Nep J Environ Sci (2025), 13(2), 93-102
https://doi.org/10.3126/njes.v13i2.85039

ISSN 2350-8647 (Print) 2542-2901 (Online)

Research Article

Antimicrobial Evaluation of Polyaniline-Coated Argeli Paper for Sustainable Packaging

Yub Narayan Thapa[1,2,3], Prakash Dhungana[4], Bimal Rajchal[1,2,5], Deepshikha Karki[1,2], Babin Khanal[1], Achyut Nepal[6], Sven Henning[6], Sashi Joshi[4*], Devesh Kumar Avasthi[7], Rameshwar Adhikari[1,2*]

[1]Research Centre for Applied Science and Technology, Tribhuvan University, Kirtipur, Kathmandu 44618, Nepal
[2]Central Department of Chemistry, Tribhuvan University, Kirtipur, Kathmandu 44618, Nepal
[3]Tribhuvan Multiple Campus, Tribhuvan University, Tansen, Palpa 32500, Nepal
[4]Department of Chemistry, Amrit Science Campus, Tribhuvan University, Lainchour, Kathmandu 44600, Nepal
[5]Department of Chemistry, Bhaktapur Multiple Campus, Tribhuvan University, Bhaktapur 44800, Nepal
[6]Fraunhofer Institute for Microstructure of Materials and Systems (IMWS), Walter-Hülse-Straße 1, 06120 Halle/Saale, Germany
[7]Centre of Interdisciplinary Research and Innovation, University of Petroleum and Energy Studies (UPES), Dehradun 248007, Uttarakhand, India

(Received: 04 October 2025; Revised: 29 December 2025; Accepted: 30 December 2025)

Abstract

Polyaniline (PANI)-coated traditional Nepali paper composites were synthesized *via* an *in situ* chemical oxidative polymerization process. Fourier Transform Infra-red Spectroscopy (FTIR), X-ray Diffraction (XRD), Optical Microscopy (OM) and Scanning Electron Microscopy (SEM) confirmed the PANI-coating. Additionally, the synthesized PANI powder (emeraldine salt) exhibited good antibacterial activity against *Bacillus subtilis* and *Escherichia coli (*zone of inhibition, ZOI: 0.5 cm each) and antifungal activity against *Candida albicans* (ZOI: 0.4 cm). In contrast, PANI-coated NK exhibited lower antimicrobial activity compared to powdered PANI. It was more effective against *E. coli* (ZOI: 0.3 cm) than against *B. subtilis* (ZOI: 0.2 cm). The antifungal activity against *C. albicans* was the lowest, with a ZOI of 0.1 cm. These findings demonstrate the potential of the composites for sustainable packaging applications with modest antimicrobial properties. Optimizing the polymerization process and material formulation could enhance their efficacy for eco-friendly active packaging systems.

Keywords: Antibacterial, conjugated polymer, traditional Nepali paper, PANI, zone of Inhibition

Introduction

Sustainable packaging solutions have significantly increased due to increased awareness of environmental issues and the need for biodegradable materials. On the top, introduction of antimicrobial property to these sustainable and biodegradable packaging materials would be more effective for preserving the food, pharmaceutical, medical devices, personal care and cosmetics (Sung et al., 2013; Vermeiren et al., 2002). Traditional antimicrobial packaging materials incorporate antimicrobial agents into the synthetic polymers such as polypropylene (PP), polystyrene (PS), polyethylene terephthalate (PET), polymethyl methacrylate (PMMA), polyvinyl chloride (PVC), and polyethene (PE). Most commonly used antimicrobial agents are nanoclays, metal and metal oxide nanoparticles, including silver (Ag), zinc oxide (ZnO), and titanium dioxide (TiO_2) (Brandelli, 2024; Duda-Chodak et al., 2023; Hasanin et al., 2023; Lai et al., 2022). However, the use of conventional antimicrobial agents, for instance, Ag nanoparticles, can lead to environmental and health risks as their working mechanism often involves the leaching of ions (Kwon et al., 2021). By contrast, Polyaniline (PANI), a class of conjugated polymer (CP), is a safer choice. It possesses unique properties including high proton dopability, excellent redox cyclability and environmental stability (Maruthapandi et al., 2022). The antimicrobial action of PANI involves the electrostatic interaction and oxidative stress generation. PANI attracts the negatively charged bacterial cells and makes physical contact with them. This contact leads to an enhanced antimicrobial activity (Jose et al., 2023; Robertson et al., 2018). Simultaneously, the excessive use of such synthetic polymer matrices imposes serious environmental issues of non-degradable plastic waste.

A significant number of works have been carried out in the development of PANI-based antimicrobial composites using different substrates. For instance, Rehim et al. (2020) fabricated PMMA-Cellulose nanocrystal (CNC) nanocomposite through physical blending. To the surface of this composite film, a PANI layer was deposited. The resultant composite exhibited notable antimicrobial activity against *Bacillus cereus* and *Salmonella typhimurium*. Similarly, Ao et al. (2025) used melt blending to incorporate PANI and graphene into a PP matrix in the presence of nanosilica and epoxy-functionalized ethyl orthosilicate. The composite with 0.5 wt. % filler loading showed complete (100 %) antibacterial activity against both *Escherichia coli* and *Staphylococcus aureus*. This further shows the effectiveness of PANI-based systems in developing antimicrobial packaging materials. However, the use of synthetic and non-biodegradable polymers such as PP and PMMA raises significant environmental challenges. In this context, cellulose-based materials are a great choice due to their inherent biodegradability, natural abundance and eco-friendly nature. Shalini et al. (2016) synthesized a PANI-cellulose composite by extracting cellulose from sugarcane bagasse and subsequently incorporating PANI through chemical polymerization. The prepared

Corresponding authors: shashi1joshi@yahoo.com (SJ) and nepalpolymer@yahoo.com (RA)

composite exhibited improved antimicrobial activity against *S. aureus* and *E. coli*, with zones of inhibition (ZOI) of 16 ± 0.43 mm and 13 ± 0.45 mm, respectively. Alternatively, Various natural plant fibres, including Argeli, Lokta, bamboo, sisal, agave, kenaf, jute, etc, have been directly used to make biodegradable and sustainable biocomposites (Adhikari et al., 2012; Gautam, Groβmann, Basyal, et al., 2024; Giri et al., 2024). Maráková et al. (2017) developed PANI-coated cotton fabrics through *in situ* polymerization, followed by silver (Ag) nanoparticle incorporation *via* immersion in silver nitrate solution of different concentrations. The cotton-PANI-Ag composite prepared using 0.05 M $AgNO_3$ solution demonstrated antimicrobial activity against *S. aureus* (ZOI: 1 mm) and *E. coli* (ZOI: 4 mm). These studies underscore the potential of incorporating PANI in the cellulose substrate to achieve an antimicrobial effect that could be used for packaging applications. While plant fibres can be incorporated into composites in various forms, the direct utilization of cellulose-based paper represents a particularly straightforward and cost-effective approach.

Traditionally, Nepali handmade papers are generally made from the bast fibres of Lokta (*Daphne papyracea* and *Daphne bholua*) and Argeli (*Edgeworthia gardneri*) (Deuba, 2015). These papers are known for their inherent unique properties of high porosity, flexibility, strength and insect repellent since ancient times in Nepal (Aryal et al., 2022). In particular, Argeli fibres possess high cellulose content, good durability and modest tensile strength (Gautam, Groβmann, Pradhan, et al., 2024). Additionally, Argeli grows comparatively faster than the Lokta plant and holds potential to fulfil the demands of raw material for Nepali paper.

Nepal has long traditional green packaging practices using biodegradable local resources based on its culture and traditions. In connection to this, bamboo baskets (*Perungo*), leaf plates (*Duna*, *Tapari*), banana leaf, etc., have been used as sustainable and green packaging materials (K.C. et al., 2024; Kalina et al., 2024; Tirpude & Singh, 2025). Traditionally handmade Nepali papers (NK), which have long been used in official government documentation, have now been used in various other sectors as well. It includes eco-friendly bags, boxes and packaging papers. Studies show that cellulose-based substrates have been effectively used for the development of PANI-cellulose composites for antimicrobial packaging materials (Ramos et al., 2019; Youssef et al., 2012). Nevertheless, the use of traditional handmade Nepali papers as substrates for PANI-based antimicrobial composites remains unexplored. The objective of this study is to extend this approach to NK and assess the potential of the composite as an antimicrobial packaging material. Considering this, NK was coated with PANI *via* a facile *in situ* chemical oxidative polymerization process. Fourier Transform Infrared Spectroscopy (FTIR), X-ray Diffraction (XRD), and Scanning Electron Microscope (SEM) analysis were carried out to analyze the effective incorporation of PANI onto the paper. Finally, the antimicrobial efficacy of the coated paper was evaluated against Gram-positive and Gram-negative bacteria and a fungus using the agar diffusion method.

Materials and Methods

Materials and sample preparation

Laboratory grade Aniline with purity 99.5 %, Hydrochloric (HCl) acid at 37 % and Ammonium persulphate (APS) with purity 99 % were obtained from Qualigens Fine Chemicals, Mumbai, India, and used as received. Traditionally handmade Nepali paper (NK), made from Argeli bast fibres, was purchased from Taplejung Koseli Ghar, Phugling 57500, Taplejung, Nepal. The thickness of the paper ranged from 0.9 mm to 0.12 mm.

Synthesis of Polyaniline (PANI)

A general approach of chemical oxidative polymerization method was used to synthesize PANI using APS as an oxidant. Typically, 12.3 g of APS (equivalent to approximately 0.054 moles) were dissolved in 70 mL of 1M aqueous HCl in a 500 mL beaker. The molar ratio of monomer to oxidant was maintained at approximately 1:1. Thus, the monomer solution was prepared by dissolving 5 g of aniline in 75 mL of 1M HCl in a separate 500 mL beaker in the presence of 5x critical micelle concentration of surfactant cetyltrimethylammonium bromide (CTAB). Both solutions were maintained at a constant temperature of 25 °C. The oxidant solution was added slowly to the aniline solution for approximately 1 minute. The reaction mixture was allowed to stand for 30 minutes, during which the constant temperature was maintained at 25 °C. After the completion of polymerization, the reaction mixture was filtered, and the PANI residue was washed with distilled water. Finally, the obtained powder was washed with acetone till a clear filtrate was obtained and dried in the oven at 60 ℃ for 24 hours (Wasu & Raut, 2014).

Synthesis of PANI-coated NK composites

The NK was cut into a square shape of dimensions 5 cm × 5 cm for the subsequent coating. For the polymerization reaction, the concentrations of the oxidant, monomer and surfactant solution were kept identical to those used in the synthesis of PANI powder. A piece of NK was then dipped into a monomer solution with surfactant taken in a beaker and soaked for a few minutes. Subsequently, the oxidant was added slowly to the beaker containing NK to initiate the polymerization reaction. After the polymerization for 30 minutes, the PANI-coated NK was removed from the reaction mixture and allowed to dry overnight. On the following day, it was rinsed carefully to remove the oligomers adsorbed on the surface of NK, followed by acetone. It was further dried in the oven at 60 ℃ for 45 minutes. A schematic diagram illustrating the stepwise PANI coating process on NK is presented in Fig. 1.

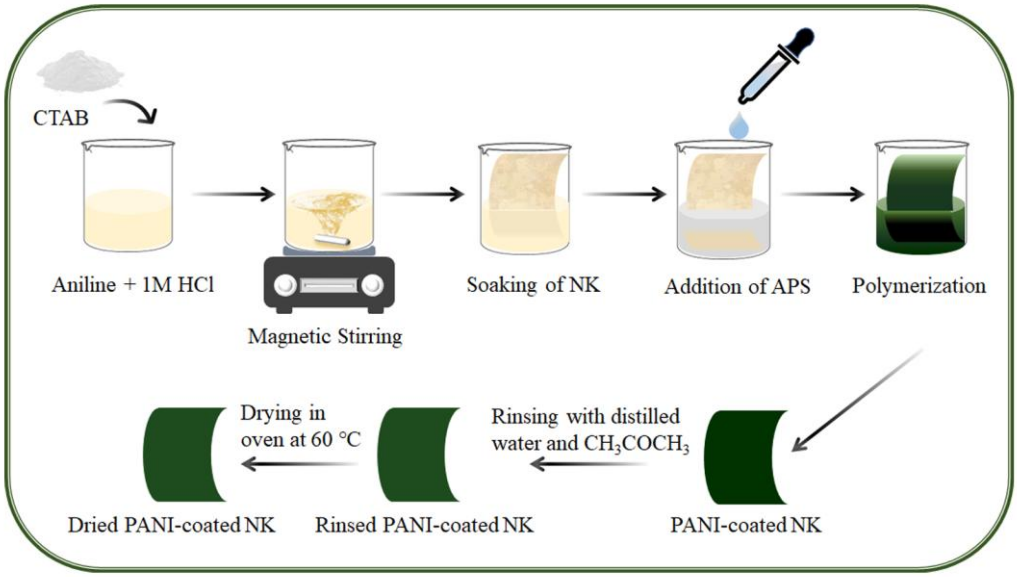

Figure 1. Schematic illustration of the *in-situ* polymerization process for PANI coating on NK

Preparation of media

Nutrient Agar (NA): Nutrient agar, a common nutrient medium, provides essential nutrients to support the growth of a wide spectrum of non-fastidious microorganisms. This medium was prepared by dissolving 28 g of nutrient agar in a litre of distilled water in an appropriately sized Erlenmeyer flask. The mixture was boiled with constant agitation to ensure complete dissolution, then sterilized by autoclaving at 121℃ under the pressure of 15 psi for 15 minutes. Subsequently, the sterilized media were cooled for approximately 50 minutes and 25 mL aliquots were aseptically poured into the sterile 90 mm petri dishes. They were properly labelled and left undisturbed to allow solidification.

Nutrient Broth (NB): Nutrient broth is a liquid culture medium identical to nutrient agar in composition, only lacking the solidifying agent, agar powder. As a result, it is in a liquid state at room temperature and is used for maintaining microbial cultures. To prepare the nutrient broth, 1.3 g of the powder was dissolved in 100 mL of distilled water using an Erlenmeyer flask, followed by sterilization *via* autoclaving at 121℃ and 15 psi for 15 minutes. After cooling at room temperature, 10 mL aliquots were aseptically transferred into a screw bottle. To ensure the aseptic storage conditions, it was further sterilized.

Mueller Hinton Agar (MHA): Mueller Hinton Agar is a culture medium composed of beef extract, starch, acid hydrolysate of casein and agar. It is primarily used for antimicrobial susceptibility testing due to its ability to support the proliferation of non-fastidious bacteria and facilitate the formation of a clear zone, particularly in the disk diffusion method developed by Kirby-Bauer. The medium was prepared by adding 3.8 g of powdered MHA to 100 mL of distilled water. The mixture was heated with frequent stirring and boiled for one minute to make the medium clear. Subsequently, the medium was sterilized by autoclaving at 121℃ under 15 psi pressure for 15 minutes. After cooling the medium at room temperature, 10 mL of medium was poured aseptically into sterile 90 mm petri dishes placed horizontally to ensure a uniform depth. Finally, the plates were left undisturbed at room temperature, allowing the agar to solidify.

Potato Dextrose Agar (PDA): Potato dextrose agar is a widely used general-purpose medium for culturing fungi, including yeasts and moulds and can be supplemented with acids and antibiotics to suppress bacterial contamination. It primarily consists of dehydrated potato infusion and dextrose, which promotes favourable fungal growth with agar as a solidifying agent. It was prepared by adding 3.9 g of commercially available PDA powder to 100 mL of distilled water and heating continuously with constant stirring until fully dissolved. After cooling to about 50 ℃, 10 mL of aliquots were aseptically dispensed into sterile petri dishes. Finally, the plates were left undisturbed at room temperature to allow the medium to solidify.

Collection of test organisms

Three microbial strains, comprising two bacteria and one fungus, were employed for the antimicrobial activity study. These strains were supplied by Himalayan Research Institute of Biotechnology Pvt. Ltd., Radhe, Bhaktapur. The study included two bacterial strains and one fungal strain. The bacterial strains comprised a Gram-positive strain, *Bacillus subtilis* ATCC 6051, and a Gram-negative strain, *Escherichia coli* ATCC 8739. For antifungal evaluation, *Candida albicans* ATCC 2091 was used as a test organism.

Preparation of Standard Culture Inocula

The microbial inocula were prepared from primary culture plates as described above. An isolated colony of each bacterial strain was aseptically transferred using a sterile inoculating loop and sub-cultured onto Mueller-

Hinton Agar (MHA) plates. Fresh cultures for testing were obtained by incubating the plates at 37 ℃ for 12 hours. Kanamycin sulphate (5 µL) was used as a standard antibiotic control.

Characterization Techniques

Optical Microscopy (OM)

The first impression of the incorporation of PANI into the paper was observed by the optical microscope Boeco BM-700, Germany. The samples were observed in the bright field mode at different magnifications (10x and 40x).

Fourier-Transform Infrared (FTIR) Spectroscopy

The functional group identification and the interaction of PANI with the NK were assessed by FTIR analysis in an attenuated total reflectance (ATR) mode using a spectrometer (IRAffinity-1S, Shimadzu, Japan).

The samples were dried and sectioned into small pieces before mounting on the ATR crystal. Subsequently, the spectra were obtained in the range of 4500 to 500 cm^{-1} using a resolution of 4 cm^{-1}. For a better signal-to-noise ratio, each sample was scanned 40 times.

X-ray Diffraction (XRD)

The structure of NK and PANI-NK composites was analyzed by X-ray Diffraction using a Brucker D8 Advance Eco diffractometer. The measurements were carried out using Cu-K□ radiation (λ= 0.154056 nm) at 40 kV and 30 mA, with diffraction patterns recorded in the 2θ range of 5-80° at a step size of 0.02°. The crystallinity index (CI) was determined using equation (1):

$$CI = \left(\frac{A_{cr}}{A_t}\right) \times 100\ \% \qquad (1)$$

where, A_{cr} represents the area of the crystalline diffraction peaks, and A_t denotes the total peak area of both crystalline and amorphous contributions.

Scanning Electron Microscopy (SEM)

The surface morphology of PANI-coated composites was examined using a scanning electron microscope (FEI Quanta 650 ESEM-FEG). A conductive palladium layer of ~3 nm thickness was deposited onto the samples by sputter coating. The samples were mounted on the SEM stubs using double-sided conductive adhesive tape. Images were acquired by operating the instrument with an accelerating voltage of 15 kV.

Antimicrobial Activity

Antimicrobial Susceptibility Testing (AST): Antimicrobial susceptibility testing evaluates the ability of an antimicrobial agent to suppress the growth of microorganisms, including bacteria and fungi. In this study, the antimicrobial activity of various samples, including Nepali paper (NK), PANI and PANI-coated NK, was assessed by measuring the average diameter of the zone of inhibition (ZOI) formed around the sample on an agar surface inoculated with specific pathogenic bacteria.

Antibacterial test

Sterile Muller-Hinton Agar Plates were dried to remove any residual moisture on the surface. A spreader (bent glass rod) was sterilized by dipping it into 95 % ethanol. After this, 5µ L of the standardized bacterial culture was carefully pipetted onto the centre of each labelled agar plate. The inoculum was spread across the surface of agar by rotating the plate at about 60° intervals, ensuring even coverage. After inoculation, the plates were covered and left undisturbed for a few minutes to dry.

Sterile filter paper discs, each 5 mm in diameter, were carefully placed onto the surface of the inoculated agar plates. The test samples were applied onto the respective discs using the sterile tweezers. The plates were then covered and left at room temperature for 30 minutes. Following this, the plates were subjected to incubation at 37 ℃ for 24 hours. After it, the ZOI surrounding the discs was measured using a ruler and recorded for further analysis.

Antifungal test

A broth culture of the fungal strain was uniformly spread over the surface of pre-sterilized PDA plates. Sterile filter paper discs were kept on the surface of agar. The samples were aseptically introduced onto the respective discs with the help of sterile tweezers. The plates were kept covered at room temperature for 30 minutes, providing sufficient time to diffuse into the medium. Thereafter, the plates were incubated at 28 ℃ for 24 hours and after which the ZOI was measured.

Results and Discussion

Structural and morphological analysis

Figure 2 depicts the FTIR spectra of neat NK, PANI powder, and PANI-coated NK. The characteristic peaks of PANI corresponding to C═C stretching of quinonoid and benzenoid structure were observed at 1560 cm^{-1} and 1483 cm^{-1}. Consistent results are documented in the literature (Lee et al., 2012; Razak et al., 2014). Additionally, the peaks centred at 1287 cm^{-1} and 1237 cm^{-1}, attributed to C─N stretching vibration of quinonoid and benzenoid structures of PANI backbone, were recorded in the spectrum, comparable with the results reported by Deng et al. (2016) and Tang et al. (2011). Similarly, the peak at 1110 cm^{-1} associated with the quinonoid ring of PANI was observed, as reported elsewhere (Turkten et al., 2023). Furthermore, the spectrum showed a peak associated with flexural vibrations of the aromatic C─H plane at 789 cm^{-1}. This observation is in agreement with the peak reported by Manjunatha et al. (2019).

The FTIR spectrum of NK displayed characteristic peaks of cellulose, as expected. A strong, broad and distinctive peak of O─H stretching vibrations from the hydroxyl group was observed, ranging between 3000-3600 cm^{-1} (Gautam, Groβmann, Pradhan, et al., 2024). Furthermore, a peak associated with C─H stretching in aliphatic hydrocarbons was recorded at 2920 cm^{-1} (Poletto et al., 2014). The lignin present in NK was

evidenced by the appearance of a peak at ~1600 cm^{-1}, which is attributed to the C=O stretching vibration of conjugated lignin structures. A similar observation was reported by Tanpichai et al. (2019). Similarly, the peak of C—O, C=C and C—C—O stretching vibrations characteristic of lignin, cellulose and hemicellulose, respectively, was observed at ~1015 cm^{-1} (Aryal et al., 2022).

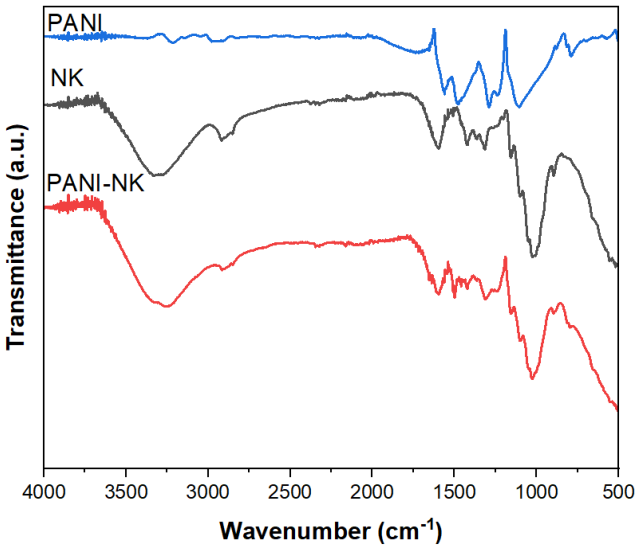

Figure 2. FTIR spectra of PANI powder, neat NK and PANI-coated NK

Similar to neat NK, PANI-coated NK also exhibited the broad band of O—H in the same range. However, the incorporation of PANI decreased the intensity of the band. A new peak at ~1499 cm^{-1} was observed, which is associated with the C—C stretching vibration of the benzenoid structure of PANI. This peak position in the composite was found to be shifted by 16 cm^{-1} to a higher wavenumber compared to that of neat PANI. This indicates the interaction of PANI with the cellulose matrix of NK and the successful incorporation of PANI into the paper. Moreover, a subtle change in the peak around 1235 cm^{-1} was observed; however, other distinct peaks characteristic of PANI were not clearly resolved due to the overlapping with the cellulose peaks, making them indistinguishable in the composite spectrum.

Overall, the FTIR analysis confirms the characteristic vibrational modes of PANI, indicating its successful synthesis and incorporation of PANI into the NK substrate.

Figure 3 depicts the XRD spectra of neat and PANI-coated NK. The cellulose type I in the NK was evident from the 2θ values of 15.5° and 22.6° corresponding to (100) and (200) crystallographic planes, respectively (Yang et al., 2015; Zhang et al., 2015). The incorporation of PANI into NK didn't introduce any diffraction peaks attributable to PANI. The same characteristic peaks of NK were retained in the composite, indicating that the PANI molecules are intercalated into the cellulose matrix without disrupting the fundamental crystalline arrangement of cellulose (Youssef et al., 2012). However, the intensity of the respective peaks was decreased compared to the neat NK. The appearance of a characteristic peak of PANI in the FTIR of the composite, but its absence in XRD, suggests that PANI is in amorphous form, lacking the long-range order in the composite. Furthermore, the crystallinity index (CI) of PANI-NK (52.42 %) was approximately 5 % lower than that of neat NK (57.49 %). The reduction in CI is expected as the incorporation of amorphous PANI disrupts the ordered crystalline structure of NK. A similar observation was reported by Hajlaoui et al. (2020).

Figures 4a and 4b show the digital photographs of neat NK and PANI-coated NK, respectively. A distinct change in colour indicated the incorporation of PANI onto the paper. At higher magnification in the optical micrograph, the random fibre distributions of different thicknesses before polymerization were distinctly visible (Fig. 4c). Upon polymerization, PANI particles were seen incorporated into these fibres (Fig. 4d). Further, the SEM micrograph complemented the detailed morphology of the coated paper with high-resolution insights. Fig. 5 shows the surface morphology of *in situ* polymerized PANI-coated NK. The fibrous structure of cellulose was visible, indicating the underlying substrate of NK was intact. However, these individual fibres appeared to be modified and completely covered by a granular layer of PANI. The coating of PANI appeared throughout the substrate. This is likely to be due to a porous substrate that enabled effective diffusion and retention of monomers and oxidant throughout the network (Ke et al., 2019). Additionally, the pores also offer more surface area, which results in greater active sites for nucleation. The granular texture of PANI suggests the agglomeration of the polymer during the polymerization.

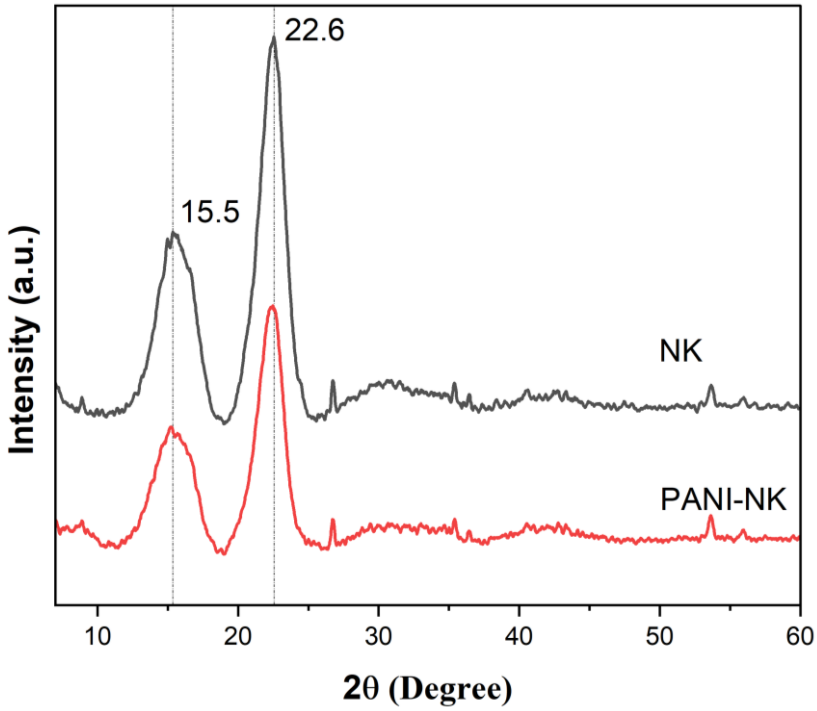

Figure 3. XRD diffraction patterns of neat NK and PANI-coated NK composite

Antimicrobial analysis

The antimicrobial activity of neat NK, PANI and PANI-coated NK was evaluated by measuring the zone of inhibition (ZOI) against *Bacillus subtilis*, *Escherichia coli*, and *Candida albicans*. The results demonstrated that PANI powder had a ZOI of 0.4-0.5 cm against all three test organisms (**Fig. 6**). The neat NK exhibited no antimicrobial activity, marked by a ZOI of 0 cm against *Bacillus subtilis* and *Escherichia coli*, and no visible effect against *Candida albicans*. PANI-coated NK had a ZOI of 0.1-0.3 cm against all three test organisms. The standard Kanamycin had a ZOI of 1.0 cm. The complete set of inhibition zone measurements is summarized in **Table 1**. The data suggest that PANI powder has the highest antibacterial activity against all tested organisms compared to Argeli paper and PANI-coated NK.

The neat NK exhibits no antimicrobial and antifungal effect, as the cellulose paper doesn't possess inherent antimicrobial and antifungal properties. This served as the negative control, affirming that any antimicrobial and antifungal activity by the composite is attributed to the PANI coating. PANI has significant antimicrobial properties, which are consistent with the literature reports. The positively charged PANI interacts electrostatically with negatively charged bacteria and disrupts the bacterial activities required for bacterial survival, growth and proliferation. Furthermore, the PANI synthesized using APS possesses a molecular weight of approximately 100 kD, capable of penetrating the cell membrane, leading to cell lysis (Maruthapandi et al., 2022; Shi et al., 2006). The antimicrobial activity of PANI is also involved with the generation of reactive oxygen species that damage the essential biomolecules and contribute to the death of the bacterial cell (Robertson et al., 2018). However, the antimicrobial efficacy of PANI is significantly influenced by its physicochemical properties, particularly the chain length (Gizdavic-Nikolaidis et al., 2012).

The lower efficacy of PANI towards fungi in comparison to bacteria is attributed to their cellular structures. The complex cell wall structure of *C. albicans,* containing glucans and mannans, acts as a robust barrier to the antimicrobial agents, making them more resistant to oxidative and disruptive effects (Iconaru et al., 2021). In contrast, the thinner peptidoglycan layers of bacterial cell walls are more vulnerable to the antimicrobial agents (Aizamddin et al., 2022). Moreover, the PANI-coated paper demonstrated extremely low antimicrobial action towards the tested microorganisms compared to the neat powdered PANI. This reduced efficacy is expected when the composites contain low PANI loading. Additionally, incomplete coverage, uneven distribution of PANI and the reduced exposed sites of PANI in the composites further lower the antimicrobial efficacy. Furthermore, the antimicrobial activity in composite materials is greatly influenced by the diffusion of antimicrobial agents from the surface of the composite (Green et al., 2011). The interaction of PANI with the substrate may induce the diffusion constraints that limit the release of PANI from the surface of the composite thereby reducing its antimicrobial efficacy. In spite of the similar antimicrobial activity of powdered PANI against both bacterial strains, the PANI-coated NK demonstrated lower activity against *B. subtilis*. This indicates the inhomogeneous PANI coating in NK, thereby affecting its antimicrobial performance.

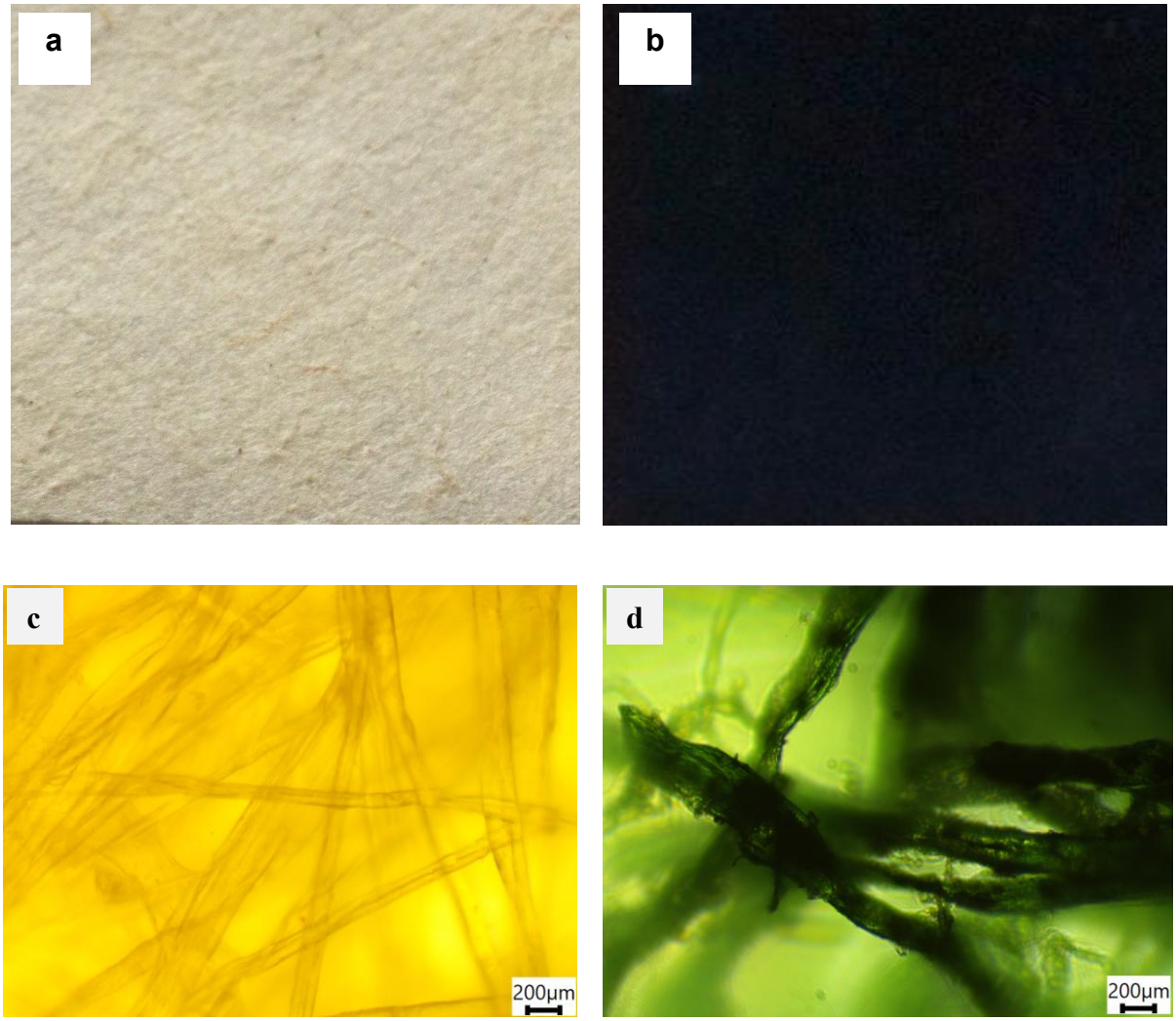

Fig. 4 Macroscopic and microscopic appearance of NK before and after PANI coating: digital photographs (a, b), and Optical micrographs (c, d) of neat and PANI-coated NK, respectively

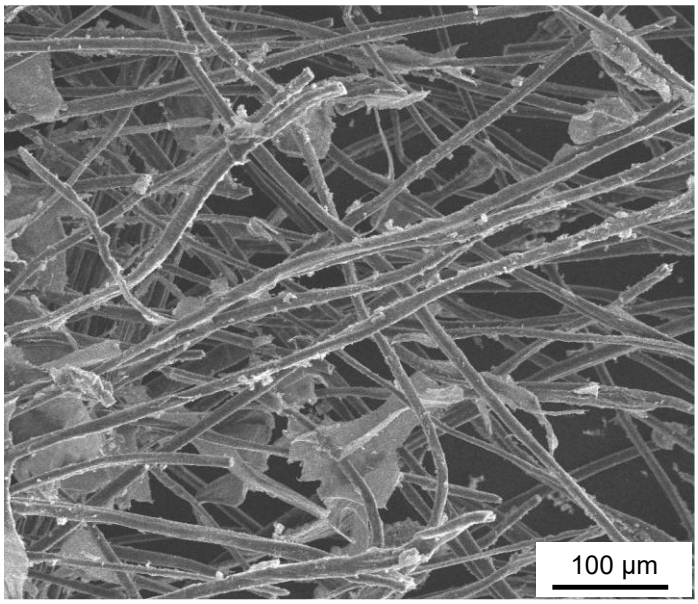

Figure 5. SEM micrograph of PANI-coated NK showing deposition of granular PANI on the surface of the fibres

Table 1. Zone of inhibition observed for neat NK, PANI and PANI-coated NK against different strains

Bacterial Strain	Reference culture	Type	List of items	ZOI of (cm)
Bacillus subtilis	ATCC 6051	Gram +ve	(a) Polyaniline powder (b) Argeli paper (c) PANI-coated paper	0.5 0 0.2
Escherichia coli	ATCC 8739	Gram –ve	(a) Polyaniline powder (b) Argeli paper (c) PANI-coated paper	0.5 0 0.3
Candida albicans	ATCC 2091	Fungi	(a) Polyaniline powder (b) Argeli paper (c) PANI-coated paper	0.4 0 0.1
Standard Kanamycin (ZOI:1.0 cm)				

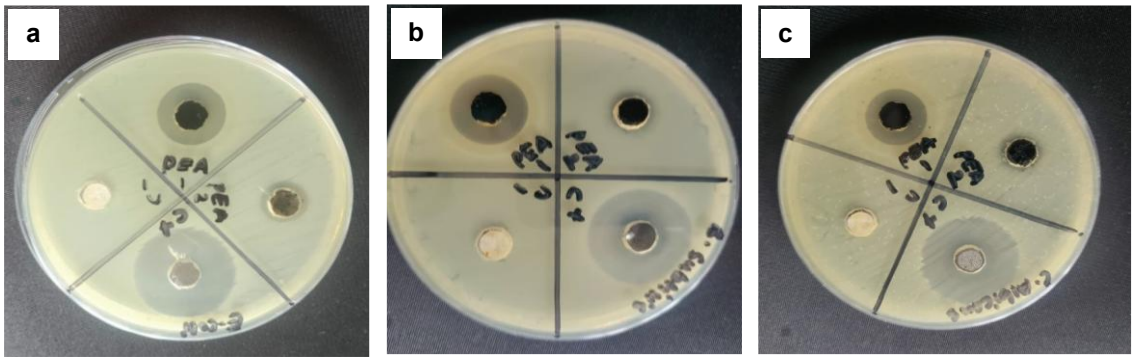

Fig. 6 Antimicrobial effect of neat NK, PANI powder and PANI-coated NK against *E. coli* (a), *B. subtilis* (b) and antifungal effect on *C. albicans* (c)

Conclusions

Traditionally handmade Nepali paper (NK), made from Argeli Bast fibre, was functionalized by incorporating PANI through an *in-situ* polymerization reaction. FTIR and XRD analysis assured the successful deposition of PANI, which was further supported by OM and SEM analysis. Flexible, porous and mechanically strong NK functioned as a good substrate for the deposition of PANI. Synthesised PANI powder exhibited antimicrobial activity against *B. subtilis and E. coli,* with slightly lower effectiveness against the fungus *C. albicans*. The variations in the cell wall composition of these microorganisms resulted in differences in their resistance to PANI's antimicrobial effects, with the robust fungal structure being more resistant compared to the thinner bacterial peptidoglycans. The PANI-coated NK exhibited lower antimicrobial activity than neat PANI powder, being least effective against *C. albicans*. The low loading of PANI, limited active sites and non-uniform distribution of PANI, and diffusion constraints reduced the effectiveness of this coated paper. Nonetheless, this work highlights the potential of PANI-coated NK for antimicrobial packaging applications.

While PANI coating on cellulosic substrates has been widely explored, this study extended its application to traditionally handmade Nepali paper, highlighting the potential of indigenous materials for sustainable packaging. Use of such locally available resources could reduce dependence on non-biodegradable plastics, support traditional craftmanship and minimize plastic waste generation in the packaging industry. To further enhance antimicrobial efficacy, strategies such as functionalization of PANI, controlling the chain length, uniform coating and inclusion of other nanoparticles into it could be explored. Additionally, incorporating water-resistant and enhancing fire-retardant properties would further advance the applicability of these materials in packaging technology.

Acknowledgements: Himalayan Research Institute of Biotechnology Pvt. Ltd. is thanked for conducting the antimicrobial tests, and the Central Instrumentation Centre (CIC), UPES, Dehradun, India, for performing XRD tests.

Funding Resources: YNT is grateful to the University Grants Commission, Bhaktapur, Nepal, for the financial assistance through a PhD fellowship and research support. This project was partly supported by Tribhuvan University, Research Directorate, in the frame of "Comprehensive Identification and Characterization of Lignocellulosic Fibers of Nepal".

Authors Contribution: YNT: supervision, analysis of results, writing the first draft; PD: Investigation, data acquisition; BR: formal analysis, graphics and plots preparation, DK: results analysis and validation; BK: sample collection, results validation; AN: sample preparation, results analysis; SH: microscopic analysis and validation; SJ: supervision, review and editing; DKA: arrangement of electron microscopic investigation, supervision, review and editing; RA: conceptualization, supervision, review and editing.

Conflict of Interest: The authors declare no conflict of interest.

Data Availability: Data will be available through the corresponding author upon reasonable request.

References

Adhikari, R., Bhandari, N.L., Causin, V., Le, H.H., Radusch, H., Michler, G.H., & Saiter, J.M. (2012). Study of morphology, mechanical properties, and thermal behavior of green aliphatic–aromatic copolyester/bamboo flour composites. *Polymer Engineering & Science*, 52(11), 2296–2303. https://doi.org/0.1002/pen.23335.

Aizamddin, M.F., Mahat, M.M., Ariffin, Z.Z., Nawawi, M.A., Jani, N.A., Amdan, N.A.N., & Sadasivuni, K.K. (2022). Antibacterial performance of protonated polyaniline-integrated polyester fabrics. *Polymers*, 14(13). https://doi.org/10.3390/polym14132617.

Ao, X., Liu, X., Dai, Z., & Zhu, A. (2025). The Preparation of polyaniline/graphene/polypropylene nanocomposite and its novel antibacterial activity. *Polymer Composites*, 46(3), 2794–2802. https://doi.org/10.1002/pc.29140.

Aryal, G.M., Kandel, K.P., Bhattarai, R.K., Giri, B., Adhikari, M., Ware, A., Han, S., George, G., Luo, Z., Gautam, B.R., & Neupane, B.B. (2022). Material properties of traditional handmade paper samples fabricated from cellulosic fiber of Lokta bushes. *ACS Omega*, 7(36), 32717–32726. https://doi.org/10.1021/acsomega.2c04398.

Brandelli, A. (2024). Nanocomposites and their application in antimicrobial packaging. *Frontiers in Chemistry*, 12, 1–13. https://doi.org/10.3389/fchem.2024.1356304.

Deng, Y., Tang, L., Zeng, G., Dong, H., Yan, M., Wang, J., Hu, W., Wang, J., Zhou, Y., & Tang, J. (2016). Enhanced visible light photocatalytic performance of polyaniline modified mesoporous single crystal TiO2 microsphere. *Applied Surface Science*, 387, 882–893. https://doi.org/10.1016/j.apsusc.2016.07.026.

Deuba, Y. (2015). *Export barriers to internationalization: An investigation on Nepalese handmade paper industries*. University of Nordland.

Duda-Chodak, A., Tarko, T., & Petka-Poniatowska, K. (2023). Antimicrobial compounds in food packaging. *International Journal of Molecular Sciences*, 24(3). https://doi.org/10.3390/ijms24032457.

Gautam, P., Groβmann, L., Basyal, O.P., Pradhan, S., Bhandari, N.L., Henning, S., Nase, M., & Adhikari, R. (2024). Argeli bast fiber as wonder reinforcing agent for biodegradable polymer composites. *Nepal Journal of Environmental Science*, 12(2), 1–8. https://doi.org/10.3126/njes.v12i2.68409.

Gautam, P., Groβmann, L., Pradhan, S., Bhandari, N.L., Nase, M., & Adhikari, R. (2024). Physicochemical and structural investigation of Argeli (*Edgeworthia gardneri*) bast fibers. *Journal of Research Updates in Polymer Science*, 13, 54–65. https://doi.org/10.6000/1929-5995.2024.13.07.

Giri, J., Adhikari, R., & Sapkota, J. (2024). Comparative study of polymer composites with cellulose microfibers from different plant resources. *Advances in Polymer Technology*, 2024, 1–11. https://doi.org/10.1155/2024/2396318.

Gizdavic-Nikolaidis, M.R., Bennett, J., Zujovic, Z., Swift, S., & Bowmaker, G.A. (2012). Characterization and antimicrobial efficacy of acetone extracted aniline oligomers. *Synthetic Metals*, 162(13–14), 1114–1119. https://doi.org/10.1016/j.synthmet.2012.04.031.

Green, J.B.D., Fulghum, T., & Nordhaus, M.A. (2011). A review of immobilized antimicrobial agents and methods for testing. *Biointerphases*, 6(4), MR13–MR28. https://doi.org/10.1116/1.3645195.

Hajlaoui, O., Khiari, R., Ajili, L., Batis, N., & Bergaoui, L. (2020). Design and characterization of Type I cellulose-polyaniline composites from various cellulose sources: A comparative study. Chemistry Africa, 3(3), 783–792. https://doi.org/10.1007/s42250-020-00148-1.

Hasanin, M.S., El Saied, H., Morsy, F.A., & Hassan Abdel Latif Rokbaa, H. (2023). Green nanocoating-based polysaccharides decorated with ZnONPs doped Egyptian Kaolinite for antimicrobial coating paper. *Scientific Reports*, 13(1), 1–14. https://doi.org/10.1038/s41598-023-38467-1.

Iconaru, S.L., Predoi, M.V., Chapon, P., Gaiaschi, S., Rokosz, K., Raaen, S., Motelica-Heino, M., & Predoi, D. (2021). Investigation of spin coating cerium-doped hydroxyapatite thin films with antifungal properties. *Coatings*, 11(4). https://doi.org/10.3390/coatings11040464.

Jose, A., Gizdavic-Nikolaidis, M., & Swift, S. (2023). Antimicrobial coatings: Reviewing options for healthcare applications. *Applied Microbiology*, 3(1), 145–174. https://doi.org/10.3390/applmicrobiol3010012.

Kalina, S., Kapilan, R., Wickramasinghe, I., & Navaratne, S.B. (2024). Potential use of plant leaves and sheath as food packaging materials in tackling plastic pollution: A review. *Ceylon Journal of Science*, 53(1), 21–37. https://doi.org/10.4038/cjs.v53i1.8145.

K.C., B.M., Lamichhane, J., Khanal, S.N., & Gauchan, D.P. (2024). Traditional utilization of bamboo in the Central Siwalik Region, Nepal. *PLoS ONE*, 19(1). https://doi.org/10.1371/journal.pone.0296886.

Ke, S., Ouyang, T., Zhang, K., Nong, Y., Mo, Y., Mo, Q., Wei, Y., & Cheng, F. (2019). Highly conductive cellulose network/polyaniline composites prepared by wood fractionation and in situ polymerization of aniline. *Macromolecular Materials and Engineering*, 304(7), 1900112. https://doi.org/10.1002/mame.201900112.

Kwon, S., Lee, W., Choi, J. W., Bumbudsanpharoke, N., & Ko, S. (2021). A facile green fabrication and characterization of cellulose-silver nanoparticle composite sheets for an antimicrobial food packaging. *Frontiers in Nutrition*, 8, 1–8. https://doi.org/10.3389/fnut.2021.778310.

Lai, T.T., Pham, T.T.H., van Lingen, M., Desaulniers, G., Njamen, G., Tolnai, B., Jabrane, T., Moineau, S., & Barnabé, S. (2022). Development of antimicrobial

paper coatings containing bacteriophages and silver nanoparticles for control of foodborne pathogens. *Viruses*, 14(11). https://doi.org/10.3390/v14112478.

Lee, B.H., Kim, H.J., & Yang, H.S. (2012). Polymerization of aniline on bacterial cellulose and characterization of bacterial cellulose/polyaniline nanocomposite films. *Current Applied Physics*, 12(1), 75–80. https://doi.org/10.1016/j.cap.2011.04.045.

Manjunatha, S., Machappa, T., Ravikiran, Y.T., Chethan, B., & Sunilkumar, A. (2019). Polyaniline based stable humidity sensor operable at room temperature. *Physica B: Condensed Matter*, 561, 170–178. https://doi.org/10.1016/j.physb.2019.02.063.

Maráková, N., Humpolíček, P., Kašpárková, V., Capáková, Z., Martinková, L., Bober, P., Trchová, M., & Stejskal, J. (2017). Antimicrobial activity and cytotoxicity of cotton fabric coated with conducting polymers, polyaniline or polypyrrole, and with deposited silver nanoparticles. *Applied Surface Science*, 396, 169–176. https://doi.org/10.1016/j.apsusc.2016.11.024.

Maruthapandi, M., Saravanan, A., Gupta, A., Luong, J.H.T., & Gedanken, A. (2022). Antimicrobial activities of conducting polymers and their composites. *Macromol*, 2(1), 78–99. https://doi.org/10.3390/macromol2010005.

Poletto, M., Ornaghi Júnior, H.L., & Zattera, A.J. (2014). Native cellulose: structure, characterization and thermal properties. *Materials*, 7(9), 6105–6119. https://doi.org/10.3390/ma7096105.

Ramos, A.R., Tapia, A.K.G., Piñol, C.M.N., Lantican, N.B., del Mundo, M.L.F., Manalo, R.D., & Herrera, M.U. (2019). Morphological, electrical and antimicrobial properties of polyaniline-coated paper prepared via a two-pot layer-by-layer technique. *Materials Chemistry and Physics*, 238, 121972. https://doi.org/10.1016/j.matchemphys.2019.121972.

Razak, S.I.A., Sharif, N.F.A., & Nayan, N.H.M. (2014). Electrically conductive paper of polyaniline modified pineapple leaf fiber. *Fibers and Polymers*, 15(6), 1107–1111. https://doi.org/10.1007/s12221-014-1107-x.

Rehim, A.M.H., Yassin, M.A., Zahran, H., Kamel, S., Moharam, M.E., & Turky, G. (2020). Rational design of active packaging films based on polyaniline-coated polymethyl methacrylate/nanocellulose composites. *Polymer Bulletin*, 77(5), 2485–2499. https://doi.org/10.1007/s00289-019-02866-0.

Robertson, J., Gizdavic-Nikolaidis, M., Nieuwoudt, M. K., & Swift, S. (2018). The antimicrobial action of polyaniline involves production of oxidative stress while functionalisation of polyaniline introduces additional mechanisms. *PeerJ*, 2018(6), 1–36. https://doi.org/10.7717/peerj.5135.

Shalini, A., Nishanthi, R., Palani, P., & Jaisankar, V. (2016). One pot synthesis, characterization of polyaniline and cellulose/polyaniline nanocomposites: application towards in vitro measurements of antibacterial activity. *Materials Today: Proceedings*, 3(6), 1633–1642. https://doi.org/10.1016/j.matpr.2016.04.053.

Shi, N., Guo, X., Jing, H., Gong, J., Sun, C., & Yang, K. (2006). Antibacterial effect of the conducting polyaniline. *Journal of Materials Science & Technology*, 22(3), 289-290.

Sung, S.Y., Sin, L.T., Tee, T.T., Bee, S.T., Rahmat, A.R., Rahman, W.A.W.A., Tan, A.C., & Vikhraman, M. (2013). Antimicrobial agents for food packaging applications. *Trends in Food Science and Technology*, 33(2), 110–123. https://doi.org/10.1016/j.tifs.2013.08.001

Tang, S.J., Wang, A.T., Lin, S.Y., Huang, K.Y., Yang, C.C., Yeh, J.M., & Chiu, K.C. (2011). Polymerization of aniline under various concentrations of APS and HCl. *Polymer Journal*, 43(8), 667–675. https://doi.org/10.1038/pj.2011.43.

Tanpichai, S., Witayakran, S., Srimarut, Y., Woraprayote, W., & Malila, Y. (2019). Porosity, density and mechanical properties of the paper of steam exploded bamboo microfibers controlled by nanofibrillated cellulose. *Journal of Materials Research and Technology*, 8(4), 3612–3622. https://doi.org/10.1016/j.jmrt.2019.05.024.

Tirpude, R., & Singh, S. (2025). A comprehensive study of use of plant leaves in product packaging. *Journal of Packaging Technology and Research*, 9(2), 77–86. https://doi.org/10.1007/s41783-025-00184-7.

Turkten, N., Karatas, Y., Uyguner-Demirel, C.S., & Bekbolet, M. (2023). Preparation of PANI modified TiO_2 and characterization under pre- and post-photocatalytic conditions. *Environmental Science and Pollution Research*, 30(51), 111182–111207. https://doi.org/10.1007/s11356-023-30090-x.

Vermeiren, L., Devlieghere, F., & Debevere, J. (2002). Effectiveness of some recent antimicrobial packaging concepts. *Food Additives and Contaminants*, 19, 163–171. https://doi.org/10.1080/02652030110104852.

Wasu, M.B., & Raut, A.R. (2014). Synthesis and characterization of polyaniline based conducting polymers. *Journal of Chemistry and Chemical Sciences*, 5(1), 70–117.

Yang, C., Chen, C., Pan, Y., Li, S., Wang, F., Li, J., Li, N., Li, X., Zhang, Y., & Li, D. (2015). Flexible highly specific capacitance aerogel electrodes based on cellulose nanofibers, carbon nanotubes and polyaniline. *Electrochimica Acta*, 182, 264–271. https://doi.org/10.1016/j.electacta.2015.09.096.

Youssef, A.M., El-Samahy, M.A., & Abdel Rehim, M.H. (2012). Preparation of conductive paper composites based on natural cellulosic fibers for packaging applications. *Carbohydrate Polymers*, 89(4), 1027–1032. https://doi.org/10.1016/j.carbpol.2012.03.044.

Zhang, F., Pang, Z., Dong, C., & Liu, Z. (2015). Preparing cationic cotton linter cellulose with high substitution degree by ultrasonic treatment. *Carbohydrate Polymers*, 132, 214–220. https://doi.org/10.1016/j.carbpol.2015.06.055.