CELLULOSE DERIVATIVES-BASED FILMS WITH HONEY BEE PROPOLIS EXTRACT: A NOVEL APPROACH TO ANTIBACTERIAL AND ANTIOXIDANT MATERIALS

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The research study aims to examine the incorporation of propolis extract and its effects on the biological properties of the thin films. These films are composed of non-ionic cellulose derivatives, specifically hydroxypropyl cellulose (HPC) and hydroxyethyl cellulose (HEC), combined with polyvinylpyrrolidone (PVP). The magnetic stirring method was used to prepare propolis extract (PE) from raw honey bee using an ethanol solution. The surface morphologies were analyzed by scanning electron microscopy. Antibacterial activity was examined against *Staphylococcus aureus* and it was observed that antibacterial activity significantly improved after the addition of the propolis extract. The 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical assay was employed to evaluate antioxidant properties. The significant antibacterial and antioxidant properties of the material recommend it for potential biomedical applications.

Keywords: propolis extract, cellulose derivatives, polyvinylpyrrolidone, antibacterial activity, cytotoxicity test

INTRODUCTION

Bioactive materials are notable for biomedical applications, such as in tissue engineering and wound healing areas.[1] Cellulose is a natural polymer, considered a sustainable and eco-friendly organic material. Cellulose derivatives exhibit superior properties compared to the cellulose itself, including improved solubility, viscosity, and film-forming ability. These modifications to the cellulose structure through derivatisation are crucial in enhancing the practical and functional aspects of cellulose-based materials, making them suitable for various applications.[2] In recent years, cellulose derivatives have been widely studied for application in various areas, including food, cosmetics, biomedical, and pharmaceutical industries.[3,4,5,6]

Cellulose ethers possess high molecular weight and have a broad range of applications in the pharmaceutical industry. The cellulose ethers that are most commonly utilized include sodium carboxymethylcellulose (NaCMC), hydroxypropylmethylcellulose (HPMC), methyl-cellulose (MC), hydroxyethylcellulose (HEC), ethylcellulose (EC), hydroxypropyl cellulose (HPC), hydroxyethyl methylcellulose (HEMC), and benzyl cellulose (BC).[7]

Hydroxypropyl cellulose (HPC) is a non-ionic cellulose derivative, with widespread use in the pharmaceutical industry. It has good film forming capacity, due to which it is often used to develop materials such as wound dressings.[7] The thin films produced from HPC have good flexibility and high-water content, being biocompatible, biodegradable, and bioadhesive.[8]

Hydroxyethyl cellulose (HEC), another cellulose ether derivative with non-ionic properties, also has wide applicability due to its non-toxic nature. The polymer's key attributes include biocompatibility and hydrophilicity, making it suitable for various uses, such as coatings, biomedical applications, pharmaceuticals, and food packaging.[9]

Honey bee propolis is a natural resinous substance produced by honey bees to enhance the

structural integrity of beehives. It is a remarkable material, with a wide range of beneficial properties, due to which, it is often used in traditional medicine. Due to its rich content of bioactive components, the propolis is a potent antioxidant and antimicrobial,[8] while it also has anti-inflammatory, immunomodulatory, antiulcer, antitumor and wound-healing effects.[9] Therefore, it has been considered as an active agent for incorporation into wound dressings. However, from this perspective, several aspects should be taken into consideration: the interaction between propolis and the polymers could affect the film's stability and hydration characteristics; also, the release profile of the propolis should be considered – all these considerations are crucial for applications such as drug delivery or wound healing.[10]

In the literature, it has been reported that polysaccharides/propolis blends have proved to be highly useful in wound treatments.[13] For example, Kapare *et al.*[18] reported that their propolis/PVA hydrogel formulation exhibited wound healing properties. Such hydrogels could also be used as a drug delivery system, where synergistic activities of propolis constituents are achieved.

Polyvinylpyrrolidone (PVP) is an amorphous non-ionic polymer. It is one of the most notable polymers showing high biodegradability and biocompatibility properties, produced from monomer N-vinylpyrrolidone.[11] The carbonyl group present in PVP can form hydrogen bonds with the hydroxyl groups of water, alcohol, and polymers to form complexes. Due to its versatility, PVP is one of the polymers in pharmaceutical applications.[12]

The present work aimed to prepare thin films using cellulose derivatives HPC and HEC loaded with honey bee propolis extract (PE). The selection of the polymers is based on their solubility, film-forming ability, degradation temperature and hygroscopicity.[15,16] The prepared thin films were characterized using FT-IR and X-ray diffraction techniques. Additionally, scanning electron microscopy (SEM) was used to analyse the surface morphology of the prepared thin films. The investigation also included cytotoxicity tests, antibacterial and antioxidant studies.

EXPERIMENTAL

Materials

Hydroxypropyl cellulose (HPC), hydroxyethyl cellulose (HEC), and ethanol were procured from Research Lab-Fine Chem Industries, located in Mumbai, India. A powdered sample of polyvinyl pyrrolidone (PVP) was obtained from Sisco Research Laboratories in Maharashtra, India. Bee propolis was acquired from Nature Honey Pvt. Ltd. in Chennai. Vero cells were sourced from the National Centre for Cell Sciences in Pune. The microorganism *Staphylococcus aureus* was utilized to evaluate antibacterial activity, and standard Amphotericin was employed at a dosage of 20 µL per disc.

Preparation of honey bee propolis extract (PE)

A quantity of 5 grams of raw propolis sourced from honey bees was accurately measured and solubilized in a 75% ethanolic solution. The resultant solution was subjected to magnetic stirring for a duration of 24 hours at ambient temperature. After the stirring process, the solution was filtered and was subsequently preserved in a vacuum flask. The obtained extract of propolis was utilized for subsequent analytical procedures.

Preparation of cellulose derivatives/PVP thin films

Two types of thin films were fabricated based on HPC/PVP and HEC/PVP, respectively, by the solvent casting technique and the propolis extract was incorporated.

In order to prepare the films, first, solutions of 5 wt% hydroxypropyl cellulose (HPC), 2 wt% hydroxyethyl cellulose (HEC) and 2 wt% PVP were made. Then, a solution mixture of HPC and PVP was prepared in a weight ratio of 50:50. The resultant solution was subjected to continuous mixing with a magnetic stirrer for 45 minutes at a temperature of 50 °C to ensure homogenous integration within the blend matrix. The resultant homogeneous solution was subsequently cast into a Teflon Petri dish and subjected to ambient drying conditions, after which the films were extracted for characterization. An analogous methodology was employed for the synthesis of the HEC/PVP thin film.

Preparation of thin film of cellulose derivatives/PVP/PE

A known concentration of 5% extracted honey bee propolis was added to the cellulose derivatives/PVP thin film formulations. The mixture was stirred for 3-4 hours in a magnetic stirrer. The resulting homogeneous solution was cast onto a Teflon Petri dish and dried at room temperature. The prepared films appeared smooth, flexible, and transparent. The final formulations and their corresponding denotations are shown in Table 1. Photographs of the thin films are shown in Figures 1 and 2.

Antibacterial assay

The antibacterial properties of cellulose derivatives/PVP and cellulose derivatives/PVP/PE thin films were investigated against *Staphylococcus aureus* using Mueller Hinton agar (MHA) medium through the agar disc diffusion methodology.[17,18]

After the solidification of the medium, the inoculum was uniformly dispersed on the solidified plates employing sterile swabs that had been moistened with the bacterial suspension. Subsequently, the discs were strategically positioned on the MHA plates. The thin films were dissolved in DMSO and placed on an agar plate inoculated with bacteria at various concentrations, specifically 1000 µg, 750 µg, and 500 µg, and were introduced onto each disc made up of high-quality absorbent filter paper. The plates were incubated at a temperature of 37 ºC for a duration of 24 hours. The diameter of the zone of inhibition was measured to evaluate the antimicrobial efficacy.

Table 1
Formulations of cellulose derivatives/PVP thin films

Formulation	HPC (5 wt%)	HEC (2 wt%)	PVP (2 wt%)	PE (5%)
HPC/PVP/PE	25 mL	-	25 mL	2.5 mL
HEC/PVP/PE	-	25 mL	25 mL	2.5 mL

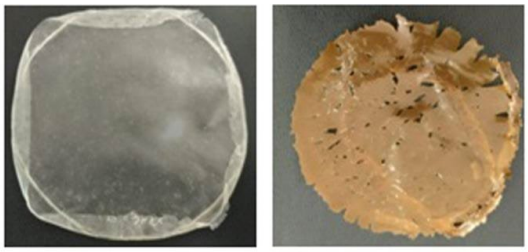

Figure 1: Appearance of HEC/PVP and HEC/PVP/PE thin films

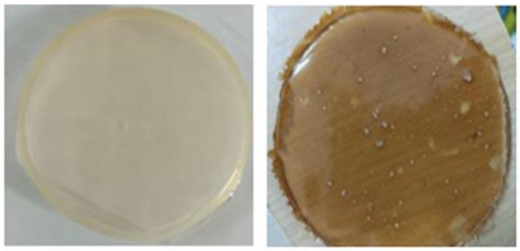

Figure 2: Appearance of HPC/PVP and HPC/PVP/PE thin films

Antioxidant activity

The free radical scavenging activity of the synthesized thin films was evaluated utilizing 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical as a model.[19] The prepared thin films were dissolved in methanol at varying concentrations. Methanol serves as solvent to maintain proper concentration of DPPH and provides a stable environment for the reaction.[20]

An aliquot of 3.7 mL of absolute methanol was introduced into all experimental tubes, while 3.8 mL of absolute methanol was used for the blank. A volume of 100 µL of BHT (butylated hydroxytoluene) was employed as a standard reference, and 100 µL of the corresponding samples was added to all the designated test samples. Subsequently, 200 µL of the DPPH reagent was administered to each test tube, including the blank. All test tubes were subjected to incubation at ambient temperature in the dark for a duration of 30 minutes. The absorbance values for all samples were measured at a wavelength of 517 nm.

The percentage of inhibition was computed in accordance with the following equation:

$$\%\ \text{of DPPH}^{\cdot}\ \text{radical inhibition} = (\text{AControl} - \text{ASample})/\text{AControl} \times 100 \quad (1)$$

where $A_{Control}$ denotes the absorbance of the DPPH radical in methanol, while A_{Sample} represents the absorbance of the DPPH radical in conjunction with the thin films.

***In vitro* assay for cytotoxicity activity**

Initially, the thin films were dissolved in DMSO at a concentration of 1 mg/mL, and then, diluted to various concentrations using DMEM (Dulbecco's Modified Eagle Medium). The specific concentrations used in the study were: 7.8, 15.6, 31.25, 62.5, 125, 250, 500, and 1000 µg/mL. Vero cells (1×10^5/well) were plated in 24-well plates and incubated at 37 °C with 5% CO_2 condition.

After the cells reached confluence, the various concentrations of the samples were added and incubated for 24 h. After incubation, the samples were removed from the well and washed with phosphate-buffered saline (pH 7.4). 100 µL/well (5 mg/mL) of 0.5% 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-tetrazolium bromide (MTT) was added and incubated for 4 hours. After incubation, 1 mL of DMSO was added in all the wells. The absorbance at 570 nm was measured with a UV spectrophotometer, using DMSO as the blank, for the various concentrations of the samples. The % cell viability was calculated using the following formula:

$$\%\ \text{Cell viability} = \text{A570 of treated cells} / \text{A570 of control cells} \times 100 \quad (2)$$

Measurements

The absorption spectrum of the extracted propolis was obtained utilizing a UV-Vis spectrophotometer (Shimadzu UV 5600 Plus). The extract was scanned between 200 and 500 nm. The Fourier-transform infrared (FT-IR) spectroscopy technique was applied to analyze the functional groups present in the produced thin films, using the Thermo Nicolet iS50 with an integrated attenuated total reflectance (ATR) spectrometer across the spectral range of 500–4000 cm^{-1}. X-ray diffraction (XRD) analyses were conducted on a Bruker D8 Advance apparatus, using

CuKα radiation at 40 kV and 40 mA over the 2θ range from 10° to 90°, with a scanning rate of 5% per minute. The surface morphologies of the synthesized thin films were scrutinized employing scanning electron microscopy (SEM) utilizing a Thermo Fisher FEI Quanta 250 FEG microscope, with a thin layer of gold deposited onto the sample prior to imaging.

RESULTS AND DISCUSSION

UV-Vis spectral analysis

UV-Vis spectrum of honey bee propolis extract is shown in Figure 3. As may be noted, a specific peak appears at 287 nm, which corresponds to the presence of phenolic compounds, which usually show an absorption peak between 250 and 350 nm in the UV light spectrum.[21,22] The phenolic and flavonoid compounds from the composition of propolis have also been reported to show peaks at the wavelength of 285 nm.[23]

FT-IR spectroscopy

FT-IR spectrometry serves as a critical analytical tool for the investigation of interactions among polymeric materials. The chemical interaction observed between cellulose derivatives and PVP polymer corresponds to the formation of miscible polymer blends. Figure 4 presents the FT-IR spectra corresponding to the propolis extract, as well as those of the HPC/PVP and HEC/PVP thin films infused with propolis extract.

The characteristic absorbance peak for propolis extract at 3355 cm^{-1}, indicative of the -OH stretching vibrations associated with phenolic compounds,[24] is distinctly visible in Figure 4 (a). Furthermore, the absorbance peaks appearing at 2974 cm^{-1} and 2919 cm^{-1} are attributed to the symmetric stretching of CH_3 and the asymmetric stretching of CH_2, respectively.[25]

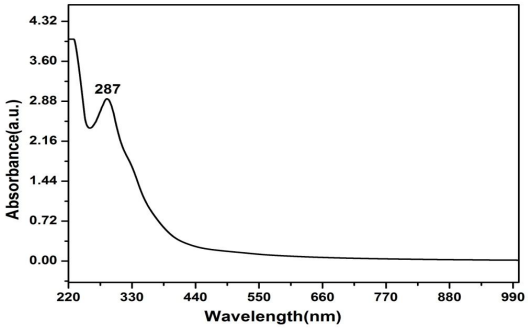

Figure 3: UV-Vis spectrum of propolis extract

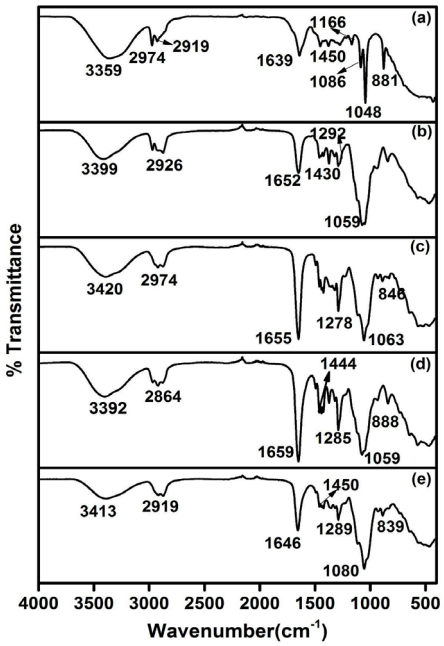

Figure 4: FT-IR spectra of (a) propolis extract, (b) HEC/PVP, (c) HPC/PVP, (d) HEC/PVP/PE, and (e) HPC/PVP/PE thin films

The peaks observed at 1639 cm^{-1} and 1450 cm^{-1} are associated with the aromatic rings of the phenolic compounds found in the propolis extract.[26] Additionally, the bands observed at 1166, 1048, and 881 cm^{-1} are ascribed to C=C stretching vibrations, C–O–C aromatic ether bonds, and the C–H wagging vibrations of the phenolic compounds.[16]

On the other hand, the FT-IR spectra of the HEC/PVP and HPC/PVP thin films reveal broad absorbance bands at 3399 cm^{-1} and 3420 cm^{-1}, respectively,[27] which arise from the O–H stretching vibrations inherent in both PVP and the cellulose derivatives, as illustrated in Figure 4 (b) and (c). The bands noted at 2926 and 2974 cm^{-1}, in conjunction with those at 1652 and 1655 cm^{-1}, are ascribed to the C–H stretching and C=O stretching vibrations of the HEC/PVP and HPC/PVP thin films, respectively.[28] Furthermore, the bands at 1063 and 1059 cm^{-1} correspond to the C–O stretching vibrations of the HPC/PVP and HEC/PVP thin films, respectively. The characteristic peaks within the range of 1278-1292 cm^{-1} indicate the presence of the N–C stretching group. An absorption band at 1652-1655 cm^{-1} in the PVP indicates the C=O stretching mode characteristic of an amide.[29]

The FT-IR spectra of the HPC/PVP/PE and HEC/PVP/PE thin films are illustrated in Figure 4 (d) and (e). The peaks noted for both films are associated with the cellulose derivatives and PVP, albeit with some modifications after the addition of propolis extract. Specifically, after the addition of propolis extract, the O–H stretching bands have shifted to 3392 and 3413 cm^{-1} for the HEC/PVP/PE and HPC/PVP/PE thin films, respectively. Additionally, the C=O stretching bands are observed at 1659 cm^{-1} for the HPC/PVP/PE sample and at 1646 cm^{-1} for the HEC/PVP/PE sample. The peaks at 1444–1450 cm^{-1} indicate the presence of aromatic compounds C-H of PE and the peaks formed at 888 and 839 cm^{-1} indicate the presence of $C=CH_2$ stretching vibrations of PE, which indicates the presence of propolis in the thin film.[30]

XRD study

XRD was performed to investigate the crystalline nature after the addition of PVP and propolis extract to the cellulose derivatives. The XRD patterns of the prepared thin films were compared with those of the cellulose derivatives and PVP. Figure 5 (a and b) shows that there were two broad peaks at 2θ = 20.29° and 27.76°, but in the HEC/PVP/PE thin film, there was a sharp peak at 2θ = 20.88° along with the peak at 28.89°, indicating an increase in crystallinity.

The XRD pattern of the HPC/PVP thin film presented in Figure 5 revealed a small peak at 2θ = 11.07° and broad peaks around at 2θ = 19.82°, whereas for the HEC/PVP/PE thin film, the peaks were observed at 20.89°. The small peak that appeared at 2θ = 11.07° became very weak after the addition of propolis. Also, a small peak (2θ = 11.07°) in the HPC/PVP/PE thin film disappeared. These changes showed the interactions between HPC/PVP and the OH groups of PE, confirming the good miscibility between the components.

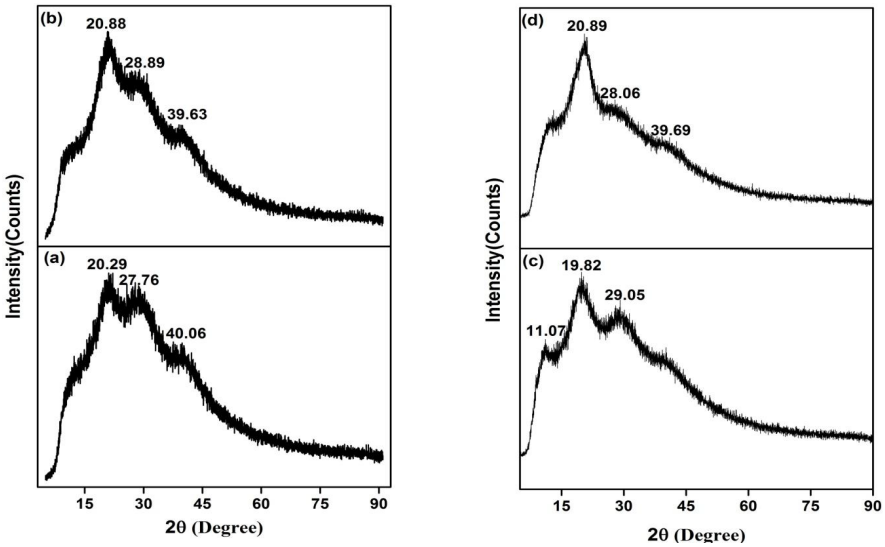

Figure 5: XRD patterns of (a) HEC/PVP, (b) HEC/PVP/PE, (c) HPC/PVP and (d) HPC/PVP/PE thin films

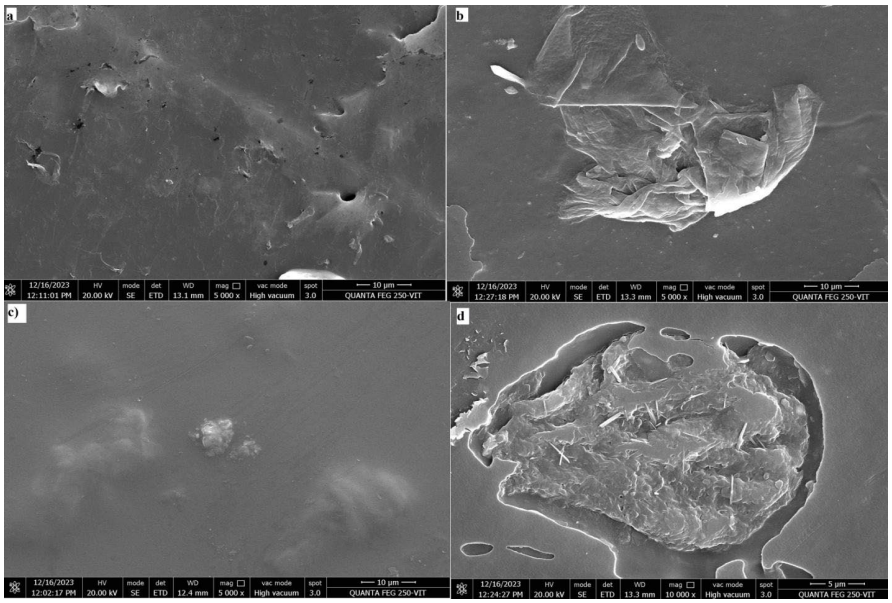

Figure 6: SEM images of (a) HEC/PVP, (b) HEC/PVP/PE, (c) HPC/PVP and (d) HPC/PVP/PE thin films

SEM analysis

One of the methods to observe the compatibility of components in a blend is through the surface morphological study. SEM images were recorded and compared following the addition of propolis extract to the cellulose derivatives/PVP thin films.

The SEM images in Figure 6 show that the hydroxy derivatives (HEC and HPC) blended with PVP are smooth and homogeneous. The surface morphology of the HEC/PVP/PE and HPC/PVP/PE thin films is illustrated in Figure 6 (b and d). It can be noted that the surface morphology of the thin films is significantly influenced by the addition of the propolis extract.[31] The surface of the films has become notably rougher, indicating a clear influence of the extract on the film structure.[32]

Also, the formation of cracks and lumps after the addition of honey bee propolis extract is noted. These significant changes that appeared in the surface of the films indicated the presence of volatile chemicals in the propolis extract. The surface of the thin films also exhibited uneven texture, attributed to the incorporation of the propolis extract.[33]

Antibacterial studies

The antibacterial behaviour of the prepared films, with and without propolis extract, against *Staphylococcus aureus* was examined and compared with that of Amphotericin as standard. The zone of inhibition of the films are shown in Table 2 at various concentrations. In Figure 7 (a-d), it is observed that the cellulose derivatives/PVP loaded with honey bee propolis extract was found to possess considerable antimicrobial activity against *Staphylococcus aureus*, but the zone of inhibition of HEC/PVP/PE is more significant than that of HPC/PVP/PE.

The inhibition zone percentage values of the prepared films against *S. aureus*, at three different concentrations, are shown in Figure 8. Propolis significantly enhances the antimicrobial activity of both films, with HEC/PVP/Propolis showing a higher increase, of 12%, in the inhibition zone, compared to 8% for HPC/PVP/Propolis, especially at the lower concentration of 500 µg/mL. This can be attributed to the existence of flavonoids and cinnamic acid derivatives found within the extract of honey bee propolis.[33,34,35]

Antioxidant activity using the DPPH assay

The DPPH scavenging assay was implemented to investigate the antioxidant characteristics of the synthesized thin films. DPPH represents a stable free radical that can be readily quenched, leading to its decolorization and a decrease in absorbance values attributed to antioxidants.[36,37] The compounds exhibiting antioxidant characteristics interact with DPPH radicals via hydrogen atom transfer or a single-electron transfer mechanism, which may be followed by a proton transfer mechanism.[38]

The mechanism of the DPPH scavenging assay can be depicted as follows:

$$DPPH^{\cdot} + RH \rightarrow DPPH - H + R^{\cdot} \quad (3)$$

The free radical DPPH interacts with the antioxidant donor molecule (RH), resulting in the formation of a neutral DPPH-H molecule. A hydrogen atom transfer reaction occurs due to electron transfer followed by proton transfer. The resultant free radical induces decolorization as the accumulation of electrons increases. The neutralization of the DPPH radical occurs through hydrogen atom transfer, which is followed by a colorimetric change in the solution.[39] The presence of DPPH causes a violet hue in a methanol solution, which fades to yellow due to the interaction with antioxidants.

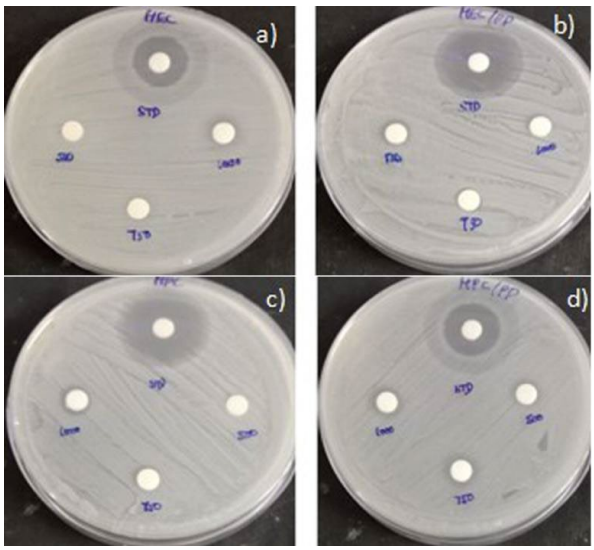

Figure 7: Antibacterial activity of the prepared films against *Staphylococcus aureus*; (a) HEC/PVP, (b) HEC/PVP/PE, (c) HPC/PVP and (d) HPC/PVP/PE thin films

Table 2
Zone of inhibition of bacterial development activity of the prepared films at various concentrations

Organisms	Cellulose derivatives based thin films	Zone of inhibition (mm)			
		Sample (µg/mL)			Standard (Amphotericin)
		1000	750	500	
Staphylococcus aureus	HPC/PVP	9	8	7	25
	HPC/PVP/PE	9	9	9	25
	HEC/PVP	9	8	7	25
	HEC/PVP/PE	11	11	10	25

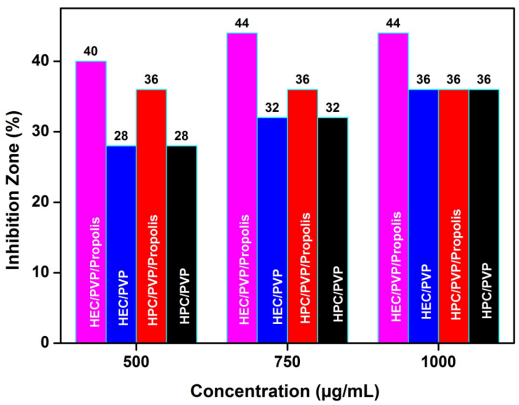

Figure 8: Antibacterial efficiency of the prepared thin films, with and without propolis extract

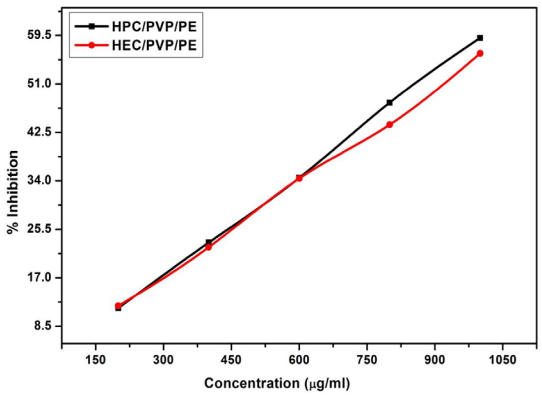

Figure 9: Antioxidant activity of HEC/PVP/PE and HEC/PVP/PE thin films

The antioxidant efficacy of HEC/PVP and HPC/PVP/PE thin films, recorded at a wavelength of 517 nm, is illustrated in Figure 9. The results indicated that an increase in the concentration of the cellulose derivative/PVP/PE thin films corresponded with an enhancement in DPPH scavenging activity. The scavenging activity induced by the DPPH radical in the synthesized thin films exhibited a concentration-dependent relationship.[40] The scavenging activity demonstrated a linear increase in correlation with the concentration of the thin films,[41] as depicted in Figure 9. HPC/PVP/PE and HEC/PVP/PE were found to exhibit a significant antioxidant activity with respect to the DPPH radical, recorded at 56.35% and 59.05%, respectively.

The EC50 value can be calculated from the graph using the principle of right-angled triangle.[42] The EC50 value for the HEC/PVP/PE thin film was determined to be 59 µg/mL, whereas that for HPC/PVP/PE was found to be 61, indicating that the HEC/PVP/PE thin film material demonstrates better antioxidant activity compared to the HPC/PVP/PE thin film. The presence of flavonoids and phenolic acids enhances the pronounced antioxidant activity, rendering them more suitable for antioxidant, anti-inflammatory, anticarcinogenic, and antibacterial applications.[41] Consequently, the results obtained suggest that the synthesized thin films are efficient in scavenging free radicals.[43,44]

Cytotoxicity assay

Vero cells were subjected to incubation on the synthesized films, with integrated propolis extract. The phenomenon of cell adhesion to the thin films was meticulously monitored for a duration of 24 hours subsequent to cell seeding. The quantitative assessment of cell adhesion was performed utilizing the MTT assay. The optical density (O.D.) values corresponding to the control and thin films at varying concentrations are delineated. The results demonstrated that both thin films exhibited a comparable pattern of concentration- and time-dependent cytotoxicity towards Vero cells, as evidenced by the observable decline in the percentage of cell viability. The cytotoxicity effect of prepared thin films at various concentrations on Vero cell line is shown in Table 3.

The graphs were plotted with the percentage of cell viability in relation to the concentration of the samples. Cell control and sample control were incorporated in each assay to ascertain the overall cell viability.[45] Figures 10 and 11 illustrate that the Vero control cells (untreated) present compact morphology, are healthy, with minimal necrotic or apoptotic bodies. Likewise, the Vero cells subjected to treatment with the prepared films infused with propolis extract exhibit a similar morphological appearance.

Table 3
Cytotoxicity of prepared thin films on Vero cell line

S.No.	Concentration (µg/mL)	Absorbance (O.D.)	HEC/PVP/PE Cell viability (%)	HPC/PVP/PE Cell viability (%)
1	1000	0.354	53.47	65.68
2	500	0.392	59.21	69.84
3	250	0.431	65.10	74.31
4	125	0.469	70.84	77.76
5	62.5	0.509	76.88	82.13
6	31.2	0.551	83.23	86.39
7	15.6	0.593	89.57	89.84
8	7.8	0.638	96.37	93.90
9	Control	0.662	100	100

Figure 10: Morphology of Vero cells after 24 h of contact with HEC/PVP/PE of different concentrations

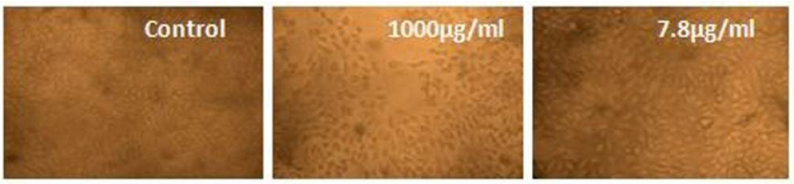

Figure 11: Morphology of Vero cells after 24 h of contact with HPC/PVP/PE of different concentrations

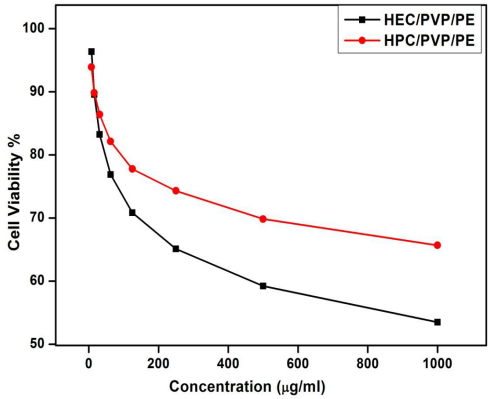

Figure 12: MTT assay results for HEC/PVP/PE and HPC/PVP/PE thin films of various concentrations

The cell viability of Vero cells is observed to increase as the concentration decreases. From Figure 12, it is discerned that at lower concentrations (7.8 µg/mL), the HEC thin film demonstrates 96% cell viability, whereas the HPC thin film displays 94% cell viability. This finding suggests that the sample is non-cytotoxic towards Vero cells. However, at concentrations above 500 µg/mL, both HPC/PVP/PE and HEC/PVP/PE thin films exhibit significant inhibitory effects and increased cytotoxicity. The percentage of cell viability is observed to decrease simultaneously with an increase in the concentration of the thin films.[46]

Normal Vero cell viability decreases with increasing the concentration of the thin films, which indicates that at higher concentrations, the thin films may cause toxicity in Vero cells.[16] However, at lower concentrations, both the prepared thin films exhibit the ability to promote the Vero cell growth. The cell viability remains above 90%, at lower concentration implying non-cytotoxicity and suitability for biomedical application.[47] Similar investigations have also been documented in the literature.[51]

Notably, the HEC/PVP/PE thin film exhibits diminished cytotoxicity towards Vero cells when compared with the HPC/PVP/PE thin film at lower concentrations. The morphological characteristics of the normal Vero cell lines treated with HPC and HEC thin films are presented in Figures 10 and 11, confirming that cell proliferation is markedly inhibited at elevated concentrations. These results demonstrate that the synthesized thin films exhibit minimal toxicity towards normal Vero cells at lower concentrations. The cell viability at higher concentration of 1000 µg/mL is between 53-65%, whereas at lower concentration of 7.8 µg/mL, the cell viability is around 90%. Hence, the synthesised thin films exhibit minimal toxicity towards normal Vero cells at lower concentrations.

CONCLUSION

This work develops novel bioactive polymeric materials based on two different cellulose derivatives (HPC and HEC) and an inorganic material like PVP. Honey bee propolis extract was integrated into the formulations. The biological properties of HEC and HPC based thin films were examined. The antioxidant activity results demonstrate that the HPC/PVP/PE and HEC/PVP/PE thin films are effective scavengers of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals. More than 50% antioxidant activity at the concentration of 1000 µg/mL against DPPH radicals was observed, indicating that the prepared polymeric thin films are suitable for bioactive food packaging. The cytotoxicity study by the MTT assay on Vero cells indicated that the prepared films showed higher cell viability at lower concentration, while at higher concentrations, some toxicity to Vero cells was recorded. These

findings reveal that the prepared thin films have potential in the development of bioactive materials.

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