Research Article

Study of Antimicrobial Potency of Synthesized Cellulose-Based Nanocomposite Films Incorporating Bi-Fe-Sn Trimetallic Microcrystalline Using Terminalia arjuna Leaf Extract for Packaging and Medicinal Applications

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In this work, cellulose-based nanocomposite films having trimetallic (Bi, Fe, and Sn) nanoparticles were prepared by green adaptive methodology using Terminalia arjuna leaf extract as a reducing and stabilizing agent. Then, they were characterized by FTIR and SEM. The color change of microcrystalline cellulose films revealed the formation of the trimetallic (Bi, Fe, Sn) nanoparticles. Characteristics absorption peaks for reducing functional groups indicated the presence and role of the plant material used; moreover, the presence of various bands in FTIR spectra below 1000 cm⁻¹ was indicative of the formation of (Bi, Fe, and Sn) nanocomposites. These synthesized nanomaterials were also tested for their antimicrobial potency against Escherichia coli and Pseudomonas aeruginosa. Positive outcomes designated their potential to be adopted for biomedical applications and in food packaging as an alternative of synthetic plastics to control pollution.

1. Introduction

In recent years after COVID-19, the outbreak of antimicrobial resistance is a serious worldwide health threat for both humans and animals. It is becoming difficult and sometimes even impossible to treat common infectious diseases as antibiotics become less effective. In order to overcome this problem, the development of antimicrobial polymeric nanocomposite films as an antibiotic has attracted much attention due to their wide range of applications which includes detoxification, biomedicine, and antibacterial activity in biomedical science [1, 2]. Along with this, the incorporation of metals within the films has provided a powerful antibacterial solution in the healthcare sector, including wound healing applications, water treatment, surgical instruments, and food processing [3, 4]. Metals, such as silver, gold, selenium, magnesium, manganese, copper, zinc, bismuth, and iron at the nanoscale, provide
a unique class of antimicrobial agent with broad spectrum antimicrobial activity and low toxicity to humans [5, 6]. Methods via metal-based cellulose nanocomposites have been prepared are either in situ techniques or ex situ [7]. The ex situ green synthesis method involves the formation of nanocomposites via a direct dispersion of nanoparticles into the polymer. This approach is more effective for large-scale industrial implementations [8], but the drawback of this method is that at larger concentrations, there is a significant risk of agglomeration or poor dispersion of nanomaterials in the matrix. However, the in situ method takes some time to generate polymeric nanocomposites because they prevent metal particle aggregation [9], while also keeping a uniform distribution in the polymer matrix [10]. Several other green methods using biopolymeric nanoparticles (NPs) [11] such as Ag/CuO [12], Ag/ZnO [13], Ag2S-ZnO/GO [14], PdNPs [15], Mn-doped TiO2 NPs [16], Ag/ NiO [17], and Ag/cel lulose nanoparticles [18] are also reported.

There are various chemical and physical ways of synthesizing metal nanoparticles but most of these methods are costly and hazardous to the environment as they use harmful and toxic chemicals. In such a case, the green method has caught the attention of many researchers for being inexpensive, nontoxic, and environmental-friendly. Many researchers have reported the synthesis of metal nanoparticles in polymer matrices, as well as cotton fabrics utilizing various leaf extracts as reducing agents [17], such as Aloe vera [19], Terminalia catappa [20], Cassia atta [21], Azadirachta indica [22], tamarind nut powder [23], red sanders powder [24], Pongamia pinnata [25], Moringa oleifera [26], and Tinospora cordifolia [27]. For the sake of improvement in the properties of individual metal nanoparticles, a systemic trial was recently conducted, such as the synthesis of bimetallic [28] and trimetallic [29] nanoparticles. Trinmetallic nanoparticles have lately received significant importance [30] due to their brilliant properties and novel applications as catalyst [31], food packaging material [32], medicinal, antibacterial, and sensor [33]. Trinmetallic nanoparticles outperform mono- and bimetallic nanoparticles in terms of catalytic activity and efficiency in various fields.

Various types of plant extracts are used by many researchers as capping [17], stabilizing, and reducing agents in plant-mediated systems for the synthesis of trimetallic nanoparticles of different shapes, sizes, and structures. Capping agents act as surface modifiers of NPs [34] that anchor to their surface through electrostatic interaction or covalent bonding and act similar to a dendrite material by enhancing its chelation sites and conductive nature [35]. In the recent few years, many researchers have reported a reliable biosynthetic method of producing Au- Pt-Ag trimetallic nanoparticles using Lamii albi fos aqueous extract [36], Au-ZnO-Ag trimetallic nanoparticles using Melliotti officinalis extract [37], Ag-Au-Pd trimetallic nanoparticles using Aegle marmelos leaves, and Syzygium aromaticum buds [38] and Cu-Cr-Ni trimetallic oxide nanoparticles using Froiepia subpinnata and Eryngium campestr leaf extracts [39]. These nanoparticles revealed excellent antibacterial potential against E. coli and S. aureus [38–40]. As the demand for secure food packaging, preservatives, and disinfectant preparations for clinical supplies, it is necessary to create recyclable packaging materials with better qualities and a broad range of antimicrobial potency. When nanomaterials are integrated within trimetallic nanoparticles, the improved microbial potential of trimetallic nanoparticles, along with many other properties are anticipated, which made them ideal to be used in food and healthcare appliances. So, in this work, trimetallic Bi-Fe-Sn nanoparticles were synthesized in the microcrystalline cellulose matrix using Terminalia arjuna leaf extract as a reducer and stabilizer [41]. Terminalia arjuna, also known as Arjuna, is a member of the Combretaceae family and is found all across India, particularly in the Sub-Himalayan and eastern regions. The leaves and flowers of this tree contain glucosides, calcium, and magnesium salts, which have been used in Ayurvedic formulations. Arjuna, which consists of antioxidant and antibacterial properties, aids in sustaining cholesterol levels [42]. The extract of arjuna leaves is quite known for its antioxidant, antimicrobial, and anticancer properties [43]. Its primary components are polyphenols, flavonoids, triterpenoids, sterols, glycosides, and minerals. These phyto-constituents such as tannins, saponins, and flavonoids mainly have anticancer potency and triterpenoids have cardio-tonic applicability such as arjunolic acid has antiplatelet effect and casuarinins has antiviral potency [44]. So, its extract is used, which not only reduce the trimetallic (Bi, Fe, Sn) ions into nanoparticles but also act as capping agents which help to reduce aggregations, thus adjusting the surface characteristics and boosting the bioactivities [45]. The synthesized trimetallic Bi-Fe-Sn microcrystalline cellulose nanocomposite films were characterized by FTIR spectroscopy to study the structural features and thermal stability. Antimicrobial tests were carried out to evaluate their antimicrobial activity. The main objective of this work is to prepare and characterize these trimetallic Bi-Fe-Sn microcrystalline cellulose-based nanocomposite films which can be used in various fields similar to reported ones in surgical aprons [46], bandage cloths to clean wounds [47], antibacterial napkins, bed-sheets in operation theaters [48] and in ultrasound clinics, in personal care products [49], as a disinfectant for bacteria in the medical field [50], nursing care [51], and for food packaging processes [52].

2. Materials and Methods

2.1. Materials. In the current work, microcrystalline cellulose (MCC) and urea were supplied from the Riedel-deHaen. NaOH and H2SO4 were received from Sigma-Aldrich while Bi(NO3)3, FeCl3·6H2O, and SnCl2·2H2O were obtained from Germiston Chemicals. Fresh leaves of Terminalia arjuna were collected from arjuna trees in Punjab University Campus, Lahore, Pakistan.
2.2. Preparation of Trimetallic Bi-Fe-Sn Microcrystalline Cellulose-Based Films

Step 1. Terminalia arjuna leaf extract preparation.

The collected fresh leaves of Terminalia arjuna were washed to remove all foreign impurities. The clean leaves were then dried at room temperature and chopped into small pieces. For the preparation of the extract, 20 g of the leaf was added to 200 mL of distilled water in a glass beaker using a 1:10 ratio of plant leaves mass to volume of distilled water. It was heated at 90°C with stirring for 30 minutes and then cooled to room temperature. The extract was then filtered by using a sieve. The filtrate was stored in a refrigerator for a week since storing it up for too long resulted in the growth of the fungal culture. It is summarized in Figure 1 [53].


The microcrystalline cellulose solution of different concentrations (5% and 7%) was prepared [18]. Initially, an aqueous solution of 14 wt% sodium hydroxide and 24 wt% urea was prepared. About 5 g of microcrystalline cellulose was dispersed in already prepared 47.5 g 14 wt% NaOH and 47.5 g 24 wt% urea aqueous solution and stirred thoroughly for 2 minutes. The mixture was then allowed to cool down at −79°C for half an hour and again stirred vigorously to fully dissolve the solution. The resulting transparent and viscous solution of microcrystalline cellulose was centrifuged at 6000 rpm for 15 minutes to get a clear solution without any air bubbles. In this way, a 5 wt% microcrystalline cellulose solution was prepared [54]. The obtained clear solution was then stored in a refrigerator until it was used. Similarly, 7 wt % microcrystalline cellulose solution was prepared by dissolving 7 g microcrystalline cellulose solution in 46.5 g 14 wt % NaOH aqueous solution and 46.5 g 24 wt% urea aqueous solution [55].

Step 3. Preparation of leaf extract-infused microcrystalline cellulose film.

For this, clear microcrystalline cellulose solution was poured on the glass slide uniformly and allowed to settle down for about 5 minutes. Then, this slide was dipped in the Petri plate containing 5% H₂SO₄ solution as a coagulation bath. The obtained films were washed thoroughly with distilled water to remove excess alcohol if present in them and then dried at 25°C. The cleaned microcrystalline cellulose films were then dipped in the prepared leaf extract (10%) in a beaker for about 24 hours to get uniform diffusion into the films [56].

Step 4. In situ generation of trimetallic (Bi, Fe, and Sn) nanoparticles using leaf extract.

In situ method was used in this work to produce trimetallic (Bi, Fe, and Sn) polymer-based nanocomposites using leaf extract. Initially, solutions of bismuth nitrate, iron chloride, and tin chloride were prepared. For the preparation of 0.1 molar bismuth nitrate solution, 1.975 g of bismuth nitrate was dissolved in little quantity of water in a 50 mL volumetric flask, the volume of solution was then made up to the mark. Similarly, a 0.1 molar aqueous solution of iron chloride and tin chloride was prepared by dissolving 1.352 g of iron chloride and 1.128 g of tin chloride in a 50 mL volumetric flask. Now, for the formation of trimetallic nanoparticles, the prepared aqueous bismuth nitrate, iron chloride, and tin chloride solutions of 0.1 M were mixed in a reaction beaker. The light brown colored microcrystalline cellulose films infused with leaf extract were then placed in a reaction beaker containing a solution mixture for about 12 hours. The color of microcrystalline cellulose films was changed from light brown to off-white [57]. This color change indicated that the trimetallic (Bi, Fe, and Sn) nanoparticles were generated on the leaf extract infused microcrystalline cellulose films (matrix). This entire scheme is summarized in Figure 2.

3. Characterization by FTIR and SEM

The synthesized samples were characterized via Fourier transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM), and antimicrobial tests [58]. The FTIR spectra were recorded by using Agilent Technologies Cary model number 630 in the range of 4000 to 650 cm⁻¹. Scanning electron microscope operated at a voltage of 15 kV was used to obtain the SEM images of the samples and the Kirby Bauer agar diffusion method was used to study the antimicrobial [59].

3.1. Antimicrobial Activity. These studies were carried out in the Conservation Biological Lab, Institute of Zoology, University of the Punjab. The Kirby Bauer agar diffusion method was used to study the antimicrobial activity of nanocomposite films. The experiment was carried out on agar plates inoculated with strains of Escherichia coli and Pseudomonas aeruginosa with the help of swab sticks. A small circular disc of synthesized composites was placed on these agar plates with sterilized tweezers and then incubated at 37°C for 48 hours. Streptomycin and Azithromycin were used as conventional antibiotics (control). The inhibitory zones were formed, and the diameter for each of the zones was measured and compared with that of conventional antibiotics.
4. Results and Discussion

4.1. Nature of Microcrystalline Cellulose Film. The comparative pictures of microcrystalline cellulose, microcrystalline cellulose films infused with leaf extract (matrix), and trimetallic (Bi, Fe, and Sn) microcrystalline cellulose-based nanocomposite films are shown in Figure 3. The formation of trimetallic (Bi, Fe, and Sn) nanoparticles on microcrystalline cellulose films can be demonstrated by the color change. The picture of microcrystalline cellulose, which appeared colorless as in Figure 3(a), while Figure 3(b) indicating that leaf extract diffused into cellulose matrix due to the appearance of brown colouration. Figure 3(c) showed a picture of microcrystalline cellulose composite film with in situ generated trimetallic (Bi, Fe, and Sn) nanoparticles. The composite film turned into an off-white color which points to the formation of nanoparticles on the composite.

4.2. FTIR Analysis. FTIR spectra of microcrystalline cellulose films and nanocomposite films were taken over the range of 4000–650 cm\(^{-1}\) to evaluate the chemical reactions between the matrix and nanoparticles present in the nanocomposites. Figure 4(a) shows that the FTIR band of microcrystalline cellulose films at about 3334 cm\(^{-1}\) correlates to the presence of the alcoholic OH group while a band at 2900 cm\(^{-1}\) relates to methylene (-CH\(_2\)) groups, a band at 1636 cm\(^{-1}\) refers to carbonyl groups; peaks around 1424 and 1363 cm\(^{-1}\) corresponds to CH\(_2\) deformation modes and the
strong band at 1027 and 896 cm\(^{-1}\) confirms the presence of C-O-C bond and indicates C-OH groups of cellulose. However, the shift in the bands in the FTIR spectra for nanocomposite films in Figures 4(b) and 4(c) shows the chemical interaction of metal nanoparticles and leaf elements.
4.3 SEM Analysis. SEM analysis results are presented in Figure 5. They indicated that pore size decreases further in cellulose films (Figure 5(a)), when its nanocomposites were synthesized (Figures 5(b) and 5(c)). Its enhanced porosity is helpful in its antibacterial efficiency due to entrapping effect that can chelate more with surface of bacterial species and enhances its antibacterial potential and stability. These products were stable and can be stored in air tight glass container up to one year easily.

![SEM images](image1.png)  
**Figure 5:** (a) SEM images of microcrystalline cellulose film, for (b) nanocomposite films of 5% concentration and (c) nanocomposite films of 7% concentration.

![Bacterial plate](image2.png)  
**Figure 6:** Bacterial plate after incubation showing activity against *Escherichia coli*.

![Bacterial plate](image3.png)  
**Figure 7:** Bacterial plate after incubation showing activity against *Pseudomonas aeruginosa*.

4.4 Antimicrobial Activity. The antibacterial activity of nanocomposites against *Escherichia Coli* and *Pseudomonas aeruginosa* was investigated in this study. For ease, the samples were coded as a (Streptomycin), b (synthesized trimetallic Cellulose nanocomposite disk-5%), c (synthesized trimetallic Cellulose nanocomposite disk-7%), and d (Azithromycin). The test results are shown in Figures 6 and
7. From them, it is evident that trimetallic (Bi, Fe, and Sn) cellulose-based nanocomposite films showed intermediate antimicrobial activity against *Pseudomonas aeruginosa* and *Escherichia coli*. The zone of inhibition against both bacterial strains was measured and compared with the inhibition zone of conventional antibiotics in Table 1.

Potential outcomes of this work indicated that cellulose-based thin films can be a suitable alternative for plastic packaging if processed further [60]. Along with that, they can be used in herbal medicine related to skin diseases due to their antimicrobial efficacy, as wet wipes [61], edible packaging [62], and wound healing tendency [47], because they are obtained from natural resources [63], such as fruit peels [64], leaves [65, 66], and biodegradable in nature [67]. So, they can have various potential applications in various healthcare systems [68], energy storage devices [69], and wastewater treatment [70]. They are summarized in Figure 8.

### 5. Conclusion

The microcrystalline cellulose-based nanocomposite films were successfully synthesized using *Terminalia arjuna* leaf extract as a reducing and stabilizing agent. An in situ approach was adopted to prepare trimetallic (Bi, Fe, and Sn) nanoparticles which are integrated into a microcrystalline cellulose matrix. The resulting nanocomposite films were characterized using FTIR and SEM. The color change of microcrystalline cellulose films revealed the formation of the trimetallic (Bi, Fe, and Sn) nanoparticles. The presence of reducing groups and their oxidation was confirmed through FTIR comparative analysis. Moreover, antimicrobial activity against both *Escherichia coli* and *Pseudomonas aeruginosa* demonstrated the potential application of the resulting nanocomposite films.

### Data Availability

The data used in this study are included within the article.

### Conflicts of Interest

The authors declare that they have no conflicts of interest.

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### References


