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ORIGINAL PAPER

Active bio composites films based on PLA/olive wood flour (*Olea europaea* L.)/cinnamon essential oil

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Abstract

In this study, activated PLA/olive wood flour (OWF) composite films were prepared by a solvent casting process with different wood flour contents (0, 1, 3, 5 and 10 wt%). 3% Cinnamon Essential Oil (CEO) is incorporated into the biocomposite film to impart antimicrobial activity. OWF and CEO properties were examined using FTIR and total phenol quantification. Biocomposite films with and without CEO were tested by FTIR and tensile strength. The antimicrobial activity of biocomposite films was tested against four types of fungi and five types of bacteria. CEO and OWF extracts physically interact with PLA, affecting its mechanical and antimicrobial properties. In addition, only 1% of OWF and CEO achieved better performance. The best antifungal inhibition against *A. flavus* and *F. oxysporum* was observed in the case of 10% of OWF, and the best antibacterial activity for *P. aeruginosa* and *S. aureus* was observed for OWC0 films. The combined use of OWF and CEO showed synergistic antifungal properties. Overall, low content of OWF and CEO could bring antibacterial and antifungal activities to biocomposite PLA/OWF biofilms with satisfactory mechanical properties. The primary use of these films is cereal packaging applications.

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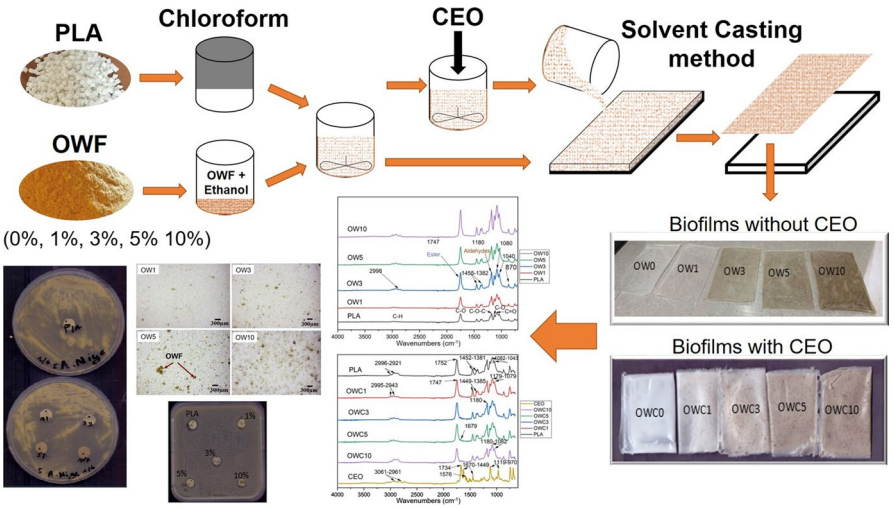
Graphical abstract



Keywords Antimicrobial activity · Cinnamon essential oil · Packaging film · PLA · Olive wood flour

Introduction

Biofilms used in active packaging have attracted the attention of the scientific community to extend food shelf life and warn consumers [1]. The African country is known for its humid and warm weather that allows the fungus to grow. Cereal products make up the diet of African populations. *Aspergillus, Penicillium, Fusarium*, and *Alternaria* grow on stored and freshly harvested grains [2]. In recent years, PLA-based biodegradable biocomposite films have been favored due to their renewability, biodegradability, and non-toxicity [3, 4], low cost, reproducibility, good physical and mechanical properties, and environmental friendliness, extensively studied for food packaging [5, 6]. The trend to use local waste to formulate new biocomposites is widely reported in the literature. Souissi et al. [7] highlighted the potential of using olive wood flour to enhance PP matrices, as well as Ou et al. [8] used cellulose isolated from rubberwood waste in a PLA biocomposite film. Wardhono et al. [9] formulated a PLA biocomposite membrane reinforced with bacterial cellulose nanocrystals isolated from commercial coconuts.

The olive tree (*Olea europaea* L.) is the most famous tree in Tunisia [10]: In 2018, more than 90 million olive trees covered 1.8 million hectares [11]. Wood is formed from a large number of carbohydrates such as cellulose, lignin and hemicelluloses [12]. Many interesting compounds have been isolated from olive wood, including extractive compounds which are terpenes, major constituents of conifer

resins, waxes, phenolic compounds such as lignans, stilbenes, flavonoids and tannins [13].

These phenolic compounds, including thujol, tropolone, and cinnamaldehyde, showed significant antifungal activity in vitro against biotic and abiotic attacks. The best known are troponoid and phenylpropanoid, which inhibit the growth of fungi against *Penicillium citrinum, Aspergillus niger, Postia placenta, Aspergillus flavus, L. sulphureus, T. versicolor* [14].

Olive wood extracts have high antibacterial activity against *Listeria monocytogenes*, the most deadly foodborne pathogen [15]. The antibacterial and antifungal properties may be closely related to phenolic compounds present in olive wood, such as oleuropein, hydroxytyrosol, tyrosol, vanillin, protocatechuic acid, benzoic acid, and p-coumaric acid [16]. Therefore, OWFs can be used as natural bioactive agents for dietetic, pharmacological and, more recently, food packaging applications.

A widely studied alternative to active packaging is the use of natural antimicrobial products, especially essential oils from herbs and spices [17], which are known for their antimicrobial effects and can be used to extend the shelf life of food products [18]. Its biological activity is related to the main functional components of EO (alcohols, phenols, terpenoids, and ketones) and their synergistic effects [19]. They are intended to treat candida infections, including *C. albicans, C. tropicalis, C. parapsilosis*, and *dermatophytes* [20, 21]. The antimicrobial activity of EO is closely related to the presence of phenols (i.e., cinnamaldehyde, citral, carvacrol, eugenol or thymol) [22]. Essential oils have been shown to be effective antimicrobial agents against several foodborne pathogens, including *Escherichia coli, Staphylococcus aureus,* etc. [23].

Cinnamon is a spice extracted from the inner bark of the tree. Its main constituents are cinnamaldehyde and eugenol; both are important agents of antimicrobial activity. Cinnamon is designated by the US Food and Drug Administration and is commonly used in antimicrobial food packaging [24]. Recently, a number of studies have reported the use of essential oils, including rosemary, myrtle, and thyme, in PLA composite film formulations. These biofilms were prepared by solvent casting and showed good antibacterial activity [25]. Many researchers have suggested the possible application of cinnamon essential oil (CEO) in PLA films for active packaging [26]. Suriani et al. [27] prepared active films based on PLA/chitosan/CEO. CEO showed improvements in tensile strength and antimicrobial inhibition against *Escherichia coli* and *Staphylococcus aureus*. Furthermore, Zhang et al. [28] prepared films with high antibacterial activity against *Escherichia coli* and *Listeria monocytogenes* with 100% inhibition. However, the release of cinnamaldehyde from the chitosan/alginate film was relatively slow; the total release period was only 7 days. A better strategy is to disperse sustained-release antibacterial agents into the film matrix to extend the antibacterial period of the composite.

This study is the first to evaluate the combination of olive wood flour and cinnamon essential oil in PLA biocomposite sheets for active biofilms. The first goal is to investigate the potential of OWFs in biofilm biocomposites and adapt the content in terms of mechanical, physical and antibacterial properties. The second goal was to focus on how essential oils synergistically confer antimicrobial activity with OWF. Olive wood flour and CEO were characterized by quantification

of chemical composition and phenolic content; biofilms with different formulations and OWF levels were formulated as a reference and then added with EO. We checked the interaction between the used components (CEO and OWF). The antimicrobial activity against a broad spectrum of fungi and bacteria was checked and the procedure was repeated after 14 days.

Chemicals, reagents and microorganisms

This study used polylactic acid (PLA) supplied by Nipovas in Sfax, Tunisia, chloroform (Cas 67-66-3) from Sigma-Aldrich as the solvent to dissolve PLA particles into a liquid state, and olive wood flour (OWF) from Tunisian olive wood collected from the manufacturer's wood waste. OWF has a moisture content of 15% and a size range of (50–500) µm. Ethanol with a purity of 98% was used as a dispersant for OWF.

A commercial essential Ceylon cinnamon bark (CEO) (Cinnamomumverum), with cinnamaldehyde as its major component, is certified by Ecocert FR-BIO-01. Polyoxyethylene (tween 80) is used as a solvent for essential oil.

Antimicrobial biofilm tests were performed using bacterial and fungal strains obtained from the international culture collections (ATCC).

Bacterial strains included Gram-positive bacteria: *Staphylococcus aureus* ATCC 6538, *Listeria monocytogenes* ATCC 19,117, and *Bacillus cereus* ATCC 14,579, as well as Gram-negative: *Salmonella enterica* ATCC 14,028 and *Pseudomonas aeruginosa* bacteria ATCC 9027.

Antifungal activity was also evaluated against *Aspergillus niger* (F38), *Fusarium oxysporum* CTM: 10.402, *Aspergillus flavus* and *Alternaria solani*. All the microbial strains used for the antimicrobial test come from the collection of Laboratory of Molecular Biotechnology of Eukaryotes (LBME, CBS, Tunisia).

Active biocomposites films preparation

Films were prepared by solvent casting using chloroform as solvent. We followed the same protocol used by Tang et al. [29]. Briefly, 2 g PLA was dissolved in 50 ml organic solvent, and different mass fractions (0, 1, 3, 5, and 10 wt%) of OWF powder containing 2% ethanol were added, denoted as OW0. OW1, OW3, OW5 or OW10 films. On the other hand, 3% CEO was added to obtain PLA/OWF/CEO biofilms, named OWC0, OWC1, OWC3, OWC5, and OWC10, with OWF (0, 1, 3, 5, and 10 wt%), respectively. The OWF was shaken vigorously and stirred at room temperature (25 °C) using a magnetic stirrer for 8 h. The solution was then poured into greased glass molds (170 mm × 110 mm) and allowed to dry at room temperature for 24 h. After the solvent evaporated overnight, biofilms were obtained by slowly peeling off the plates as shown in Fig. 1.

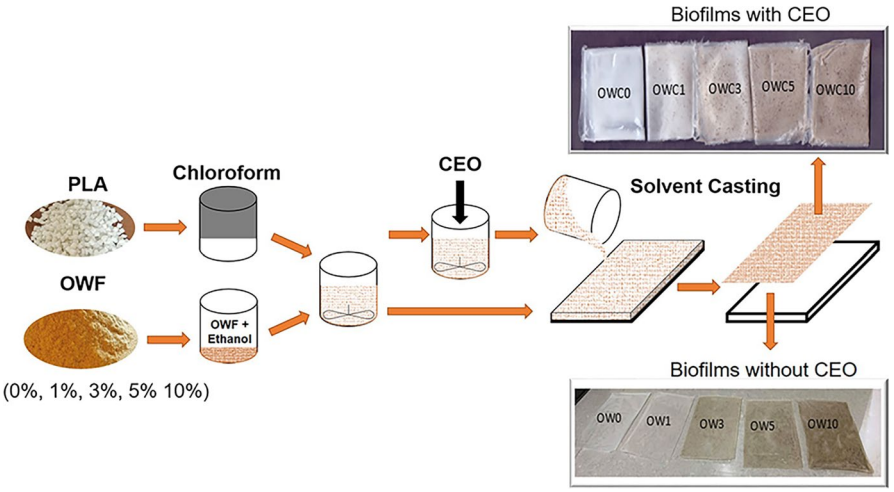


Fig. 1 Solvent casting method for the formulation of the biofilms

Chemical composition of olive wood flour

The chemical composition of OWF was determined according to ASTM standard tests [30]. The extractives were removed via an extraction process using a Heidolph VV2000 rotary evaporator from the Netherlands. The extraction was carried out using an organic solvent mixture: ethanol/toluene (1:2 v/v). The 6 g of dried OWF was treated with 300 ml of ethanol/toluene mixture: (100 ml of ethanol and 200 ml of toluene), pectin (ASTM D1110-84), lignin (ASTM D1106-96), holo-cellulose (ASTM D1104-56), alpha-cellulose (ASTM D1103-60); as for the hemicelluloses, they were determined by subtracting the holo-cellulose with α-cellulose content and ash (ASTM D1102-84).

Fourier transform infrared spectral analysis (FTIR)

The infrared spectroscopy analysis of the different biofilms, wood extractives and CEO were recorded using a Thermo Scientific Nicolet iS5 FTIR spectrophotometer from India. The samples were placed in ATR (attenuated total reflectance) mode on a germanium crystal and the scanning range was between 600 and 4000 cm^{-1} with a resolution of 2 cm^{-1}.

Determination of total phenolic content

The Total Phenolic Content (TPC) in cinnamon essential oil extracts and OWF extractives was determined using the Folin–Ciocalteau reagent (Singleton, Rossi, 1965) [31]. The CEO and OWF extractives were diluted to an appropriate

concentration for analysis. 50 µl of the 20-fold diluted extract was added to 250 µl of Folin–Ciocalteau and 500 µl of sodium carbonate (20%). After vortexing, the volume was adjusted to 5 ml with distilled water and the mixture was incubated for 30 min at room temperature. Measurement of optical density (OD) at $\lambda = 727$ mm was performed using a spectrophotometer (UV-1650PC Shimadzu, Japan). Distilled water was used as a blank to adjust the zero.

Mechanical properties

The thickness of the different biofilms was measured using an OTMT STAINLESS HARDNED digital micrometer with a sensitivity of 0.01 mm, and three random positions were taken into account for each film. Average values are used to calculate mechanical parameters. The mechanical properties of PLA biocomposite films, including UTS tensile strength (MPa), elastic modulus (MPa) and elongation at break (%), were evaluated using an Adamel Lhomargy DY35XL tensile machine manufactured in France, equipped with 250 N according to ASTM D638 [32], with a load cell at a speed of 5 mm/min. The thickness of the samples was between 0.1 and 0.3 mm. The initial distance between the clamps was 22.9 mm. The sample is 60 mm length and 30 mm width. The reported values are the average of six measurements.

Antimicrobial activity of PLA films

Antibacterial properties were tested using the agar disk method (ADM). Immerse a disk of agar in a sterile saline solution to create a uniform distribution of bacteria. Then, place the disk in a petri dish and add a sample of the bacteria to the agar. After 24 h, the bacteria are ready for testing. The process is normalized to 0.5 McFarland standards; each bacterial sample should contain 108 CFU per milliliter. The bacteria culture process is also normalized to 1% yeast extract, 0.5% peptone, and 0.5% NaCl in Luria Broth LB liquid medium. Different sizes of biofilm disks were measured in order to estimate the inhibitory effects of prepared films with different rates of OWF 1%, 3%, 5% and 10%. Films prepared with different levels of OWF were punched into 6 mm holes using paper punches under sterile conditions and placed on the support surface solid. After growth for 24 h at 30 °C, the inhibitory effect of the biofilm generated was estimated by measuring the clear zone for each strain tested minus the size of the biofilm disk [33]. In order to determine the antifungal activity of the biofilm, the fungal strains were first cultured on solid potato dextrose agar (PDA, Merck) for 72 h at 30 °C. The fungal spores produced were collected under sterile conditions, counted and suspended in a 20% glycerol solution to reach a final concentration of 10–5 CFU mL-1. 100 µl of each spore suspension were spread on PDA plates then incubated for 48 h at 30 °C. The determination of the inhibitory effect of activated biofilms was the same as in the antibacterial test.

Statistical analysis

SPSS software was used to perform the analysis of variance (ANOVA) on the experiment results obtained. Tukey's test was used to make comparisons of means at a significance level of 0.05 ($p \leq 0.05$).

Results and discussion

Chemical composition of olive wood flour

Figure 2 shows the chemical composition of the olive wood flour (OWF). The main components of wood are cellulose, hemicelluloses, and lignin.

The first thing to note is that OWF has relatively high cellulose content (40.46%). These values are similar to those in bibliography [34, 35]. The second important observation relates to the measured lignin content of OWF, which is of the order of $17\% \pm 1$, which is an important value. However, this percentage is lower than for other woods, which can reach up to 38% for mahogany and 34% for cedar [36]. Lignin is the most chemically stable part due to the strong carbon–carbon bonds. Furthermore, it also plays an important role in the protection of hemicelluloses and celluloses [36, 37]. The hemicelluloses content of olive wood was found to be 20.87%. It is also related to moisture content and wood hygroscopicity [38]. Another important link is the ash content, which accounts for 10.34%. The pectin content is 5.16%, and it is usually combined with other compounds such as cellulose and lignin [39]. The extractives represent only 7.02% of the olive wood but are an important source of diverse molecules that are putatively bioactive. This will be further confirmed by the determination of total phenols and antimicrobial analysis of the OWF extractives below. The inhibition of fungal growth is one of the most interesting properties of wood extractives in a context of wood preservation [40].

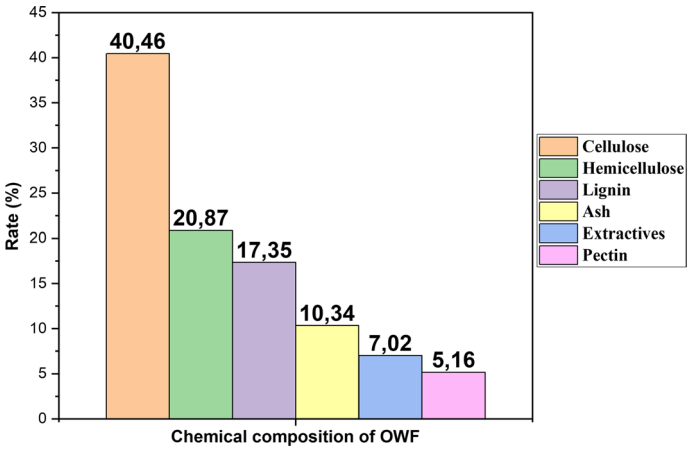


Fig. 2 Chemical composition of OWF

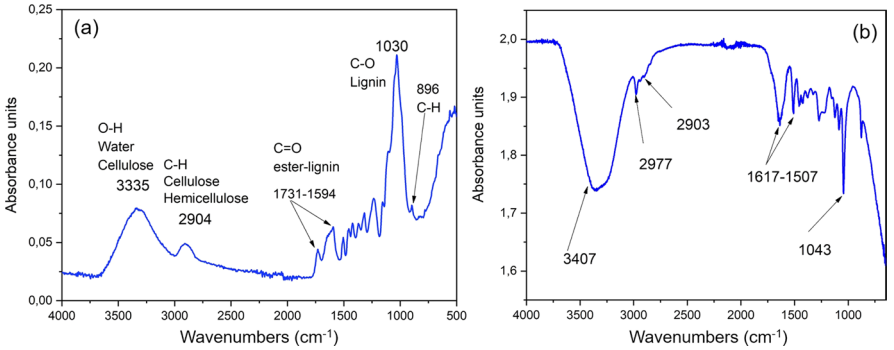


Fig. 3 Fourier transform infrared spectrum of the OWF (**A**), FTIR of wood extractives (**B**)

Table 1 Absorption bands assignments for the OWF [41–45]

Wave number (cm^{-1})	Group	Component
3000–3800	O–H	Water cellulose
2904	C–H	Cellulose hemicellulose
1731–1747	(Carbonyl, ketone and ester) C = O	Hemicellulose, lignin
1616	O–H hydroxyls	Water
1594	C = O	Lignin
1455–1422	C–H	Hemicellulose, lignin, cellulose
1370	C–H, polysaccharides	Cellulose, hemicellulose
1318	$C–H_2$	Cellulose
1235–1158	C–O–C de la liaison phénol-éther	Cellulose hemicellulose
1030	C–O	Lignin

Fourier transform infrared spectral analysis (FTIR)

Figure 3A shows the FTIR spectrum of OWF; it can be observed that there is a broad and strong band at about 3335 cm^{-1}, which is attributed to the different OH segments of water and cellulose. Another band at about 2904 cm^{-1} is associated with asymmetric and symmetric methyl stretching modes for all wood components, especially cellulose and hemicelluloses (Table 1). In fact, OWF is rich in polysaccharide and ester functions, as well as ketone and carbonyl groups due to the presence of cellulose, hemicelluloses and lignin components. The spectrum of the OWF extract (Fig. 3B) shows two images at 2977 $cm^{-1}$and 2903 cm^{-1}: This may be due to the high content of extracts from this wood, such as fatty acid methyl esters and phenolic resins compound. According to the literature, fatty acid methyl esters and phenolic acids contain methyl and methylene groups [41]. In addition, due to the complexity of its composition, multiple peaks appeared in the range of 2400–1500 cm^{-1}in Fig. 3B.

Some compounds in the extractives could be absorbed at about 1617 cm^{-1} and 1507 cm^{-1}, which may be due to the benzoic acids present in the wood tannins

that contain aromatic rings in their structure [41]. It can be seen from the literature that there is no significant peak change at the characteristic absorption peak of cellulose, hemicelluloses and lignin as shown in Table 1.

Biofilm PLA/OWF and PLA/OWF/CEO showed similar FTIR spectra to pure PLA (Fig. 4). The peak intensity at 1747 cm^{-1} increases from OW0 to OW10, assigned to ester functional groups and carbonyl groups; this is due to the increase in non-cellulosic elements, mainly lignin and hemicelluloses. Similar increases in the intensity of these peaks have been reported in previous studies [42, 43]. Interestingly, the peak intensity increases due to increasing OWF content. In fact, the interaction between OWF's cellulose and PLA matrix affects the carbonyl (C = O) bending peak. The peak shift shows the interaction between the oxygen in the carbonyl of PLA and the hydrogen in the hydroxyl of cellulose [46, 47]. The FTIR

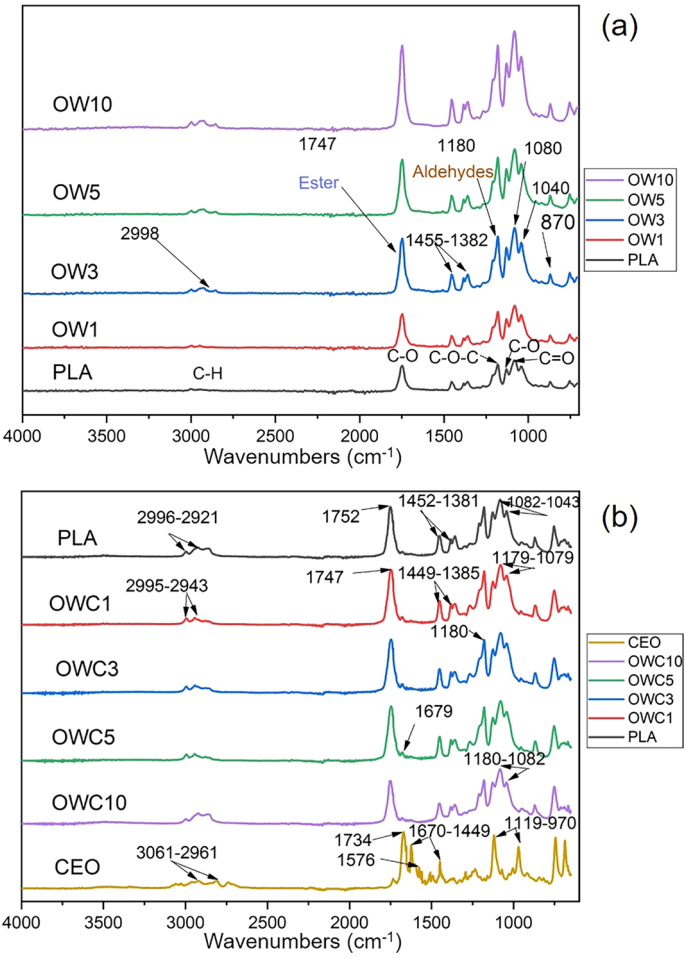


Fig. 4 FTIR spectroscopic analysis of the different films developed: PLA/OWF films (**A**), PLA/OWF/CEO films (**B**)

of different biofilms PLA/OWF/CEO was analyzed. The CEO's FTIR showed characteristic bands (Fig. 4.B). A broad spectrum is observed in the footprint region between wavelengths 1800–600 cm^{-1} and in the hydroxyl/CH region between 3500 and 2800 cm^{-1}. In this region (C–H and O–H region), the peak at 2924 cm^{-1} can be attributed to the symmetric C–H stretching vibrations that are caused by the lipids of cinnamon (essential oil) and the peaks at 3061 cm^{-1} are absorption peaks of the O–H stretching vibration. These peaks are present in biofilms OWC0 to OWC10 with some shifts that clearly show the interaction between the CH groups of the CEO lipids with the cellulose hydroxyls. The peak at 1734 cm^{-1} represents the carbonyl bond and is attributed mainly to aldehydes of saturated fatty acids. The peaks at 1679 cm^{-1} and 1626 cm^{-1} are attributed to the stretching vibration of a C═O aldehyde carbonyl, which corresponds to aldehydes such as coumarin, cinnamaldehyde, and other aldehydes found at high levels in cinnamon bark. The peak at 1734 cm^{-1} is shifted to 1747 cm^{-1} in OWC0 to OWC10 biofilms that is due to the presence of C–O esters and carbonyls with the appearance of a small peak around 1679 cm^{-1} which is due to the good CEO interaction in PLA /OWF films. The peak at 1576 cm-1 corresponds to the vibration of the C═C backbone of an aromatic ring, normally associated with eugenol, a phenol found in cinnamon oil. The peak at 1449 cm^{-1} is described by the absorption of the bending vibration of a C–OH alcohol that is observed in the different biofilms. The peak at 1248 cm^{-1} is due to the symmetric expansion of the C–O–C aromatic acid ester and the > C–OH stretching vibration of the phenols. These peaks collectively represent the characteristics of eugenol and esters in cinnamon bark [48].

Determination of total phenol content

The total phenol content of the extract was determined by the Folin-Ciocalteu test and calculated from the equation of the gallic acid standard curve ($y = 0.0017x$). The total phenolic content (TPC) of cinnamon essential oil and OWF extracts was expressed as gallic acid equivalents. The OWF extract had the highest total phenolic content of 759.23 ± 25.7 µg GAE, and the cinnamon bark had a total phenolic content of 687.69 ± 25.3 GAE, similar to that found by Chia et al. [30] (658.40 ± 4.383 µg GAE), but higher than other types of OWF [49, 50].

Mechanical testing

In order to study the effect of adding cinnamon essential oil on the mechanical properties of PLA/OWF composite films, tensile tests were carried out. The elastic modulus, elongation at break and stress at break of various composite materials are shown in Table 2. PLA is a brittle rigid polymer with very low plastic deformation capacity (≈3%). Incorporation of OWF in PLA showed mechanically different behavior from PLA.

The average thickness of the neat PLA films was 194 ± 14 µm; it increased significantly with increasing OWF ratio, resulting in films with rough surfaces. Adding 1% OWF can significantly improve the tear strength by 31.07% compared with pure

Table 2 The tensile characteristics of the PLA/OWF biocomposites

Specimens	Elastic modulus (MPa)	Tensile strength (MPa)[a]	Elongation at break (%)[b]	Thickness (µm)
OW0	1042.68 ± 98	22.62 ± 6.6	3.5 ± 0.3	170 ± 10
OW1	1199.18 ± 94	29.65 ± 11.5	3.27 ± 1.4	200 ± 5
OW3	1165.86 ± 97	17.73 ± 4.5	2.27 ± 0.5	200 ± 20
OW5	975.07 ± 100	20.73 ± 8.1	3.07 ± 0.3	240 ± 15
OW10	810.38 ± 109	17.48 ± 7.7	3.85 ± 1	250 ± 20
OWC0	1174.34 ± 95	30.2 ± 6.5	3.5 ± 0.3	150 ± 10
OWC1	1392.85 ± 90	32.9 ± 10	3.4 ± 1.4	170 ± 5
OWC3	1112.41 ± 100	16.5 ± 5	2.1 ± 1	170 ± 20
OWC5	1030.26 ± 105	22.5 ± 8.3	3 ± 0.3	190 ± 15
OWC10	931.92 ± 109	27.1 ± 7.5	4.6 ± 1	200 ± 20

According to ANOVA, analysis of variance

[a]Significant test with 99% confidence level ($\alpha = 0.01$)

[b]Test not significant ($\alpha = 0.02 < 0.05$)

PLA film (22.62 ± 6.6 MPa). This could confirm the good interfacial interaction of OWF and PLA matrix, as evidenced by FTIR analysis. However, the tensile strength started to decrease with the addition of OWF. This may be due to aggregation of the filler observed in Fig. 5. Furthermore, the addition of OWF resulted in improved Young's modulus values, especially for OW1. It reached 1199.18 ± 94 MPa (a 15% increase) before starting to drop to 810.38 ± 109 MPa for OW10. Also, adding reinforcement does not change the elongation at break, which is within the range (3% and 4%).

As can be seen in Table 2, there are no significant differences for PLA/OWF films when 3% CEO is added. However, similar behavior is observed for Young's modulus, tensile strength, and elongation at break, in which the formulations do not show statistically significant differences, except in the case of OWC1 film. The addition of CEO slightly improves the tensile strength in particular with a gain of 10.07%, compared to OW1 film (29.65 ± 11.5 MPa), and the value of Young's modulus, which reached 1392.85 ± 90 MPa (16.15% increase), then it starts to decrease to 931.92 ± 109 MPa for OWC10 as mentioned above for PLA/OWF films. In fact, OWC1 leads to the best compromise between tensile strength and Young's modulus.

Antimicrobial activity of biofilms

Using the disk method, the PLA/OWF sheet is first placed on the surface of the medium. Figure 6 shows the antifungal activity of PLA/OWF/CEO films with different OWF ratios: 1%, 3%, 5% and 10% against *A. niger* (A), *A. solani* (B), *F. oxysporum* (C) and *A. flavus* (D). The addition of 3% CEO to the PLA/OWF film showed a clear zone of inhibition around and below the film (Fig. 6). OWC10 had the best antibacterial effect on *A. flavus and F. oxysporum* with a zone of inhibition of 20 ± 2 mm; the biofilm remained active even after 14 days, but the

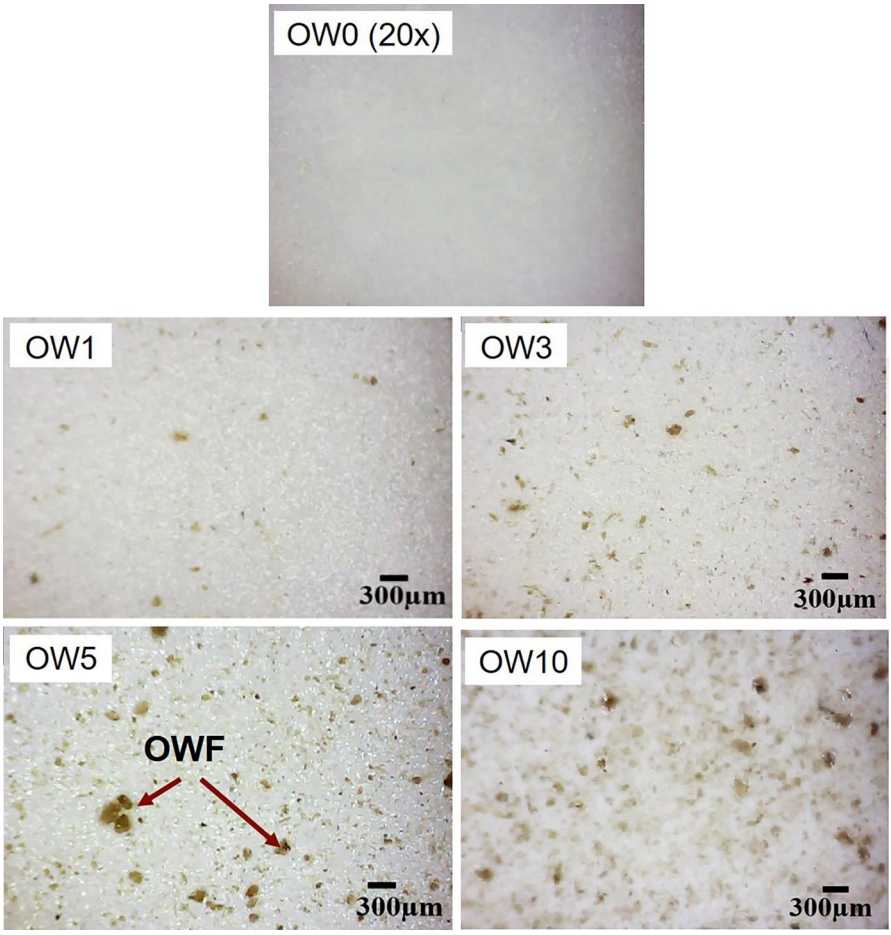


Fig. 5 Optical observation of PLA/OWF films: OW0, OW1, OW3, OW5 et OW10

decline ranged from 10 to 50%, as shown in Table 3 This is most common among CEOs, as shown in [51]. The antifungal efficacy of cinnamon essential oil against *A. flavus* has also been demonstrated in other studies [52]. With the increase in OWF content, the antifungal activity increased obviously. On the one hand, this could be due to the good interaction of PLA/OWF and CEO, justified by previous FTIR analysis, and on the other hand, due to the phenol-rich OWF composition. The wood extracts tested in Table 3 showed antifungal activity at a diameter of 13 mm, especially against *Aspergillus flavus*. These results indicated that the chemical composition of the wood flour extract contained interesting bioactive

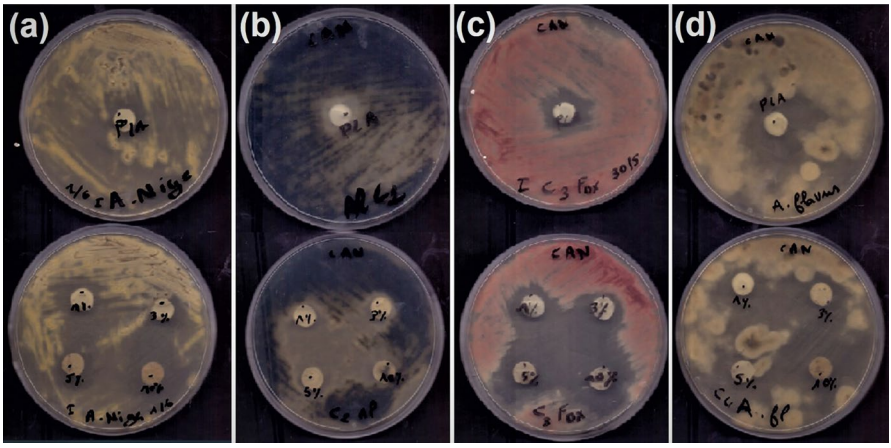


Fig. 6 Antifungal activity of PLA/OWF/CEO films with different OWF rates: 1%, 3%, 5% and 10% on *A. sniger* (**A**), *A. solani* (**B**), *F. oxysporum* (**C**), *A. flavus* (**D**)

elements, as indicated by FTIR analysis and phenol content testing [53]. However, the lignin and extraction rates in OWF were about 17.35% and 7.02%, respectively. It is well known that native lignin in plants plays an important role in plant defense by providing antibacterial, antifungal, antiviral, antioxidant, insecticidal and antifeeding properties. The origin of the antimicrobial properties of lignin has been attributed to the phenolic subunits that constitute the polyphenolic structure of lignin [54].

According to Ateş et al. [49], Flavonoids and phenolic compounds in olive extracts are good inhibitors of wood rot fungi. However, other studies have explained the increased antifungal activity because wood is influenced not only by its molecular structure, but also by a group of biological parasites that provide natural antifungal defenses [55]. In terms of antifungal activity, we can conclude that OWF and CEO act synergistically. To investigate the effect of biofilms on bacterial activity, we referred to the same method used for antifungal activity. Figure 7 illustrates the activity of PLA/OWF films with different contents of olive wood flour against *(S. aureus), (S. enterica), (P. aeruginosa), (B. cereus), and (L. monocytogenes)*. All biocomposite films without CEO are not antimicrobial against all bacteria.

However, the PLA/OWF/CEO biocomposites films show a significant antibacterial activity, especially for OWC0 (PLA/CEO) with the best inhibition observed against *P. aeruginosa* and *S. aureus* with a diameter of 17 ± 1 and 16 ± 1 mm, respectively (Table 4 and Fig. 7), which persisted even after 14 days, the decline ranged from 20 to 50%. By varying the OWF rate, no significant changes in antibacterial activity were recorded, but different formulations of the films had similar activities. However, Table 4 shows that OWF extracts have low antibacterial

Table 3 Antifungal activity of biofilms against fungus

Types of fungus	Antimicrobial agents			OWF rate	Inhibition diameter	Diameter after 14 days	Antifungal test
A. niger	CEO	Cst	10,700 ppm	OWC0	8 ± 1	7 ± 1	+
				OWC1	10 ± 1	9 ± 0.5	+
				OWC3	11 ± 1	10 ± 1	+
				OWC5	14 ± 1	11 ± 2	+ +
				OWC10	16 ± 2	9 ± 2	+ +
	Extractives			*	–	–	–
Alternariasolani	CEO	Cst	10,700 ppm	OWC0	7 ± 2	4 ± 1	+ +
				OWC1	10 ± 1	5 ± 1	+
				OWC3	7 ± 1	5 ± 1	+
				OWC5	14 ± 1	9 ± 1	+ +
				OWC10	10 ± 1	9 ± 2	+
	Extractives			*	5 ± 1	–	+
F. oxyporium	CEO	Cst	10,700 ppm	OWC0	9 ± 1	8 ± 1	+
				OWC1	10 ± 1	9 ± 1	+
				OWC3	12 ± 1	9 ± 1	+
				OWC5	17 ± 1	11 ± 1	+ +
				OWC10	20 ± 2	10 ± 2	+ +
	Extractives			*	3 ± 1	–	+
A. flavus	CEO	Cst	10,700 ppm	OWC0	15 ± 1	10 ± 1	+
				OWC1	10 ± 1	4 ± 1	+
				OWC3	15 ± 1	5 ± 1	+ +
				OWC5	15 ± 1	8 ± 1	+ +
				OWC10	20 ± 2	8 ± 2	+ +
	Extractives			*	13 ± 1	–	+

activity, especially against *S. aureus*, with a diameter of 10 ± 1 mm. Therefore, we recommend that only the CEO has primary responsibility for this activity. CEO has potential antibacterial activity against *S. aureus* which is considered the most common foodborne pathogen [56, 57]. According to Ranjbaryan et al. [44], the CEO has a significant inhibition against *S. aureus, E. coli, S. enteritidis and P. aeruginosa* used for sodium caseinate-based antibacterial films. Antimicrobial activity against Gram-positive *S. aureus* was recorded in cinnamon essential oil on PLA/chitosan/CEO films [27].

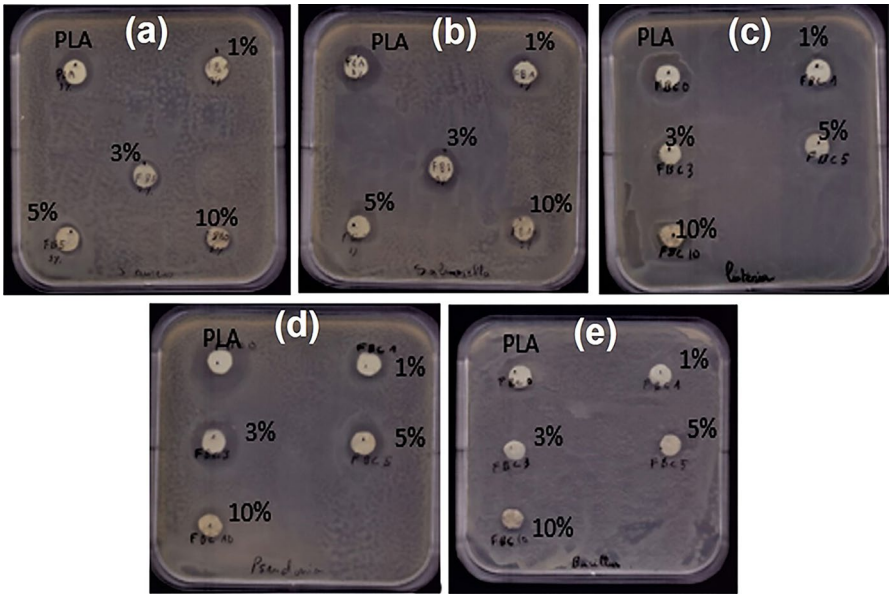


Fig. 7 Antibacterial activity of PLA/OWF/CEO films with different OWF rates: 1%, 3%, 5% and 10% on *(S. aureus), (S. enteric), (P. aeruginosa), (B. cereus), (L. monocytogenes)*

Conclusion

In this work, the potential of essential oils and olive wood flour in active biofilm properties was investigated. Addition of CEO to OW1 increases tensile strength and elastic modulus. PLA/OWF/CEO film is the active ingredient; OWC0 has the best antibacterial inhibitory effect on *P. aeruginosa and S. aureus*. OWF content did not affect this activity, although OWF extracts had antibacterial activity against *S. aureus*. The results also showed that the antifungal activity against *A. flavus and F. oxysporum* was better when the OWF content was increased to 10%, as the interaction between the PLA and OWF improved and the lignin and extractives of the OWF also increased.

The results suggest that the cinnamaldehyde in the CEO played a role in the antimicrobial activity, and that the OWF and CEO worked together to enhance the antifungal activity. The biofilms were found to remain active even after 14 days, with some decline in activity, but still showing good results overall. These results demonstrate that the combination of CEO and OWF can be a promising solution for cereal packaging materials, as it provides a good balance between mechanical properties and antimicrobial activity.

Table 4 Antibacterial activity of biofilms against bacteria

	Agents			OWF rate	Inhibition diameter	Diameter after 14 days	Antifungal test
P. aeruginosa	CEO	Cst	10,700 ppm	OWC0	17 ± 1	14 ± 1	+ +
				OWC1	10 ± 1	5 ± 0.5	+
				OWC3	10 ± 1	6 ± 1	+
				OWC5	8 ± 1	5 ± 2	+
				OWC10	5 ± 1	5 ± 2	+
	Extractives			*	2 ± 1	–	+
S. aureus	CEO	Cst	10,700 ppm	OWC0	16 ± 1	7 ± 1	+ +
				OWC1	12 ± 1	3 ± 1	+
				OWC3	6 ± 1	5 ± 1	+
				OWC5	8 ± 1	4 ± 2	+
				OWC10	10 ± 1	3 ± 2	+
	Extractives			*	10 ± 1	–	+
S. enteric	CEO	Cst	10,700 ppm	OWC0	10 ± 1	10 ± 1	+
				OWC1	5 ± 1	5 ± 1	+
				OWC3	6 ± 1	3 ± 1	+
				OWC5	5 ± 1	5 ± 1	+
				OWC10	6 ± 1	6 ± 1	+
	Extractives			*	5 ± 1	–	+
A. cereus	CEO	Cst	10,700 ppm	OWC0	10 ± 1	10 ± 1	+
				OWC1	3 ± 1	3 ± 1	+
				OWC3	3 ± 1	3 ± 1	+
				OWC5	5 ± 1	5 ± 1	+
				OWC10	3 ± 1	3 ± 1	+
	Extractives			*	5 ± 1	–	+
L. monocytogenes	CEO	Cst	10,700 ppm	OWC0	12 ± 1	10 ± 1	+ +
				OWC1	5 ± 1	5 ± 1	+
				OWC3	10 ± 1	5 ± 1	+
				OWC5	6 ± 1	5 ± 1	+
				OWC10	7 ± 1	6 ± 1	+
	Extractives			*	5 ± 1	–	+

+++ total inhibition, ++ significant inhibition, + medium inhibition, —no activity,* not defined

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Declarations

Conflict of interest The authors declare that they have no conflict of interest.

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