

# Antimicrobial properties of citrus waste-infused used cooking oil (UCO) shellac

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## ABSTRACT

The used cooking oil (UCO) and leftover citrus were combined to create a UCO shellac infused with potent antimicrobials. By converting oil and citrus waste into a value-added product, this process significantly contributes to waste reduction efforts. The efficiency of the shellac formulation was demonstrated by assessing the bactericidal activity of shellac made from UCO infused with orange (*Citrus sinensis*), lemon (*Citrus limon*), and key lime (*Citrus aurantifolia*) peel waste. Fourier-Transform Infrared Spectroscopy (FTIR) analysis revealed that the UCO shellac infused with citrus waste contained functional groups such as hydroxyl, alkyne, carboxylic acid, conjugated acid, aromatic ring, and primary alcohol, which contribute to its antimicrobial properties. Among the formulations evaluated, the lemon-infused UCO shellac had the highest ascorbic acid content (0.9 mg/L). When compared with other shellac formulations, including UCO infused with orange or lime waste, as well as a control shellac, the lemon-infused UCO shellac proved to be the most effective in inhibiting bacterial growth on the agar plate. On the plate treated with the lemon-infused UCO shellac, bacterial colonies started to form and begin expanding after 96 hours of culturing. This indicates that the infusion of discarded lemon peel into UCO yielded the shellac with the strongest antibacterial properties.

## 1. INTRODUCTION

Used cooking oils and fats are commonly sourced from the food processing industry, including restaurants, fast-food establishments, and households. Used cooking oil (UCO) is typically classified as domestic waste. Unfortunately, uninformed individuals often dispose of old cooking oil improperly by pouring it down sinks or drains, leading to clogged pipes and flash floods. Even though UCO should be properly managed to minimize its adverse effects on human health and the environment, consumers frequently dispose of it

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through sinks, bins, drains, toilet bowls, or directly into nearby water bodies and lands (Kabir et al., 2014). However, leftover cooking oil has several other potential uses, such as in the production of soap, candles, renewable energy, or even shellac. Currently, there is a lack of research on using UCO in shellac formulations, making this study significant for exploring UCO's potential in producing shellac.

Shellac is a natural resin produced by the female lac insect (*Kerria lacca*), native to Southeast Asia and India. The resin is collected, processed, and refined to create shellac, a versatile and widely used coating material. Shellac coatings have been used for centuries and remain popular for wood treatment because they enhance the natural beauty of wood grain. The resulting finish has a warm, amber tone that highlights the depth and character of the wood. Shellac can be used as a varnish, polish, or sealer (Bashir et al., 2022) and is available in various forms, including flakes, buttons, or liquid, depending on the intended use. One of the most notable advantages of shellac is its quick drying time. When applied, the alcohol solvent in shellac evaporates rapidly, leaving a hard and glossy surface. Before application, shellac flakes or buttons must be dissolved in alcohol (typically ethanol or denatured alcohol) to form a shellac solution. The ratio of shellac flakes/buttons to alcohol can be adjusted to achieve the desired strength of the solution. A common concentration, known as a 1-pound cut, involves dissolving one pound of shellac flakes in one gallon of alcohol, resulting in a solution that is easy to apply as a thin coating. Higher concentrations (e.g., 2-pound or 3-pound cuts) can be prepared for a thicker or more protective finish (Kumar et al., 2023).

Despite its many advantages, shellac has some drawbacks. It is not as durable as many synthetic coatings, and its protective properties may degrade over time. Wood, being an organic and heterogeneous material, is particularly susceptible to mold and blue-stain fungi growth under certain exposure conditions. The durability of wood-based products depends on factors such as wood quality, processing methods, structures, and end-use conditions. In end-use conditions, humidity—specifically, the amount and duration of moisture stress—combined with temperature and exposure time, are critical factors in the growth of mold and blue stain on wood surfaces. Weathering processes further reduce the resistance of wood-degraded surfaces to water movement and biological attacks by microorganisms such as fungi, insects, molds, and bacteria (Carmen-Alice Teacă et al., 2019).

Microorganisms in the environment typically do not exist as individual cells; instead, they prefer to form aggregates known as microbial granules (Liu et al., 2004). These aggregates often adhere to interfacial surfaces, such as liquid/liquid, solid/air, and air/liquid interfaces. In soil and aquatic environments, microorganisms engage in self-immobilisation processes, with cell surface hydrophobicity (CSH) playing a crucial role (Wu et al., 2012). High CSH enables microbes to attach to hydrocarbon droplets on surfaces or within cells, allowing them to move from the aqueous phase into the organic hydrocarbon phase, where waste is broken down by enzymes and biosurfactants (Kaczorek et al., 2008). A pathogen is an organism that causes disease in its host, with "virulence" referring to the severity of the disease's symptoms. Pathogens span a wide taxonomy, including bacteria, viruses, and both unicellular and multicellular organisms, and they infect every living creature. They are also targeted by specialist viruses known as phage. The World Health Organisation (WHO) (2020) has identified COVID-19 as a hazardous virus causing respiratory tract infections. According to Hajimin et al. (2020) and Shereen et al. (2020), COVID-19 is an infectious illness known as severe acute respiratory syndrome coronavirus (SARS-CoV) and Middle Eastern respiratory syndrome coronavirus (MERS-CoV). Discovered in late 2019 in Wuhan, China, the COVID-19 pandemic has since spread globally. The prolonged duration of the COVID-19 pandemic has significantly impacted politics, socioeconomics, health, and education systems in many countries, including Malaysia (Abdul Rashid et al., 2020). Viruses must infiltrate host cells to replicate, and they are adept at exploiting all of our bodies' defences. However, many of the viruses living inside our bodies do not attack our cells; instead, they target microorganisms in our microbiomes. These viruses, known as bacteriophages or phage, invade bacterial cells, utilize the internal machinery to replicate themselves, and then burst out to infect additional bacteria, destroying their host cells in the process (Pride, 2020). Therefore, this study focuses exclusively on antimicrobial properties.

Several studies have shown that vitamin C possesses antimicrobial properties, reducing the risk of infections and having immunomodulatory functions, particularly at high concentrations. The minimum inhibitory concentration (MIC) of ascorbic acid or antibiotics alone and in combination against all isolates was measured using the micro-dilution technique, following the Clinical and Laboratory Standard Institute (CLSI) criteria (Clinical and Laboratory Standard Institute (CLSI), 2019). A baseline concentration of vitamin C is necessary for a normal, well-functioning host defence mechanism, and pharmaceutical use of vitamin C is believed to enhance immune function (Mousavi et al., 2019).

According to Tiryaki et al. (2013), oils such as tung oil can be used as a finish to enhance shellac's benefits. Tung oil is well-suited for finishes that need to withstand moisture due to its water-resistant properties, which provide noticeable protection after five or six applications. Vegetable oils like tung and linseed oil offer a more organic, aesthetically pleasing finish by highlighting the colour and texture of wood while providing a protective layer without the use of hazardous chemicals found in some synthetic finishes. Growing health concerns about chemical finishes have led to a preference for natural oils; safer options include tung and linseed oils, especially for products like wood toys, where chemical exposure is a major concern (Tiryaki et al., 2013). The final finish may be influenced by the curing times and application techniques of different oils. For instance, tung oil cures more slowly than boiled linseed oil but faster than raw linseed oil, which may affect the time between applications. While tung oil can provide a durable finish, it may require more maintenance and reapplication over time than synthetic finishes. Achieving a smooth surface with tung oil can also be challenging, requiring multiple applications and sanding (Tiryaki et al., 2013).

Ascorbic acid (vitamin C) has been shown to impact microbes, including inhibiting bacterial growth and biofilm formation. It can suppress the growth of foodborne pathogens such as *Staphylococcus aureus*, *Escherichia coli*, and *Listeria monocytogenes* in the early stages of biofilm formation, with the greatest inhibition observed at 25 mg/mL concentrations, although effects vary by strain (Przekwas et al., 2020). Ascorbic acid also interacts directly with the virulence factor pyocyanin produced by *Pseudomonas aeruginosa*, modulating its structure and inhibiting its binding to DNA (Das et al., 2023). Additionally, combining ascorbic acid with a quorum sensing inhibitor has been found to enhance biofilm disruption (Das et al., 2023).

The antimicrobial-infused used cooking oil (UCO) paint (AI-UCOP) was formulated using dried citrus waste from orange (*Citrus sinensis*), lemon (*Citrus limon*), and key lime (*Citrus aurantifolia*). The selection of these citrus wastes for infusion into UCO was based on previous research by Saleem and Saeed (2020) and Prashanth et al. (2019). Saleem and Saeed (2020) found that lemon had the highest antibacterial activity among fruits, followed by orange and banana. In another study by Prashanth et al. (2019), lime peels were found to reduce bacterial colonies on agar plates more effectively than orange and kiwi peels. Additionally, a study on wild oranges (*Citrus macroptera*) showed antibacterial activity against *Bacillus* spp. and *E. coli* spp. (Hassan et al., 2022). *Citrus aurantifolia* juice was also shown to reduce bacterial colonies in a study by Ping Ooi et al. (2019).

Further research by Raspo et al. (2020) demonstrated that essential oils from grapefruit, mandarin, and lemon were effective in suppressing *E. coli* ATCC 25922. These findings suggest that citrus waste is a promising component for disinfectants, making citrus fruits a natural antibacterial option. Based on this literature, citrus waste can be utilized to formulate eco-friendly antimicrobial shellac by infusing it into UCO. This innovative approach to integrating these two waste products into an antimicrobial product should be recognized as a creative invention with environmental benefits. Therefore, in this study, UCO was used as a key component in the formulation of antimicrobial shellac, which holds potential for commercialisation. The objective of the research is to assess the antimicrobial properties of citrus waste-infused UCO shellac against airborne microorganisms.

## 2. METHODOLOGY

### 2.1 Material

In this research, waste from peeled, cleaned, and chopped orange (*Citrus sinensis*), key lime (*Citrus aurantifolia*), and lemon (*Citrus limon*) was utilized. Both the citrus fruit waste and the used cooking oil (UCO) were sourced from household waste. The UCO had been used twice for cooking and was filtered through a fine mesh bag to remove all contaminants. For the shellac formulation, standard shellac flakes (Masserini), colophony resin (Masserini), plywood, and 95% denatured alcohol (DChemie) were purchased from a local hardware store. 95% denatured alcohol was also used in the formulation process.

### 2.2 Method

#### *Preparation of citrus waste and UCO*

The citrus waste was dried in an oven at 100°C for three hours. The used cooking oil (UCO) was filtered through 25 µm pore-sized filter paper from Oil and Air Filtration to remove any foreign particles, then heated to 90 °C. Table 1 outlines the ratio of citrus waste infused into the UCO used in this experiment. After 12 hours of infusion, the mixture was filtered again. The weight/volume percentage was calculated using the following equation:

$$\text{Percentage(\%)} \text{ of } \frac{\text{weight}}{\text{volume}} \left( \frac{w}{v} \right) = \frac{\text{Mass of citrus waste (g)}}{\text{Volume of UCO (mL)}} \times 100 \quad (1)$$

Table 1. Formulation of citrus waste used in the experiment

Type of citrus waste	Ratio (% v/w)	Citrus waste (g)	UCO (mL)
Orange	12.50	25	200
Lemon	12.50	25	200
Lime	12.50	25	200

Source: Authors' own data

#### *shellac*

#### *Preparation of*

The shellac solution was prepared following the method described by Timar & Beldean (2022). Solid shellac flakes (10 g) and 10% rosin (1 g) were dissolved in ethanol (100 mL) and heated to 100 °C. The ratio of rosin to shellac flakes was maintained at 1:10, while the ratio of ethanol to shellac flakes was 10:1. The solution was then filtered through fine filter paper and stored in the lab at 20 °C until use, with the temperature maintained at approximately 20 °C by storing it in a well-air-conditioned room. The infused UCO shellac was prepared by mixing the infused UCO with ethanol at a 1:10 (v/v) ratio. This ratio was selected based on the miscibility and compatibility of essential oils and ethyl alcohol, as determined by FTIR analysis in previous research (Timar & Beldean, 2022).

#### *Antimicrobial analysis*

The agar was prepared with 28 g of nutrient agar powder (CM0003B) dissolved in 1 L of distilled water. It was completely mixed and dissolved. Then, it was autoclaved at 121 °C for 15 minutes for sterilization process. The liquid was then poured into the petri dish and allowed to harden for later use. Airborne

microorganisms on the surfaces of the door and plywood, both with and without UCO shellac coating, were swabbed onto agar plates. The plates were then sealed with parafilm and incubated at 37°C for 24 hours. Microbial growth was observed on the agar plates, and colonies were counted on plates containing fewer than 50 colonies, following the Colony Forming Unit (CFU) method as described by O'Toole (2016). The plate count procedure was performed in triplicate to ensure accuracy of the readings. Fig. 1 shows the surface of the plywood sample coated with shellac.



Fig. 1. Sample of plywood surface coated with control shellac

Source: Authors' own data

#### *Fourier-transform infrared spectroscopy (FTIR) analysis*

Fourier-transform infrared spectroscopy (FTIR) was employed to identify the functional groups present in the used cooking oil infused with citrus waste. The FTIR analysis was conducted using a Spectrum One 74630 instrument (PerkinElmer, USA) in the Instrument Laboratory at the School of Chemical Engineering, UiTM. During FTIR analysis, materials are exposed to infrared (IR) radiation, leading to interactions between the IR radiation and the sample's molecular vibrations, resulting in energy absorption and transmission (Bayu et al., 2019). The primary objective of FTIR analysis in this study was to determine the functional groups present in the citrus waste-infused used cooking oil. The infrared range identified was typically divided into far-IR (below 400  $\text{cm}^{-1}$ ), mid-IR (400–4000  $\text{cm}^{-1}$ ), and near-IR (above 4000  $\text{cm}^{-1}$ ) regions (Bayu et al., 2019). In this experiment, the mid-IR region was utilized. The mid-IR spectrum was further divided into specific regions: the single bond region (2500–4000  $\text{cm}^{-1}$ ), triple bond region (2000–2500  $\text{cm}^{-1}$ ), double bond region (1500–2000  $\text{cm}^{-1}$ ), and fingerprint region (600–1500  $\text{cm}^{-1}$ ) (Coates, 2006).

#### *UV-vis spectrophotometer method*

A UV-vis spectrophotometer was used to measure the concentration of ascorbic acid in the sample solutions at a wavelength of 266 nm. The procedure involved placing a cuvette containing distilled water (as a blank) and a cuvette containing the sample solution into the UV-vis spectrophotometer (Cary 60 UV-Vis, Agilent, United States). Standard curves of ascorbic acid were constructed using solutions with concentrations ranging from 0.1 M to 1.0 M at 0.1 M intervals. The ascorbic acid concentrations in the five formulated citrus-infused UCO shellacs were then analysed, using distilled water as the extraction solvent.

For each sample, 1 mL of solution was transferred into a cuvette, with distilled water used as the blank for zeroing. The absorbance of each solution was measured at 266 nm, and these procedures were repeated three times to ensure accuracy. A linear calibration curve for ascorbic acid was generated based on the absorbance values of the standard solutions, yielding an  $R^2$  value greater than 95%, indicating a strong correlation between absorbance and concentration.

### 3. RESULTS AND DISCUSSION

#### 3.1 Fourier-transform infrared spectroscopy (FTIR) analysis

The FTIR spectra for all samples analysed in this study are presented in Fig. 2, with the classification of functional groups for each sample detailed in Table 2. Among the samples, an alkyne group was detected in the lime-infused UCO shellac at a wavelength of  $3333\text{ cm}^{-1}$ . The hydroxyl group was identified in the control shellac, UCO shellac, and lemon-infused UCO shellac at wavelengths of  $3326\text{ cm}^{-1}$ ,  $3324\text{ cm}^{-1}$ , and  $3325\text{ cm}^{-1}$ , respectively. This observation is consistent with a previous study where the hydroxyl group was found in orange peel powder samples (Muhd Rodhi et al., 2020). The peaks observed in this study are comparable to those reported by Lim et al. (2021), who investigated the FTIR spectra of lemongrass-infused cooking oil. The similarities in the FTIR peaks suggest that the chemical composition of the samples analysed in our study may share common functional groups or constituents with those found in lemongrass-infused oil. This resemblance supports the accuracy of our findings and aligns them with established research, validating the presence of similar substances or active components in our samples. Furthermore, shellac is known to be insoluble in water, glycerol, hydrocarbon solvents, and esters but dissolves readily in alcohol, aqueous solutions of alkalis, organic acids, and ketones. This characteristic supports the conclusion that hydroxyl, carboxyl, and carbonyl groups are present in shellac (Sharma et al., 1983).

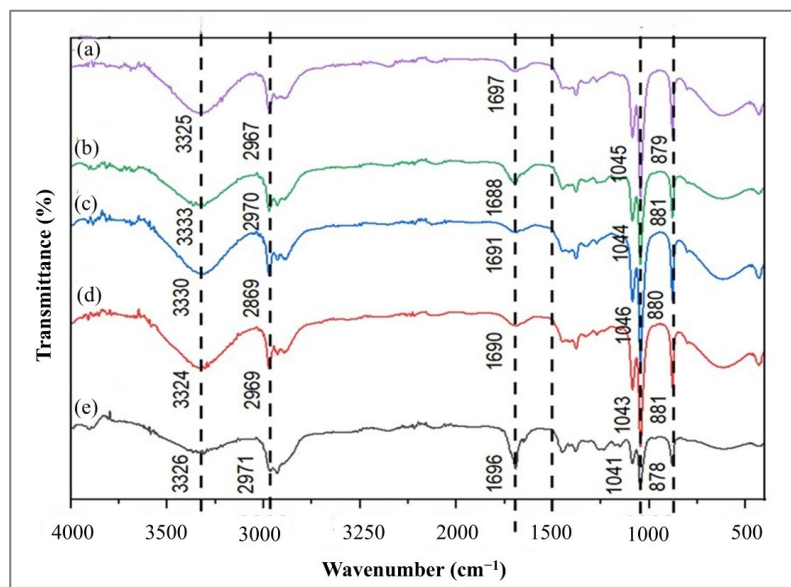


Fig. 2. FTIR analysis obtained for (a) Lemon-infused UCO shellac; (b) Lime-infused UCO shellac; (c) Orange-infused UCO shellac; (d) UCO shellac; (e) Control shellac

Source: Authors' own data

**Table 2.** Classification of functional groups obtained for citrus waste infused UCO shellac

Range Wavenumber (cm <sup>-1</sup> )	Functional Group	Wavenumber (cm <sup>-1</sup> )				
		Control Shellac	UCO Shellac	Orange-infused UCO Shellac	Lime-infused UCO Shellac	Lemon-infused UCO Shellac
3700–3200	Hydroxyl (O–H)	3326.	3324	-	-	3325
3333–3267	Alkyne	-	-	-	3333	-
3300–2500	Carboxylic Acid	2971	2969	2869	2970	2967
1710–1680	Conjugated Acid	1696	1690	1691	1688	1697
1060–1040	Primary Alcohol	1041	1043	1046	1044	1045
900–860	Aromatic Ring	878	881	880	881	879

Source: Authors' own data

The hydroxyl radical, a highly reactive oxygen species, possesses an unpaired electron, which drives it to oxidize other substances by depriving them of an electron (Sheng et al., 2015). Hydroxyl radicals are known to play a crucial role in the immune system by destroying invading bacteria through oxidation. Beyond the immune response, these radicals are also essential in the bactericidal action of various drugs. The antimicrobial effectiveness of carboxylic acids, particularly short-chain carboxylic acids (SCCAs), is strongly influenced by the surrounding pH. SCCAs exert their antimicrobial effect by lowering the ambient pH and penetrating the cell membrane, leading to intracellular acid stress (Ng et al., 2023). The antibacterial activity of conjugated bile acids against strains of lactic acid bacteria is strain-specific and involves multiple mechanisms. These include loss of cellular membrane integrity, dissipation of membrane potential ( $\Delta\Psi$ ), reduction in pH gradient ( $\Delta\text{pH}$ ), and depletion of intracellular ATP. The contribution of each mechanism to the overall antibacterial effect varies among different bacterial strains (Chai et al., 2023). Aromatic rings can increase the lipophilicity of a molecule, enhancing its ability to penetrate bacterial cell membranes. Additionally, aromatic rings can interact with bacterial DNA or enzymes, inhibiting their function and thereby exerting an antimicrobial effect (Lei et al., 2019).

### 3.2 UV-vis spectrophotometer analysis

Numerous studies have demonstrated that vitamin C exhibits antimicrobial properties, reducing the risk of infections and offering immunomodulatory benefits, particularly at high concentrations. The minimum inhibitory concentration (MIC) of ascorbic acid, both alone and in combination with antibiotics, was measured using the micro-dilution technique, following the Clinical and Laboratory Standards Institute (CLSI) criteria (CLSI, 2019). Adequate levels of vitamin C are essential for maintaining a well-functioning immune system, and its pharmaceutical use is associated with enhanced immune function (Mousavi et al., 2019). Ascorbic acid (vitamin C) has been shown to impact various microbes, including inhibiting bacterial growth and biofilm formation. It can effectively inhibit the growth of foodborne pathogens such as *Staphylococcus aureus*, *Escherichia coli*, and *Listeria monocytogenes* in the early stages of biofilm formation, with maximum inhibition observed at concentrations of 25 mg/mL, although effectiveness can vary by strain (Przekwas et al., 2020). Additionally, ascorbic acid interacts directly with the virulence factor pyocyanin produced by *Pseudomonas aeruginosa*, modulating its structure and preventing its binding to DNA (Das et al., 2023). Combining ascorbic acid with a quorum sensing inhibitor has also been found to enhance biofilm disruption (Das et al., 2023).

The concentration of ascorbic acid in each sample was determined using UV-vis spectrophotometry. Fig. 3 presents the standard concentrations of ascorbic acid ranging from 0.1 to 1.0 g/L. The correlation coefficient of 0.99 was considered excellent, as studies using titration as a reference method often report coefficients around 0.7 (Santos et al., 2016). The standard curve equation was used to calculate the concentration of ascorbic acid in the formulated samples.

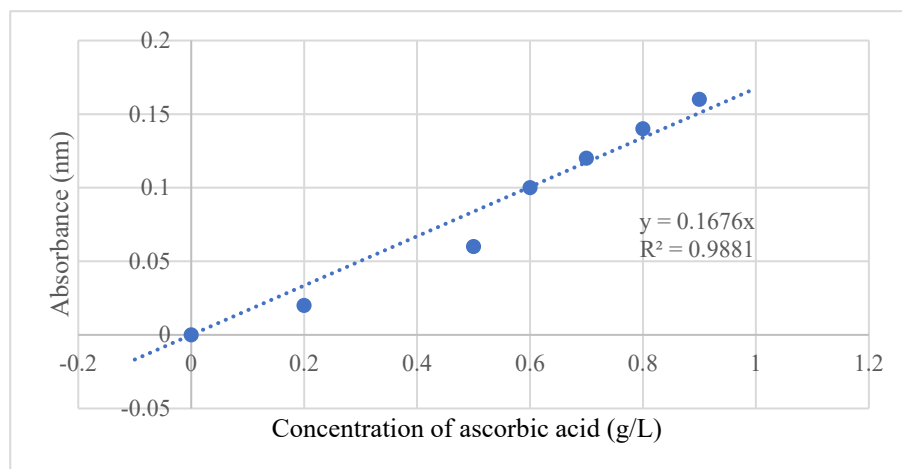


Fig. 3. Calibration curve of ascorbic acid obtained from UV-vis spectrophotometry analysis

Source: Authors' own data

Table 3 presents the ascorbic acid concentrations obtained from the UV-Vis analysis. The highest concentration of ascorbic acid was observed in the lemon-infused UCO shellac. This finding aligns with the results of Saleem and Saeed (2020), who identified lemon as having the highest antibacterial activity among citrus fruits. Several studies have confirmed that vitamin C has antimicrobial properties, which help reduce the risk of infections and offer immunomodulatory benefits, especially at high concentrations (Hong JM et al., 2016). Ascorbic acid lowers the pH of its surroundings, creating an acidic environment (Hong JM et al., 2016; Przekwas et al., 2020). This acidic condition can inhibit the growth and replication of many bacteria, as most have a specific pH range that is optimal for their development.

Table 3. Concentration of ascorbic acid in citrus waste infused UCO shellac

Sample	Absorbance (nm)	Concentration (g/L)
Lime-infused UCO shellac	0.1456	0.8687
Lemon-infused UCO shellac	0.1841	1.0984
Control shellac	0.0526	0.3138
Orange-infused UCO shellac	0.1025	0.6116
UCO shellac	0.0895	0.5340

Source: Authors' own data



### 3.3 Antimicrobial analysis

Table 4 and Fig. 4 present the results of the antimicrobial efficacy of the infused-UCO shellac. The data, derived from a 96-hour observation period, provide qualitative insights into the growth of microorganisms. Among the antimicrobial shellac formulations, the one using dried lemon demonstrated superior inhibition of microbial growth compared to those using dried orange or lime. Fig. 4 illustrates the microbial activity for the shellac formulation with dried lemon. These findings are consistent with previous research by Saleem and Saeed (2020), which also identified lemon extract as the most effective in inhibiting microorganism growth. In contrast, Edogbanya et al. (2019) reported that lemon had a lower antimicrobial effect compared to orange and lime. This discrepancy may be due to differences in the solvents used, with Edogbanya et al. employing n-hexane. Fig. 4 indicates that, after 24 hours, bacteria and fungi began to appear and grow on the control (door) sample. In contrast, plates swabbed with the antimicrobial infused-used cooking oil shellac showed significantly less microbial growth. Previous studies suggest that microbial growth typically begins between 24 to 48 hours under optimal conditions (Edogbanya et al., 2019), which was also observed in this study, with more pronounced microbial activity after 48 hours. Furthermore, the formulation using dried lemon-infused used cooking oil shellac required the longest time to form visible colonies, whereas the dried lime-infused shellac and the control sample exhibited colony formation within 24 hours. The rapid colonisation of the control surface can be attributed to frequent contact by individuals, which increases the likelihood of bacterial transfer.

Table 4: Colony Forming Unit (CFU) of citrus waste infused UCO shellac

Type of Sample	Time (Hour)	Colony Forming Unit (CFU)
Door (Control)	24	121
	48	1
Control Shellac	24	1
	48	> 50
	72	> 50
	96	> 50
UCO Shellac	24	0
	48	0
	72	0
	96	2
Lime-infused UCO Shellac	24	>50
	48	>50
	72	>50
	96	>50
Lemon-infused UCO Shellac	24	0
	48	0
	72	0
	96	1
Orange-infused UCO Shellac	24	4
	48	>50
	72	>50
	96	>50

Source: Authors' own data

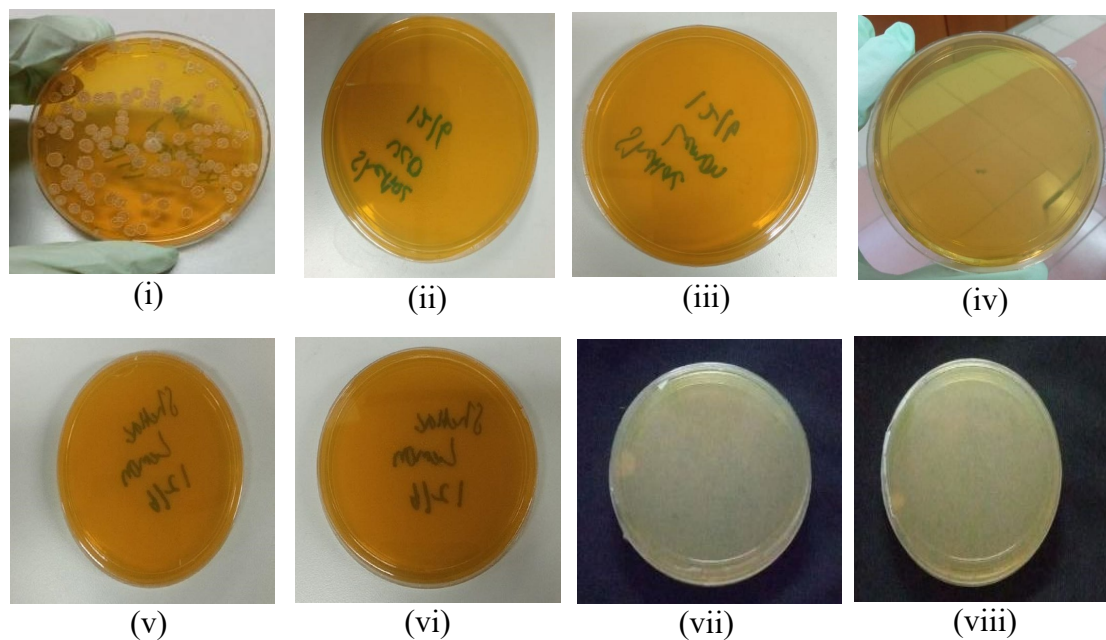


Fig. 4. Growth of airborne microorganisms determined on agar plate from (i) Wooden door (Control) after 24 hours; (ii) UCO shellac after 24 hours; (iii) Lemon-infused UCO shellac after 24 hours; (iv) UCO shellac after 48 hours; (v) Lemon-infused UCO shellac after 48 hours; (vi) Lemon-infused UCO shellac after 72 hours; (vii) Lemon-infused UCO shellac after 96 hours; (viii) UCO shellac after 96 hours

Source: Authors' own data

The component in shellac that may reduce antibacterial activity is the presence of moisture or water and the amount of moisture in shellac can have a variety of effects on how antimicrobial it is (Evangelho et al., 2019). Traditionally employed as a coating material in a variety of applications, shellac is a natural resin made from the secretions of the lac insect. Moisture can alter the characteristics of shellac, which in turn affects how effective it is against microbes. Excess moisture in shellac coatings can create a favourable environment for microbial growth. Microorganisms, such as fungi and bacteria, thrive in moist conditions. High moisture content within the shellac film may provide a suitable substrate for microbial colonisation and proliferation, reducing the antimicrobial efficacy (Talley et al., 2002).

#### 4. CONCLUSION

This study aimed to develop and evaluate the effectiveness of antimicrobial-infused used cooking oil (UCO) shellac. UV-vis analysis revealed that lemon-infused UCO shellac had the highest ascorbic acid concentration at 1.0984 g/L. FTIR analysis also proven the existing of several antimicrobial compounds in the shellac solution, including hydroxyl, alkyne, carboxylic acid, conjugated acid, primary alcohol, and aromatic acid in the lemon-infused UCO shellac. Additionally, the presence of fatty acids in the shellac solutions also contributed to its antimicrobial activity by inhibiting bacterial growth. The antimicrobial activity results indicated that lemon-infused UCO shellac exhibited prolonged bacterial suppression, with visible microbial growth only occurring after 96 hours and with a single colony formed. This suggests that shellac formulated with dried lemon is particularly effective in suppressing bacterial growth, making it a promising candidate for commercialisation, particularly in medical applications.

Several recommendations can be proposed for future research such as formulating with UCO collected from variety sources of cooking which could provide a broader understanding of the phytochemical composition and antimicrobial properties of different source of UCO. Formulating and analysing the major components in the infused oils using other raw materials in shellac making also could offer insights into their individual chemical properties and interactions that might improve the antimicrobial efficacy of the shellac.

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## CONFLICT OF INTEREST STATEMENT

The authors agree that this research was conducted in the absence of any self-benefits, commercial or financial conflicts and declare the absence of conflicting interests with the funders.

## AUTHORS CONTRIBUTIONS

**Miradatul Najwa Muhd Rodhi:** Conceptualisation, methodology, draft manuscript preparation, validation, supervision, and writing-review & editing; **Nur Zahirah ‘Ainaa’ Mohd Asri Author:** Methodology, investigation, formal analysis, and writing-original draft; **Abdullah Farhan Rozi:** Methodology, investigation, formal analysis, and writing-original draft; **Harumi Veny:** Project administration and conceptualisation and writing-review; **Fazlena Hamzah:** Project administration and conceptualisation and writing-review.

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