

Evaluation of Antibacterial and Photocatalytic Activity of Bimetallic Nanocomposite Obtained From Leave Extract of Bryophyllum Pinnatum and Bougainvillea

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Abstract:

As Nanotechnology has emerged as a practical solution to cure many diseases as well as to purify the contaminated water. So use of green materials has replaced the harmful chemicals for the fabrication methods of nanocomposites. This article is designed to elaborate the effectiveness of nanocomposite applications by combining leave extracts of two different plants viz. Bryophyllum pinnatum and Bougainvillea. The bimetallic salt combination was prepared by using Ferric Nitrate and Cerium Nitrate (FCN). Finally leave extract and bi-metallic salts were combined in appropriate ratio to obtain the desired nanocomposite (BB-FCN). FESEM and HRTEM described the attainment range of nanocomposite between 20-40 nm. FTIR spectra identified type of functional groups in the leave phytochemicals and their transformation into nanocomposite in the form of displacement of peak positions. BET disclosed the pore diameter between 2-10 nm and elaborates the ability of nanocomposite to be used as photocatalytic agent with microporous nature. XRD spectra prove the transformation of amorphous leave extract into crystalline by appearance of sharp peaks in nanocomposites. XPS graph indicate presence of peaks of expected atoms at appropriate positions. The synthesized nanocomposite was found to show excellent antibacterial as well as photocatalytic activity.

Keywords: Nanocomposite; Characterization; Antibacterial activity; Photocatalytic activity

Introduction:

Nanomaterials, including nanoparticles, nanotubes, and nanocomposites are ideal for adsorbing and removing dangerous heavy metals from water, such as lead, cadmium, mercury and arsenic due to their unique physicochemical properties that include high reactivity, a large surface area and tunable surface functionalities [1]. The use of nanotechnology in water treatment allows for rapid, affordable, and

environmentally friendly methods that often outperform conventional filtration and chemical processes in terms of removal efficiency. Moreover, magnetic nanoparticles have the advantage of being easily recovered and repurposed. By integrating nanotechnology with current treatment techniques, water contamination may be addressed and safe, clean drinking water can be guaranteed [2]. In the meantime, heavy metal pollution in particular has become a major global environmental concern. Heavy metals, such as Pb, Zn, Cu, Hg, and others, can naturally build up in the food chain and pose a serious threat to human health. Most heavy metals are considered to be very toxic and carcinogenic. Heavy metal removal from water is therefore important and has drawn a lot of interest [3-4]. To date, a number of techniques have been developed to deal with this problem, including chemical precipitation, ion exchange, adsorption, membrane filtration, electrochemical treatment and others [5-9]. Moreover, it is often the case that many approaches are combined to improve the removal result [10-11]. Among the techniques discussed above, adsorption is one of the most popular since it is low-cost and simple to implement. In recent decades, nanomaterials have attracted a lot of attention. Numerous industries, including energy, electronics, and healthcare, have made use of nanoparticles. In recent decades, the application of nanoparticles in environmental protection has increasingly increased [12-15]. Materials with a microscopic internal structure or surface or whose external dimensions are in the nanoscale (usually 1–100 nm) are commonly referred to as nanomaterials [16]. At the nanoscale, nanomaterials often exhibit distinctive properties such as a surface effect, tiny size effect, quantum effect, and large quantum tunnel effect [17]. Although chemically produced NPs have recently been suggested as an effective leishmaniasis treatment, their toxicity to macrophages has restricted their use [18–21]. However, because they are biocompatible, NPs made using green chemistry have gained importance as substitutes. It was discovered that biogenic silver nanoparticles (AgNPs) were more effective against *Leishmania amazonensis* promastigotes than chemically synthesised AgNPs. Reports in recent years have demonstrated the potential of nanostructured materials, also known as nanomaterials, in applications related to health and well-being, water treatment and sanitation, and responsible resource production and consumption [22].

The current research explain the modification of leave extract of two plants i.e. **Bryophyllum pinnatum and Bougainvillea (BB)** into respective nanocomposite (**BB-FCN**) by using mixture of two salts i.e. Ferric Nitrate and Cerium Nitrate (**FCN**). The so obtained nanocomposites were identified by various analytical and spectral instruments and further applied for photocatalysis and antimicrobial activity.

Material and Methodology:

The used chemicals were purchased from sigma aldrich and were of analytical grade. All the chemicals were utilized as such without further purification.

1.1.Synthesis of combination leave extract of Bryophyllum pinnatum and Bougainvillea (BB):

Fresh leaves of *Bryophyllum pinnatum* and *Bougainvillea* were collected from the nearby regions of District Solan, Himachal Pradesh, India. The leaves of both plants were cut into small pieces by using sharp knife. The fragments were dried in shade. The dried material was ground into fine particles using a laboratory grinder. Then 200 ml of water and 10 g of the mixture of finely divided powder of both leaves was heated in a 500 mL flat bottom flask. The heating and stirring was continued for 2 hour by maintaining temperature 25°C. The aqueous leave extract was filtered using filter paper, put into an amber bottle, and stored in the refrigerator at 4° C for further use.

1.2.Synthesis of bi-metallic nanoparticles by using Ferric Nitrate and Cerium Nitrate (FCN):

25 ml of 0.1 M solution of Ferric Nitrate and 0.1 M solution of cerium nitrate each were dissolved and

subjected for continuous stirring for two hr. Few drops of 10 % NaOH were added in the same solution to maintain basic pH with stirring. Appearance of precipitates indicates the adjustment of pH adjustment indicating formation of bi-metallic nanoparticles (FCN).

1.3.Synthesis of bi-metallic leave based nanoparticles (BB-FCN):

Metal based nanoparticles were produced using the biological synthesis approach in a 250 ml beaker. 10 mL of (BB) and 50 mL of a 0.1 M solution of Ferric Nitrate and 0.1 M solution of cerium nitrate (FCN) was added to the beaker in 1:1 ratio, which was agitated for two to two hours at 60°. 10% NaOH solution was also added dropwise in the beaker till the appearance of precipitates. After that, the solution was left to settle down. Initial recognition of formation of metal based nanocomposite was done on the basis of color change of the solution. The synthesized nanocomposite (BB-FCN) was filtered and dried at room temperature.

Characterization Techniques:

The energy-dispersive X-ray spectroscopy (EDX, BRUKER,) was used to analyze the distribution, elemental composition and purity within the nanocomposite. The morphology was then evaluated using a high-resolution transmission electron microscopy model (HR-TEM, JEM2100 plus, Jeol). The functional groups in synthesized (BB), (FCN) and (BB-FCN) were initially identified by using FTIR, PerkinElmer® Spectrum™ 400 FT-IR/NIR spectrometer, to compare the characteristics features. Scanning electron microscopy (SEM, Hitachi SU 8010 Series) was deployed to identify the surface morphology and particle boundaries. The specific surface area and distribution of pore size of the nanocomposite were measured using BET (Quantachrome Autosorb-iQ-MP/XR), and crystallinity was assessed using X-ray diffraction (XRD) analysis (X'Pert Pro XRD models).

Results and Discussions:

FTIR: This spectra provide information regarding variety of functional groups present in all three samples (**Fig. 1**). In this spectrum (**a**) describe the abundance of polyphenols, proteins, flavonoids, tri-terpenoids and tannins etc. present in the form of natural phytochemicals in leave extract (BB). All these organic molecules can be identified in the form of peaks at appropriate positions for particular functional group [23]. Such as broad and strong peak at 3346 cm^{-1} represent the hydroxyl group in combination with N-H stretching of amine or amide overlapping. Similarly peaks at 1742 cm^{-1} , 1565 cm^{-1} , 1402 cm^{-1} and 1029 cm^{-1} corresponds to availability of C=O, C=C, C-H and C-O groups of phytochemicals. On the other hand, Spectrum (**b**) investigate the spectrum of hydrated Ferric Nitrate and Cerium Nitrate (FCN) where intense broad peak at 3351 cm^{-1} represent the -OH stretching present in metal salts as they are used in hydrated form. Peaks at 1630 cm^{-1} , 1302 cm^{-1} and 835 cm^{-1} signify the H-O-H scissor bending, NO- asymmetric stretch and out of plane bending [24]. Comparison of spectrum (**a**) and (**b**) with (**c**) proves efficient formation of metal based nanocomposite (BB-FCN) by transferring the -OH shift at 3319 cm^{-1} along with other peaks for carbonyl group and C-O group at 1544 cm^{-1} and 1050 cm^{-1} . Generation of extra peaks in this spectrum at 678 cm^{-1} and 431 cm^{-1} again represent lattice vibrations of metal-oxygen confirming Ce-O, Fe-O and Fe-O-Ce bridging structures [25]. So FTIR spectra elaborate the difference between organic, inorganic and nanocomposite frameworks on the basis of functional groups.

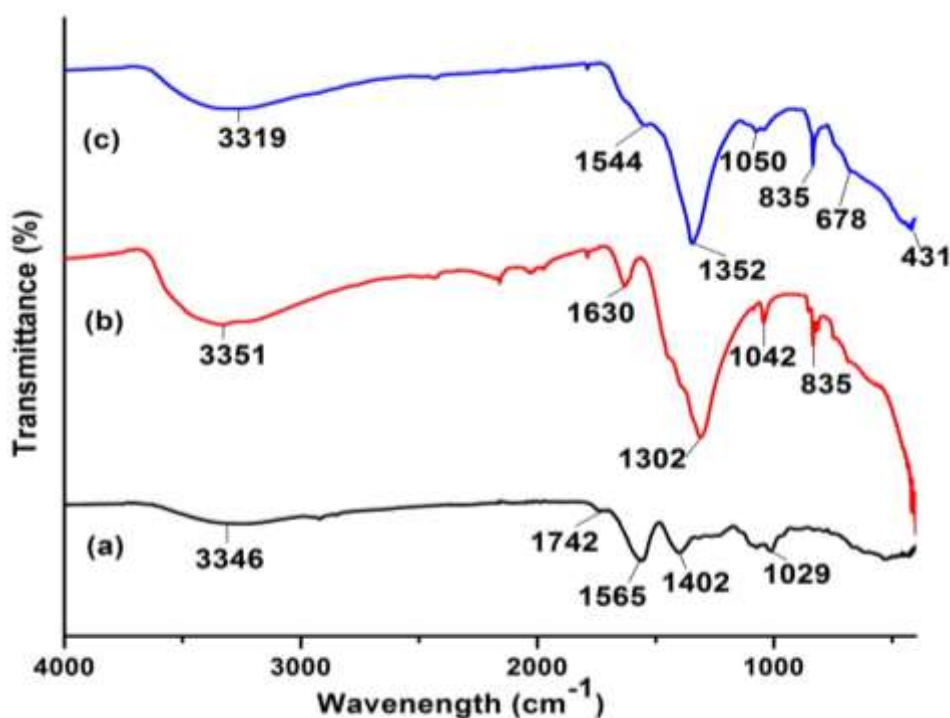


Fig.1: FTIR spectra showing peaks a) BB, b) FCN and c) BB-FCN

FESEM: Field Emission Scanning Electron Microscopy (FESEM) is utilized to monitor the transformation of leave extract (BB) in the nanocomposite (**BB-FCN**) by encapsulation of (**FCN**) Fe and Ce bi-metallic matrix (**Fig. 2**). A dense, quasi-smooth and continuous background suggested nanoporous surface (**a**) corresponding to carbonaceous dried phytochemicals [26]. Image (**b**) is displaying polygonal, brittle-edged platelet and sharply defined crystalline arrangement of bimetallic salts showing step boundaries with sharp edges confirming unreacted salt domains prior to reduction treatment [27]. Image (**c**) and (**d**) is showing the morphology of (**BB-FCN**) at different magnification i.e. encapsulation of Fe and Ce into the core of phytochemicals present in the leave extract. The rod like structure is shown in image at 200 nm magnification demonstrating the extended secondary nano-clusters. Anisotropic assembly is indicated by columnar backbone by the organic scaffolds of plant extract [28]. The image at 100 nm magnification demonstrate layered, striated and stepped composition of nanocomposite bearing nanoparticles with a range 15-40 nm due to Fe/Ce oxide spherical with densely uniform texture.

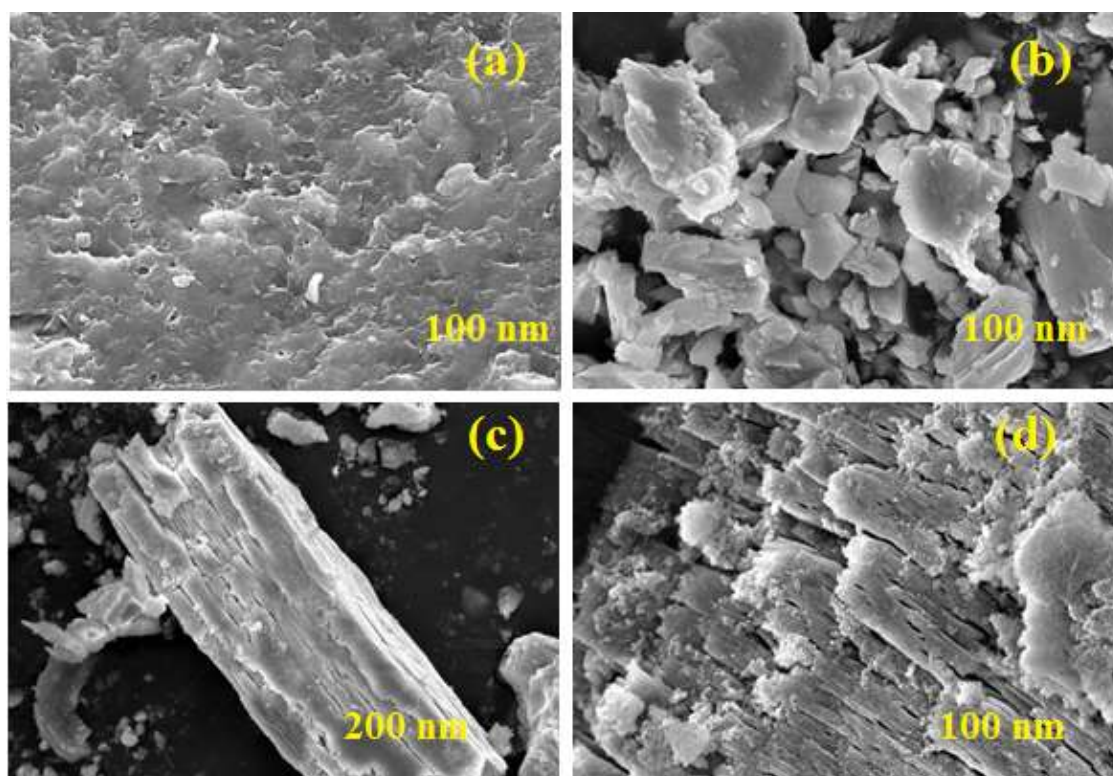


Fig. 2: FESEM images of a) BB, b) FCN and c) and d) BB-FCN

HRTEM: HRTEM monograph (Fig. 3) demonstrated agglomeration behavior, morphology, distribution and range of particle size for the fabricated biogenic Fe/Ce nanocomposite (**BB-FCN**) at different magnification [29].

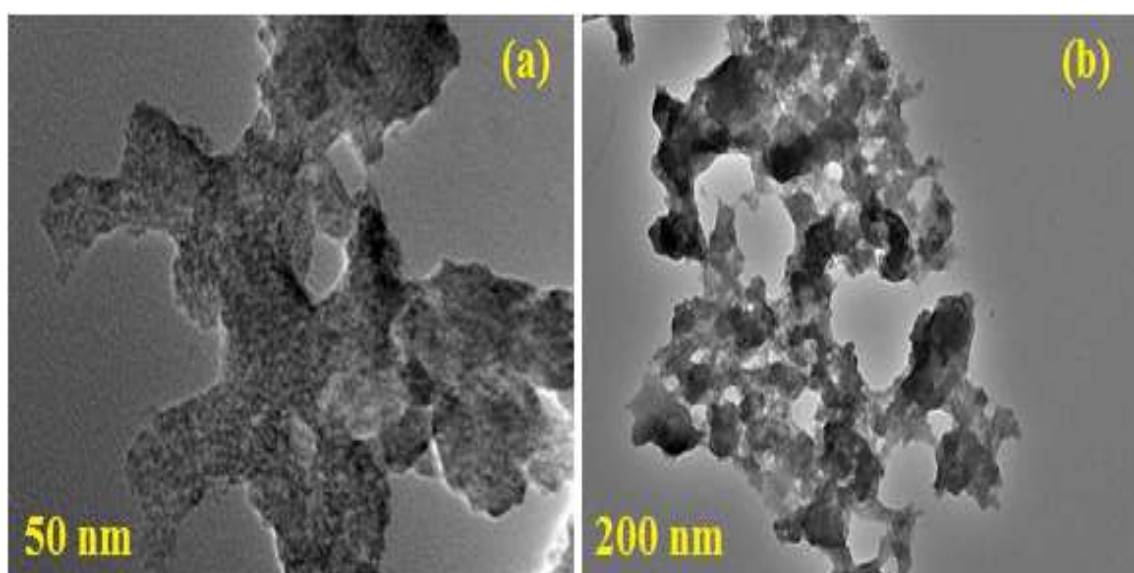


Fig.3: HRTEM images of a) and b) BB-FCN at different magnifications

The image at 200 nm describe interconnected network, branched and porous nano-clusters with quasi-spherical, dense, -three-dimensional chain-like agglomerates. This behaviour is generally driven by more energy-surface to volume ratio and dipolar or Magnetic interactions linked with iron. Image at higher magnification (a) describe more resolved nanocomposite exhibiting grain size between 10-40 nm [30].

The metal oxide is observed as dark and electron rich core containing amorphous, lighter-contrast organic material within. This matrix corresponds to efficient and active stabilizing and capping phytochemical agents recovered from the leave extract [31]. The presence of interfacial organic layer defends unwanted bulk collection and enhances the fabrication of macrospores which is found to necessary to facilitate transportation of mass in photocatalysis.

XRD: (Fig. 4) describes the XRD diffractogram of **BB**, **FCN** and corresponding nanocomposite (**BB-FCN**). The raw leave extract powder diffractogram (a) is identified with lack of discrete, sharp Bragg reflections and showing a poorly defined broad hump, characterizing organic matrix (**BB**) as amorphous [32].

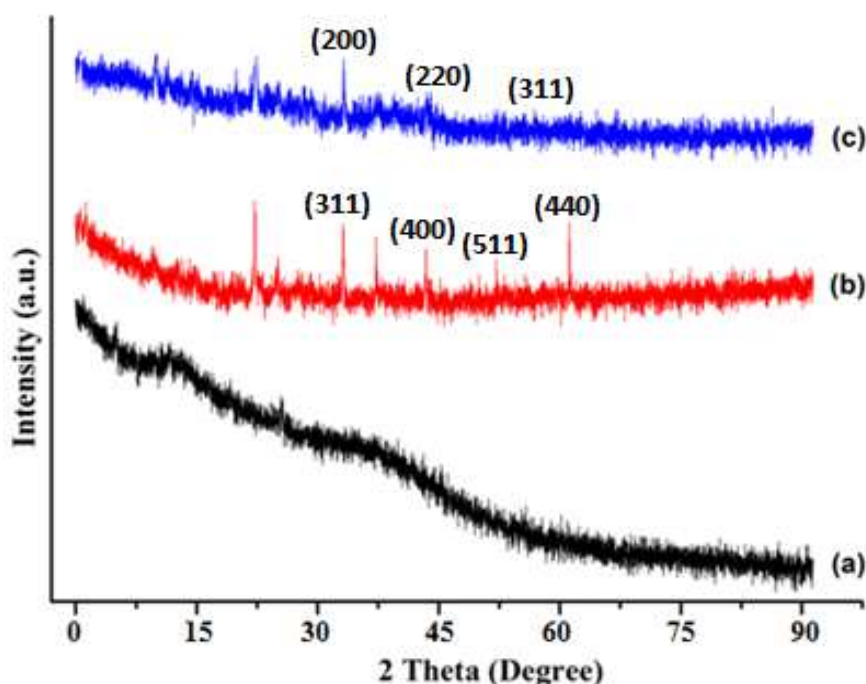


Fig.4: XRD plots of a) BB, b) FCN and c) BB-FCN

The reason might be lacking of long range order of atoms due to phytochemicals present in extract. Reversibly (b) confirm crystalline behaviour of (FCN) in the form of sharp and distinct superimposed peaks. Well organized crystalline lattice confirm pristine bimetallic composite fabricated from ferric and cerium nitrate. Peaks at $2\theta = 35.7^\circ$, 30.2° , 43.3° and 63.2° corresponds to crystal planes (311), (400), (511) and (440) represent the γ -magnetite showing cubic spinel ferric oxide [33]. The structural modification of nanocomposite (BB-FCN) is visible in pattern (c) where slight suppression of peaks in comparison to (FCN) implies the occurrence of chemical reaction between leave extract and metal salts where phytochemicals may act as capping, complexing and reducing agents. Crystal planes (200), (220) and (311) here related to peak at 33.1° , 47.5° and 56.3° represent nanocrystalline domain of cerium oxide. The transformations of amorphous to slightly crystalline nanocomposite prove the variation in core structure.

XPS: The investigation of oxidation states and elemental composition of the fabricated nanocomposite (BB-FCN) has been done by X-ray photoelectron spectroscopic method. (Fig. 5) elaborate distinct photoelectron signals for C, O, Fe, Ce and Na atoms and hence confirm their association to form nanocomposite [34].

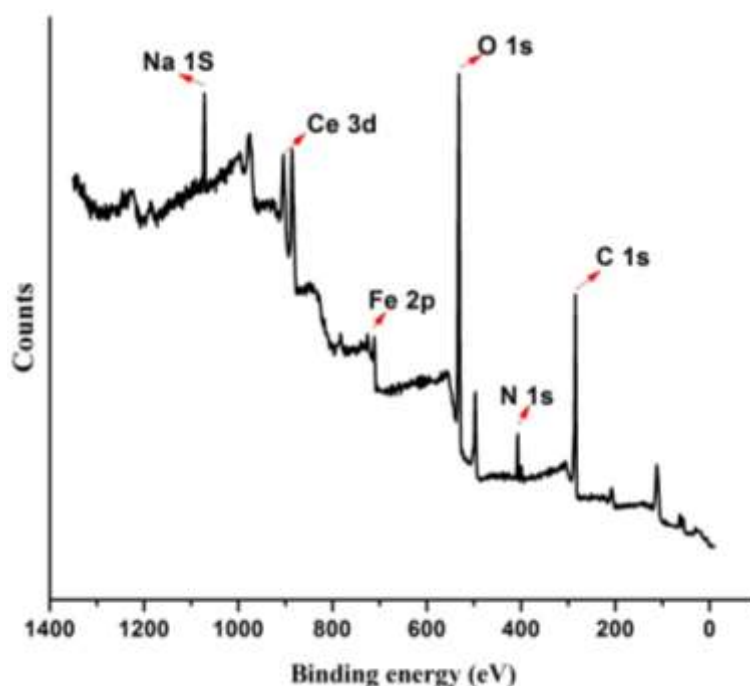


Fig.5: XPS graph showing peaks a) BB, b) FCN and c) BB-FCN

Highest purity of the resulted compound has also been verified as no extra peak for other metal is found. Intense sharp peak at 284.8 eV suggest the presence of C 1s, multiplet peaks at 880-920 e V corresponds to cerium oxide as Ce 3d while doublet around 711 eV suggest the introduction of Fe 2p atom. Moreover, peak at 400 e V is related to nitrogen 1s which might be present in the phytochemicals of leave extract as a part of pyridinic, pyrrolic graphitic-like nitrogen [35]. Oxygen in the form of O 1s is observed at 531 eV which is attributed to bind with cerium and iron to form metal-oxygen bond. The mixed valences of transition metals are confirmed in the form of Ce 3d and Fe 2p regions which play important role to strengthen the physiochemical properties of the material. All these signals for particular atoms at specific electron volt confirm indeed formation of nanocomposite.

BET: The net pore volume vs pore size graph is evaluated by using BJH method obtained from adsorption and desorption isotherm (**Fig. 6**). Total pore volume exhibited by (**BB-FCN**) is approximately 0.0225 cm³/g [36]. A sharp and vertical rise at the beginning of adsorption-desorption graph suggest the localization of pore volume within mesoporous or microporous range. But on increment in pore diameter from 20 to 240 nm, flat curve appear indicating minimum contribution of mesoporous and macroporous voids to total porosity of the nanocomposite [37]. The maximum adsorption of nitrogen gas is found at around 10 nm of pore diameter confirming mesoporous nature as it lies between 2-50 nm range which is considered as excellent range for photocatalysis and adsorption etc.

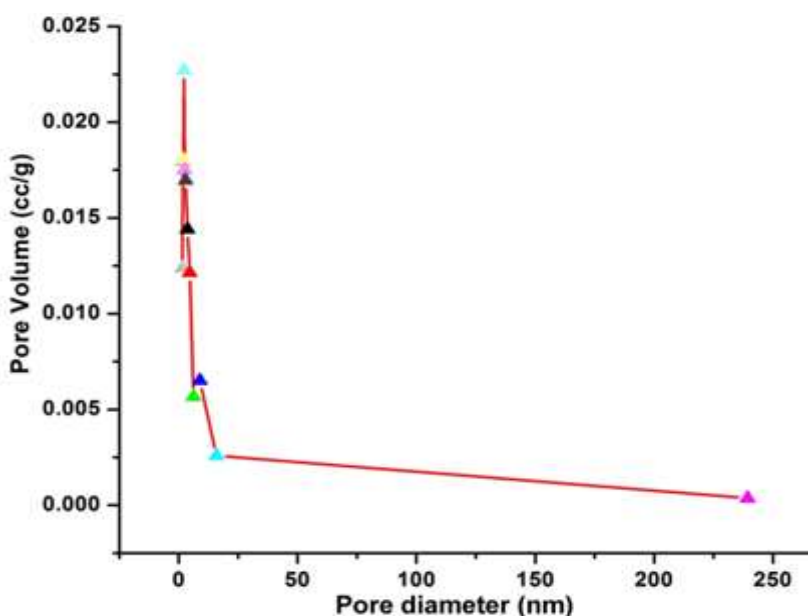


Fig. 6: BET image of BB-FCN showing pore diameter

Photocatalytic Activity

The tacq plots of BB-FCN and FCN nanocomposites for the calculations of band gap were shown in the (Fig. 7). Lower band gap value for BB-FCN nanocopmosite is due to addition extra level in photoexcitation difference between VB and CB resulting in need of less energy to reach CB of the nanocopmosite.

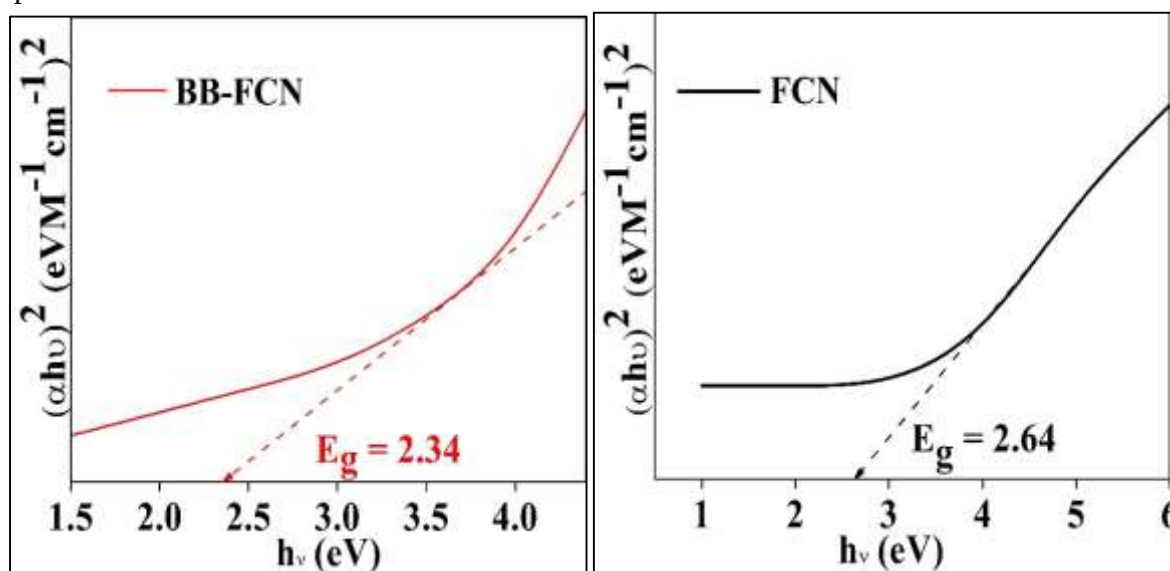


Fig. 7: Tacq plots of BB-FCN and FCN molecules.

The photocatalyst shows great photocatalytic activity against model pollutant dye congo red degradation owing to its favorable band structure and visible light active band gap energy. The detailed band structure of the composite is shown in (Fig.8). The photocatalytic action starts with the absorption of sunlight by the composite [38]. Initially, phytochemicals of the plant extract gain energy from sunlight and electrons from its HOMO (1.4 eV) get excited to LUMO (-0.9 eV). These excited electrons get shifted to CB of oxides of cerium (-0.2 eV) which further shifted to CB of oxides of Ferric (0.2 eV) by loosing excitation energy [39]. At the same time, the holes formed at valence bands and LUMO levels of

the composite (2.8 eV for oxides of cerium, 2.3 eV for oxides of Iron and 1.4 eV for LUMO of phytochemicals) get shifted to less positive value among them i.e. LUMO of phytochemicals (1.4 eV). This transfer of excited electrons and holes leads to formation of electric interfaces and heterojunctions. This prevents the process of recombining back of excited electrons and holes, which result in improve rates of photodegradation process.

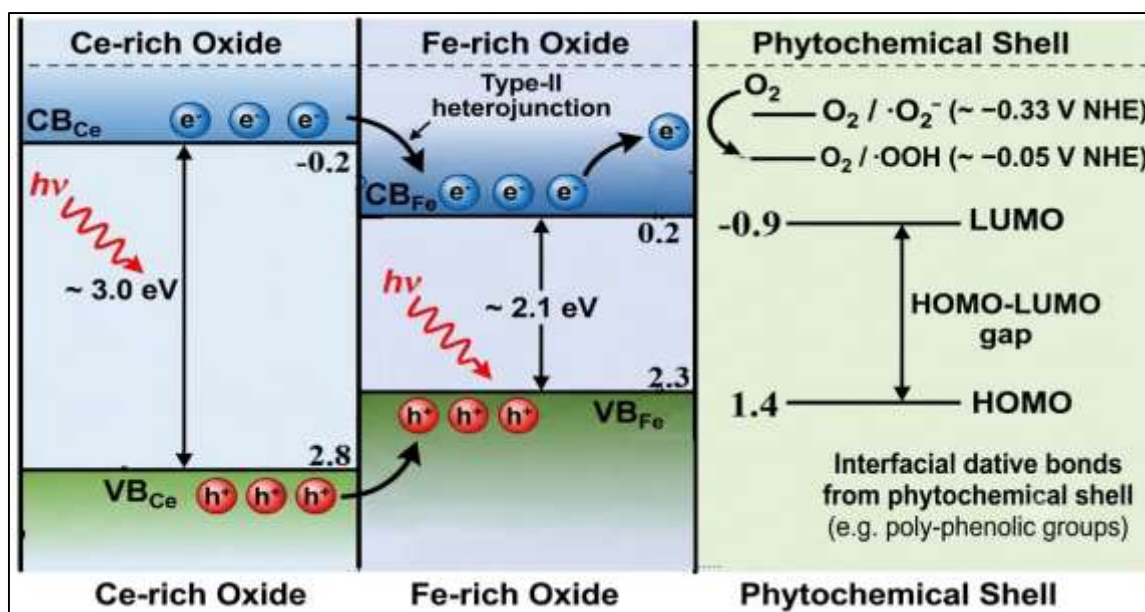
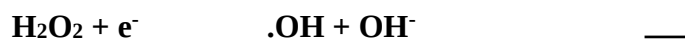


Fig. 8: Band gap structure of BB-FCN nanocomposite

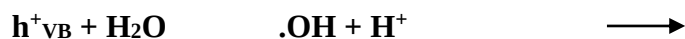
The process of generation excited electrons-holes pairs is continuous and leads to formation of reactive oxygen species (ROS) in due course of reaction. These ROS react upon the pollutant dye molecule and degrade into harmless, simpler CO₂, H₂O and inorganic ions. The details of the process are as follows:



At CB



At VB



The reaction intermediates (ROS) formed are $\cdot\text{OH}$, $\cdot\text{O}_2^-$, h^+_{VB} and these act upon pollutant dye molecule to degrade into simpler, harmless molecules as shown below.



The degradation of pollutant dye molecule congo red in the reaction mixture in presence of sunlight is studied and a graph between intensity and wavelength is plotted with UV spectrophotometer [40]. The intensity peak decreases over time showing decrease in concentration of coloured dye molecule in the reaction mixture. (Fig. 9) shows UV spectra of congo red over time intervals with BB-FCN and FCN molecule.

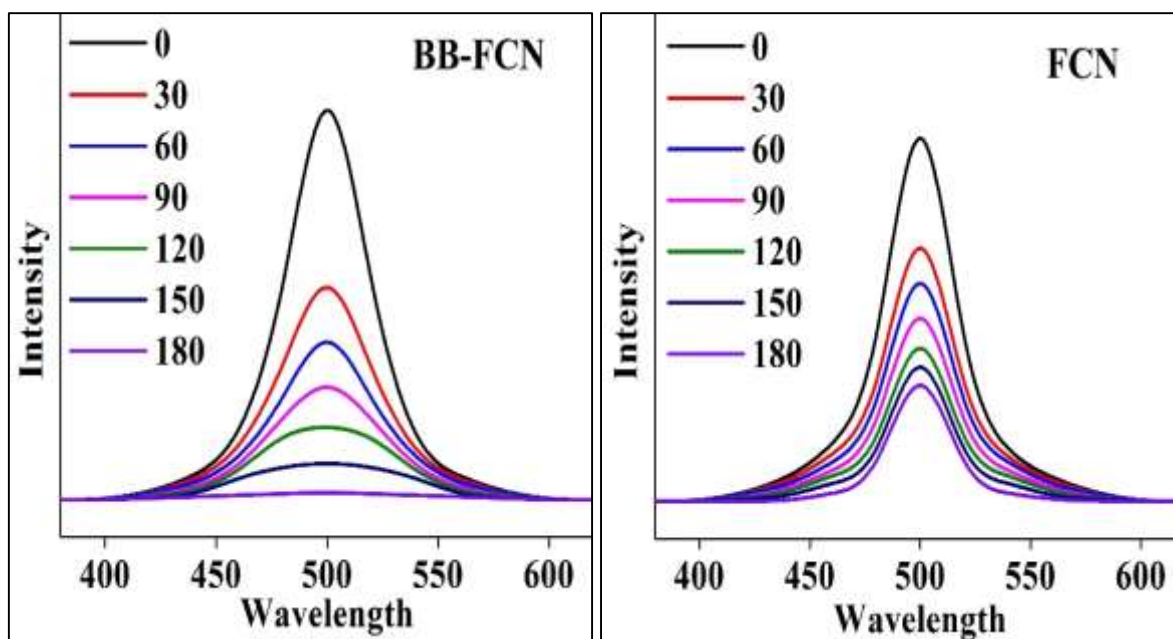


Fig. 9: Intensity graphs of BB-FCN and FCN molecules

The kinetics of the degradation reaction was studied with 1st order rate reaction. The photodegradation of congo red dye molecule with nanocomposite follows pseudo 1st order reaction with regression coefficient value of 0.993 [41]. The plot for reaction rates with BB-FCN nanocomposite and FCN molecule is shown in (Fig. 10).

The %age degradation of congo red molecule is shown in (Fig. 10). It is 90% for nanocomposite and 63% for FCN molecule. The degradation reaction results vary with variation in reaction parameters. Parameters like photocatalyst dose and dye concentration are studied further to have suitable parameters for better results.

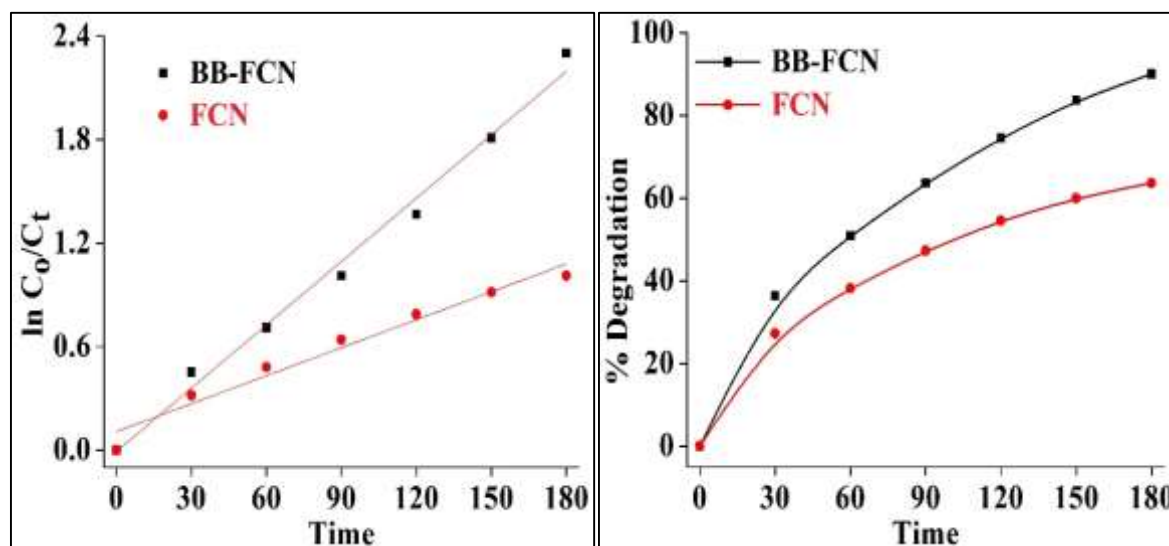


Fig. 10: Ln Co/Ct vs Time with Congo red and % degradation with BB-FCN and FCN

The amount of photocatalyst was varied in the range of 50 to 250 mg [42]. The maximum rate of congo red degradation was with 150 mg of BB-FCN loading. The rate of degradation starts to decrease over 150 mg L⁻¹ photocatalyst concentration. This was due to the fact that more amount of catalyst hinders the interaction of sunlight with pollutant due to turbidity which results in a lower rate of photodegradation. The rate values for every catalyst concentration were plotted in (Fig. 11).

The effect of congo red concentration was studied in the range of 10-90 ppm. The rate values at different dye concentrations for BB-FCN and FCN were plotted in (Fig. 12). The rate of removal of congo red was maximum for 50 ppm of congo red concentration [43]. With an increase in concentration of congo red over 50 ppm the degradation rate starts to decrease. This decline in degradation rate can be attributed to the fact that with the increase in congo red concentration the active sites on the surface of photocatalyst gets filled [44]. The increased amount of congo red remained unadsorbed on to the surface of the photocatalyst which result in lower rate of photodegradation.

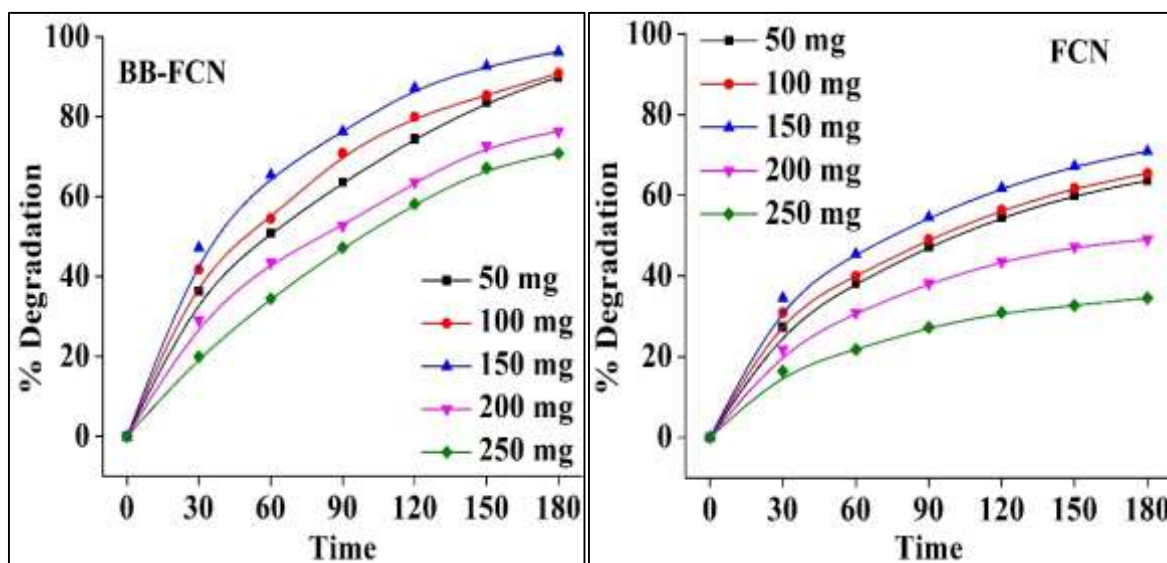


Fig. 11: Variation in %age degradation of congo red with different catalyst dose

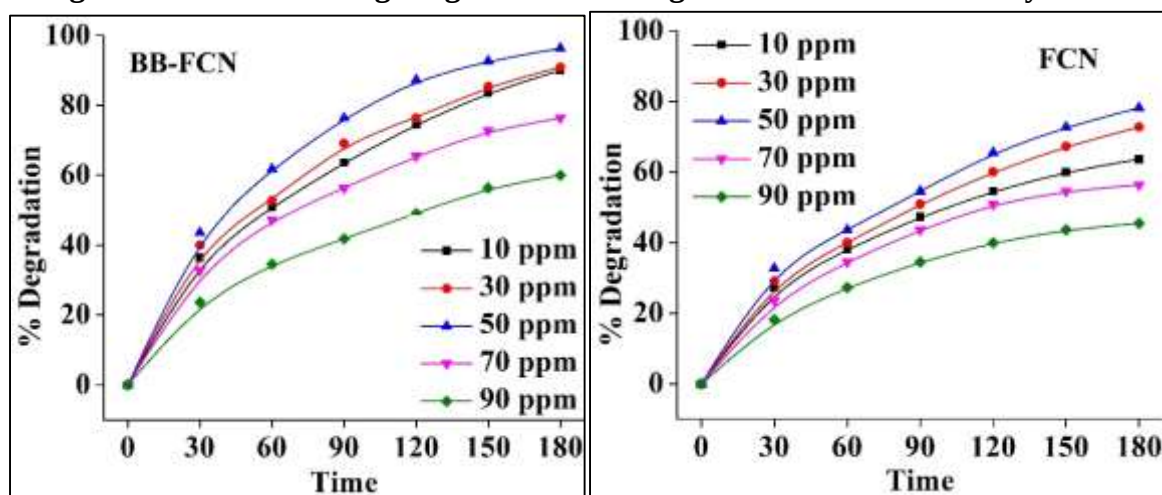


Fig. 12: Variation of %age degradation of Congo red with different dye concentration

Antibacterial activity

The result of antimicrobial activity of BB-FCN nanoparticles against gram-positive and gram-negative bacteria was observed. The inhibition zones were determined by placing BB-FCN and antibiotic in separate wells [45-47]. The antibiotic used as a positive control that inhibited the growth of both gram-positive and gram-negative bacterial strains i.e., *Bacillus subtilis* and *Pseudomonas aeruginosa*. Expected inhibitory zones were observed for positive control and no inhibitory zone was observed for negative control. Zone of inhibition was observed for *Bacillus subtilis* (27mm) and *Pseudomonas*

aeruginosa (17mm) as shown in (Table 1). BB-FCN nanoparticles showed good zone of inhibition against gram-positive bacteria as compared to gram negative bacteria as shown in (Fig. 13).

Table: Inhibition zones against bacteria.

Bacteria	NPs	Positive control	Negative control
B. subtilis	27±0.52	25±0.65	ND
P. aeruginosa	17±0.50	21±0.53	ND

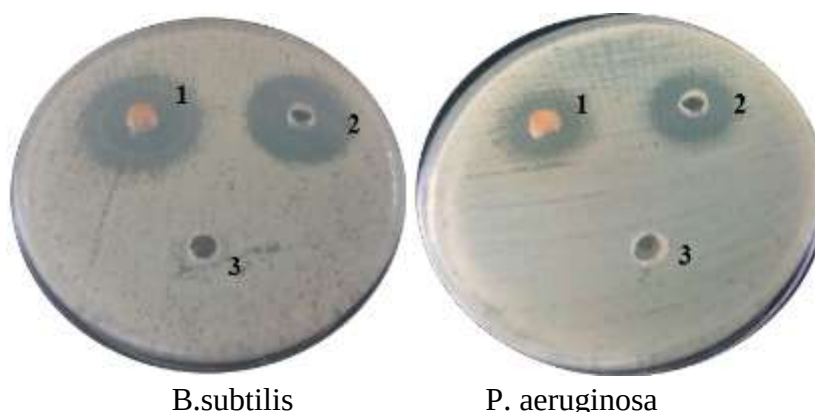


Fig. 13: Antimicrobial activity of LBG-XG@ZSPT against B. subtilis, P. aeruginosa. (In images 1) represents BB-FCN nanoparticles, 2) +ve control, 3) –ve control

Conclusion:

This research article concludes the use of waste biomaterial i.e. combination of leave of **Bryophyllum pinnatum** (common name **Miracle Leaf/Cathedral Bells**) and **Bougainvillea** to derive the leave extract which was further reacted with nitrate salts of Ferric and cerium to get corresponding nanocomposite. The main focus of this article was to analyse the antibacterial and photocatalytic property of the obtained nanocomposite and results of both analysis were tremendous. The verification of formation of nanocomposite was done on the basis of various analytical and spectral methods and all the results prove the nanocomposite manufacturing from the give leave extracts. So, the formed moieties can be used as raw material to cure the bacterial infections as well as to purify contaminated water after further study by following standard methods.

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