | - | - | - | 2922.26 | - | 585,538 |
| N4 | - | - | - | - | 2925.86 | - | 588,694 |
| N5 | - | - | - | - | 2930.31 | - | 575,694 |

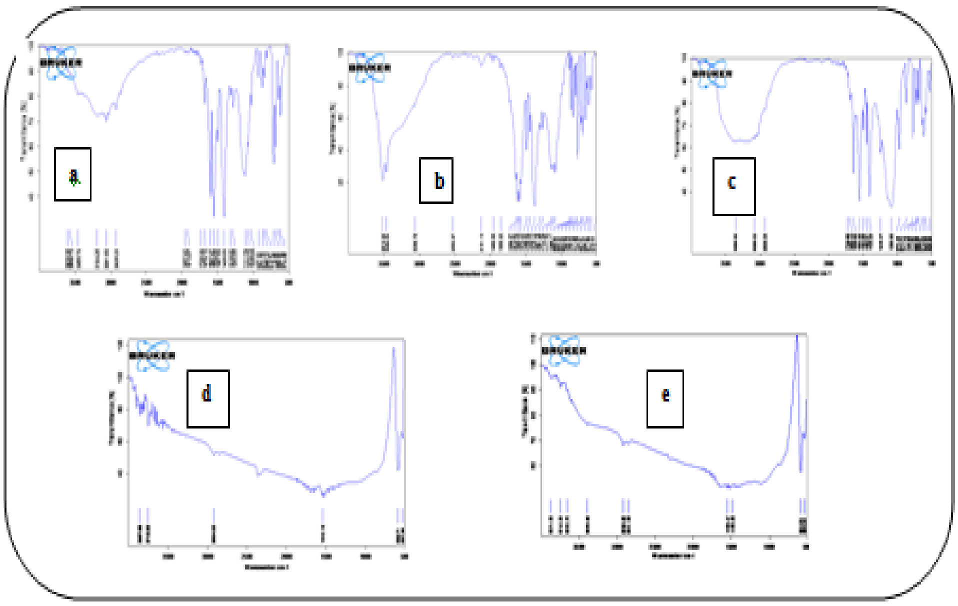



**Figure 2.(a), (b) & (c)** FT IR spectrum of Copper(II) Precursors and (d) & (e) CuONPs.

### 1.3 XRD – Analysis

XRD analysis of the prepared sample of Copper nanopowder was done by a Goniometer (Ultima 3 theta-theta gonio, under 40kV/30mA - X-Ray, 2θ / θ – Scanning mode, Fixed Monochromator). Data was taken for the 2θ range of 10 to 80 degrees with a step of 0.02 degree. Indexing process of powder diffraction pattern was done and Miller Indices (h k l) to each peak was assigned in first step [25]. Diffractogram of the entire data is in below Figure 3. The structure and chemical composition of all copper nanoparticles samples synthesized in this work are confirmed with XRD. A typical XRD pattern of all copper nanoparticles samples is shown in below figure and Table 3. The Copper nanoparticles synthesized got oxidized in due course. The XRD pattern is consistent with the spectrum of barnacle encapsulated copper and its oxide nanoparticles. The average particle size obtained from the XRD data of nanoparticles was around 22-55 nm.

**Table 3 :** Particle size of Copper nanoparticles

| S. No. | CuO Nano | D Size (nm) |
|---|---|---|
| 1 | N1 | 22.43 |
| 2 | N2 | 36.65 |
| 3 | N3 | 55.07 |
| 4 | N4 | 21.95 |
| 5 | N5 | 27.43 |

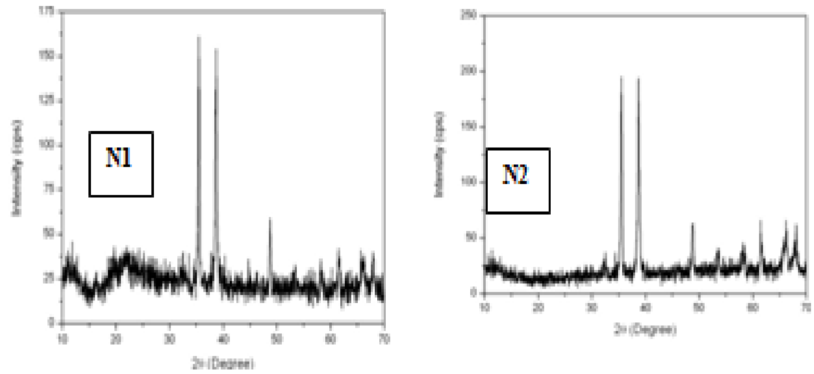



**Figure 3**. N1 & N2 XRD pattern of the barnacle encapsulated CuONPs.

### 1.4 Anti Fouling Activity

The coatings formulated have been characterized in terms of common coatings and immersed in sea water. The iron plates which are coated by encapsulated with barnacle are less damaging to the environment. These antifouling properties have been exhausted. This has a strong degree of protection against most fouling materials.

| S.No. | Test organisms | P2 | N2 | P3 | N3 | P4 | N4 | P5 | N5 | S | D |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1. | Staphylococcus aureus | 12 | 22 | 8 | 24 | 9 | 25 | 5 | 28 | - | 25 |
| 2. | Escherichia coli | 8 | 24 | - | 28 | 6 | 25 | 9 | 20 | - | 10 |
| 3. | Bacillus subtilis | 10 | 22 | 7 | 23 | 6 | 24 | 7 | 21 | - | 19 |

**Figure 4.** Antifouling activity of the barnacle encapsulated CuONPs.

**3.5. Antimicrobial Activity**

Thirty Six gram of Muller Hindon Media (Hi-Media) was mixed with distilled water and then sterilized in autoclave for 15 minutes. The sterilized media were poured intopetridishes. The solidified plates were bored with 6mm diameter cork porer. The plates with wells were used for the antibacterial studies. The stored culture of Escherichia coli, Staphylococcus aureus, Streptococcus pyogenes and Pseudomonas aeruginosa were collected from the Microbial Type Culture Collection (MTCC), The Institute of microbial Technology. Sector 39-4, Chandigarh, India. The pathogenic fungal strains Aspergillus niger, Aspergillus flavus**,** Rhizopus Sp. and Mucor indicus were collected from the Microbiological Lab, Christian Medical College, Vellore, TamilNadu, India.

The anti bacterial effect of Copper(II) precursor and CuONPs with encapsulated barnacle is Table 4 & Figure shown 5. All the synthesized precursors (P2–P5) and their corresponding nanoparticles (N2-N5) were screened in vitro for their antibacterial activity using the agar well diffusion method. Other wells supplemented with DMSO and reference ofloxacin is antibacterial drug. The plates were incubated immediately at 378°C for 24 hrs. Activity was determined by measuring the diameter of zones showing complete inhibition (mm). Growth inhibition was compared with ofloxacin is the standard drug. In order to clarify any participating role of DMSO in the biological screening, separate studies were carried out with the solutions alone of DMSO and they showed no activity against any bacterial/fungal strains.

**Table 4.** In vitro antimicrobial activity of some human pathogenic bacteria on nanoparticles by disc diffusion assay

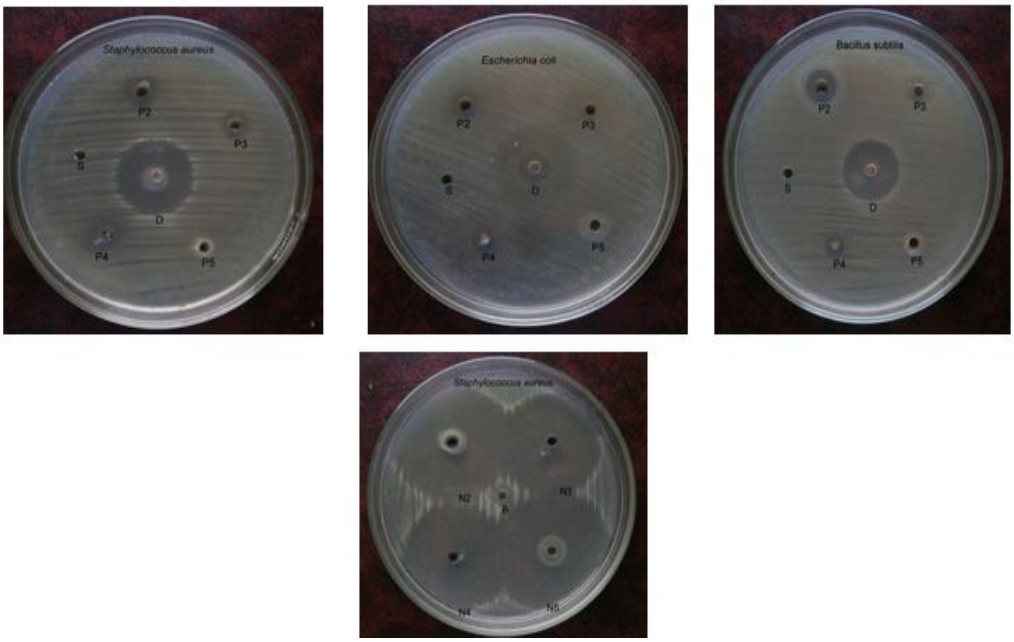



**Figure 5.** Antibacterial test (disc method): (a) Staphylococcus aureus, (b) Escherichia coli (1) DMSO S (2) Std Drug (D), (3) Copper(II) Precursor, (4) CuONPs**.**

## IV. SUMMARY

Encapsulated barnacle with copper nanoparticles was synthesized with the help of hydrazine as reducing agent is a simple technique for preparing material with uniform sized nanoparticle. In this study, copper nanoparticles have been synthesized by green mechono chemical method. The spectra taken by the different types conformed the nanoparticles formation. The IR shows reduced peaks which indicates copper nanopartcles with encapsulated with barnacle. The XRD data's clearly showed the formation of Cu, $Cu_2O$ and CuO phases and grown size. Average size of the nano formed is 22-55 nm. From the antimicrobial activity which is tested represents that the copper nanoparticle encapsulated barnacle shows better antibacterial effect on Bacillus subtilis and antifungal effect on Aspergillus flavus. The antifoulng effect of the barnacle encapsulated copper nanoparticle play a major role in the marine biofouling which act as a good antifouling agent.

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