Antimicrobial Properties of Green Disinfectant from Citrus Waste-Infused Used Cooking Oil Using Conventional Method

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Abstract: *This research aims to formulate a disinfectant from citrus waste-infused used cooking oil through the conventional process and evaluate its effectiveness in microbial elimination. Fourier Transform Infrared Spectroscopy (FTIR) and Gas Chromatography-Mass Spectrometry (GC-MS) were utilized to characterize citrus waste-infused used cooking oil. Two prominent bands belonging to the alkane (2921.93–2922.26 cm^{-1}) and ester (1743.60–1743.73 cm^{-1}) were observed on all FTIR spectra. Aside from that, through GC-MS analysis, dried orange-infused used cooking oil was discovered to have the highest percentage content of major antimicrobial compounds such as esters, oxygenated monoterpenoids, triterpenes, and alkaloids with 1.92% of the total amount of compounds found in the sample. However, the agar plate method revealed that the fresh lemon waste-infused used cooking oil disinfectant formulation was the most effective at inhibiting bacterial growth as the colony-forming detected on the agar plates dropped from 20 colonies to nearly zero and from 49 to 3 colonies for the plate swabbed with microbes from the table and doorknob surfaces, respectively. Based on the findings, the citrus waste and used cooking oil were viewed to have the potential as one of the possible ingredients in creating safer disinfectants in the future.*

Keywords: *disinfectant; citrus waste; used cooking oil; antimicrobial activity; infused oil*

INTRODUCTION

Microorganisms can either be helpful or harmful, and they are present in every single space and even the human body. The kind of organisms that are harmful and the disease-causing are called pathogens. Pathogens can make humans sick and even cause death [1]. Infectious disease outbreaks and diseases caused by disease-carrying microorganisms have always been and will continue to be a significant source of worry. As the world is now facing the disease brought by a novel coronavirus known as COVID-19, most people have developed an interest in surface hygiene. It is known that there are ways for diseases and germs to spread through direct and indirect contact, yet the indirect contact through the transfer of microorganisms on surfaces was further explored in this study. Before the virus can attack the immune system, it commonly comes from the surfaces around us until humans are contacted. The virus from an infected person can be transmitted indirectly to others who have touched the same surfaces [2]. Most people become wary and paranoid due to the pandemic and become dependent on disinfectants, sanitizers, and other bacteria-killing products whenever they are outside. Cleaning and disinfecting indoor surfaces, such as those in the home and office, is necessary for restricting and reducing microbial growth since microorganisms may survive for lengthy periods on any surface. The disinfection procedure works by destroying the cell walls of microorganisms and interfering with their metabolism to kill or inactivate them [3]. Many disinfectants are available in the market to sanitize contaminated surfaces and are composed of chemical substances capable of disinfecting microorganisms such as alcohol, aldehydes, bleach-based, and suchlike [4]. During the outbreak of COVID-19, disinfectants are extensively used to sterilize public spaces and prevent contamination. The extensive disinfection process also comes with issues of its own. Many ingredients in disinfecting products can cause unintended adverse side effects to humans and their surroundings. Furthermore, although chemical disinfectants work to kill dangerous bacteria, they typically have a strong noxious odor and may include carcinogenic substances such as quaternary ammonium chloride (QUATS) and triclosan. Ingredients like QUATS can trigger asthma in those who suffer from it, and triclosan has been correlated with decreased thyroid hormones [5-6].

Even though chemical disinfectants are excellent in killing germs, a green-based disinfectant might be equally as effective. Green-based disinfectants might be a wonderful turning point toward a safer disinfectant, given the development of the green movement across the world. Those natural disinfectants are better for adults' health and safer for children and the environment. Eco-friendly disinfecting led to a sustainable and healthy foundation for a greener future. There are tons of useful waste thrown out each year, such as used cooking oil and citrus peel waste [7]. Used cooking oil (UCO) means the remaining oil leftover from food preparation, and discarding the cooking oils in the drain is a poor idea since the oil can solidify and clog the drain. According to a study, Malaysia discards about 50,000 tons of vegetable and animal-based used cooking oils each year [8]. However, used cooking oil is not completely a useless waste. It can be reused to make other products. Many methods of re-utilizing used cooking oil are now available, such as using it as an additive. Rendering to another study, used cooking oil is comprised of triacylglycerols, glycerols, free fatty acids (FFAs), and other polymerization compounds [9]. FFAs have an antibacterial activity that could prevent or directly destroys bacteria, fungi, and other microbes by affecting several cell targets, including the cell membrane and the components located therein [10].

Other than that, citrus wastes are classified as one food waste that becomes a concern since it is likely to create environmental pollution. Due to the rising demand, its production rises every year, and the rises of production led to rises in citrus waste produced since the citrus peels waste itself is almost 50% of the fruit mass. Citrus waste can be of considerable economic importance as it contains an abundance of various flavonoids, carotenoids, dietary fiber, sugars, polyphenols, essential oils, and ascorbic acid [11]. In an earlier study, which emphasis on the antimicrobial activity of peels extract of *Citrus sinensis* (oranges) and *Citrus aurantium* (bitter oranges), both citrus fruit wastes contain a high quantity of phenolic compounds, including several flavonoid compounds [12]. The formulation using both used cooking oil and citrus waste could be the alternative for the commercial disinfectants and contribute to the zero-waste and eco-friendly movement. Thus, this study focuses on producing green disinfectants from citrus waste-infused used cooking oil using a conventional preparation process that is effective in inhibiting the growth of microbes. The antimicrobial properties of different types of citrus wastes were evaluated and explored in-depth by determining the antimicrobial compositions in the green disinfectant made from citrus waste-infused used cooking oil and its effect on microbes swabbed from surfaces. A conventional preparation process is a great approach in producing natural disinfectants owing to the pandemic. In a bigger picture, this study contributes to waste reduction by converting the citrus and oil waste into a value-added product.

■ EXPERIMENTAL SECTION

Materials

Citrus fruit wastes used in this study were from orange (*Citrus sinensis*), key lime (*Citrus aurantiifolia*), and lemon (*Citrus limon*). The waste of citrus fruits was obtained from the market in Puchong, Selangor. In the meantime, the UCOs were collected from the household's daily cooking routine. The food-grade vinegar used for the research has 5% acidity.

Instrumentation

Fourier-transform Infrared Spectroscopy (FTIR) (Perkin-Elmer, TGA/SDTA851, USA) and Gas Chromatography-Mass Spectrometry (GC-MS) (Varian 240, USA) were utilized for analysis and compounds characterization of citrus waste-infused used cooking oil. Both instruments are available in the Instrumentation Laboratory at the School of Chemical Engineering, UiTM Shah Alam.

Procedure

Sterilization of materials

The utensils and apparatus used were microwaved for 3 min [13] and boiled for 15 min for sterilization purposes.

Preparation of citrus waste

Citrus fruits were washed and peeled. The peels were then ground in a conventional blender into small particles of a size larger than 5 mm to initiate better infusion. The citrus wastes were divided into two formulations: dried and fresh citrus-infused oil. For the first formulation, the wastes were oven-dried at 100 °C for 3 h to eliminate the moisture, while in the second formulation, fresh citrus wastes were used.

Preparation of infused UCO

The UCO was first filtered using a tea filtering bag until no impurities were left. Two formulations were observed in the study based on dried and fresh citrus fruit wastes. The dried fruit wastes were infused in the UCO with a ratio of 1:5 (w/v). Every 10 g of prepared peels was added to 50 mL of filtered used cooking oil. In the meantime, the fresh fruit wastes were acidified using vinegar before being infused in the UCO to prevent bacterial growth. For both methods, the UCO was slowly heated until it reached the temperature of 90 °C before being added to the citrus wastes. UCO was heated to infuse the flavoring of citrus wastes into the UCO swiftly. The infused UCO was then capped and left to cool. Infusions were carried out for 1 to 14 d at room temperature to increase the intensity of the infused flavor over time.

Fourier-transform infrared spectroscopy (FTIR) analysis

The functional groups present in the citrus waste-infused UCO was determined using FTIR analysis based on the spectrum's wavelengths [14]. The determination was performed within the measurement band range from 4000 to 1000 cm^{-1}. The resolution of the FTIR was 4 cm^{-1}, and the scanning time was 64 s, where the spectrum takes about 2 min to complete the whole scanning cycle to be recorded. The samples were scanned after a drop of citrus waste-infused used cooking oil was placed in the opening of the top plate. Scanning the samples in an FTIR machine yielded the spectrum of the infused oil disinfectants. Acetone was employed to keep the device clean [15].

Gas-chromatography-mass spectrometry (GC-MS) analysis

Citrus waste infused UCO was pipetted in the vial prepared for GC-MS. An autosampler was installed on a Varian 240 GC-MS, which was used to detect the analytes while the separation of analytes was accomplished using a column. Helium was used as the carrier gas at a flow rate of 1 mL/min. Temperatures were kept at 250 °C for the injector and 250 °C for the interface, respectively. The initial column temperature was set at 80 °C for 2 min, followed by 15 °C/min temperature gradients to 230 °C for 2 min. The total run time was approximately 45 min. For ionization, the electron impact ionization (EI) mode was used. Full scan mode (m/z range, 50–500) was used for qualitative analysis [16].

Antimicrobial analysis

The antimicrobial activity was done using the fresh lemon-infused cooking oil disinfectants swabbed on the universal agar plate that was formerly swabbed with the sample taken from the doorknob and table. The control agar plate was only swabbed with the sample taken from the doorknob and table. All agar plates were incubated at room temperature for 24 h and the colony formed on the agar plate was counted. The colony formed on the agar with infused UCO and control plate was compared.

RESULTS AND DISCUSSION

Functional Group of Antimicrobial Compound in the Citrus Waste Infused UCO Using the FTIR Analysis

FTIR analysis involves exposing materials to infrared (IR) radiation. The IR radiations then interact with the atomic vibrations in the sample, causing energy absorption and transmission [14]. FTIR analysis aims to determine the functional group present in the citrus waste-infused UCO. Momentarily, the infrared range was parted into three wavenumber districts which were far-IR (400 cm^{-1}), mid-IR (400–4000 cm^{-1}), and close IR (4000–13000 cm^{-1}) [14]. In this investigation, the mid-IR was utilized to analyze the sample because most of the compounds to be determined exist within this range.

The FTIR spectrums obtained for all samples in this study are shown in Fig. 1–3. Meanwhile, the classification of functional groups identified through FTIR for each citrus waste infused UCO is shown in Table 1. Among all of the samples, the signal of the hydroxyl group was observed at the range of 3200–3500 cm^{-1} was only found in the dried lemon waste-infused UCO at the wavelength of 3359 cm^{-1}. This contradicted the data attained by the previous study for the hydroxyl group in orange peel powder samples [17]. The spectrum that appears for each type of citrus waste-infused oils were found to be similar to one another. The alkane, ester, aromatic, ether, and amine groups were discovered in each sample. At the vibrations of 3000–3800 cm^{-1}, the orange and dried lemon samples coincide with the OH and NH_2 functional groups. There were two intense bands shown on all FTIR spectrum which belongs to alkane (Methylene) and ester groups. The first band, a methylene group or alkane, was observed at 2921.93 and 2922.26 cm^{-1}. The other intense band was observed between 1743.60 and 1743.73 cm^{-1} caused by C=O vibration, indicating the presence of esters. Triglyceride (TGA) was another name for this category of functional group, which is the most abundant component in fats and oils [18]. The peaks obtained from the present study were seen to have similar esters and aromatic groups with

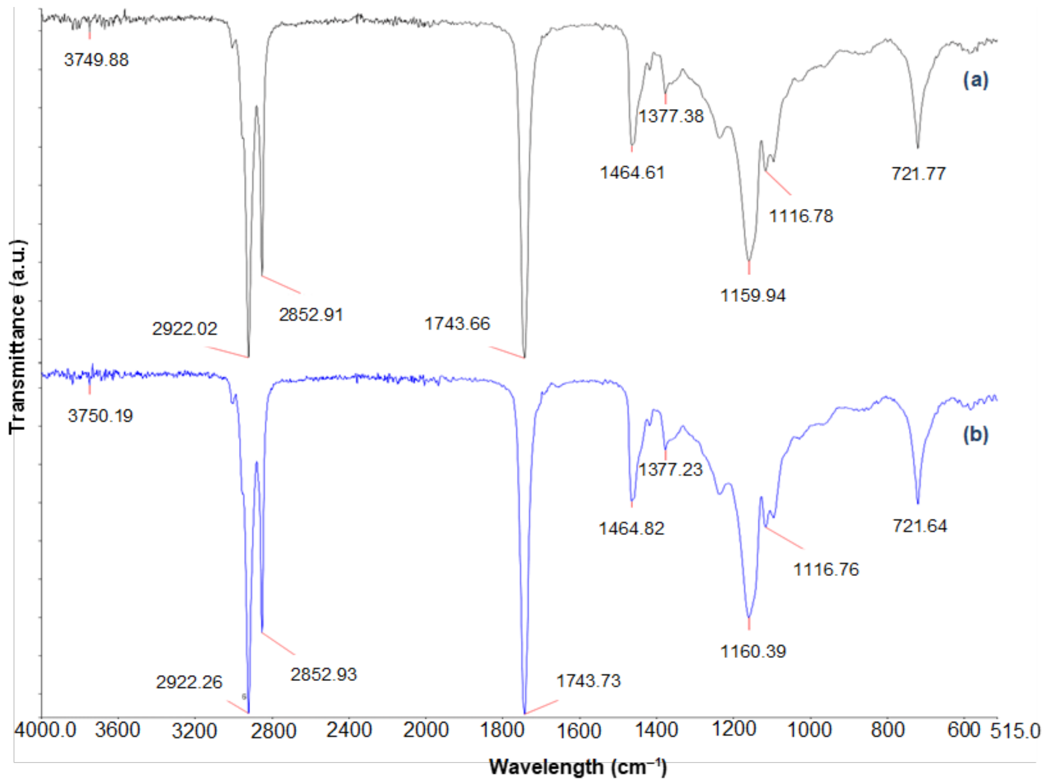

Fig 1. FTIR spectrum of orange waste - infused UCO disinfectants; (a) dried orange waste-infused oil, (b) fresh orange waste-infused oil

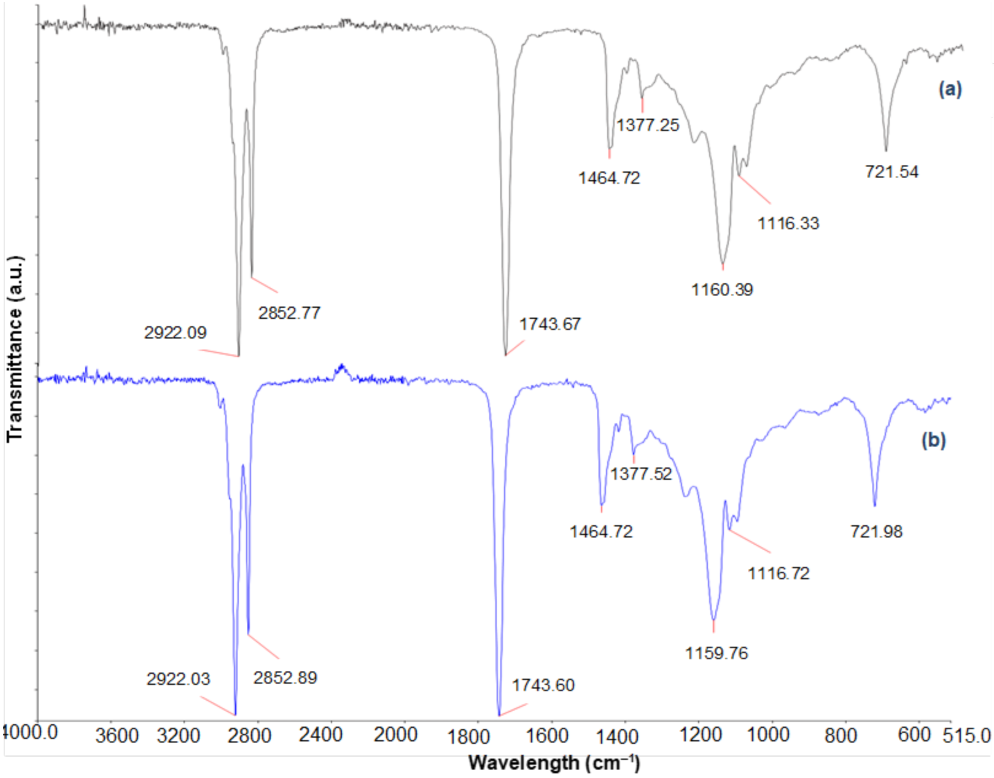

Fig 2. FTIR spectrum of key lime waste - infused UCO disinfectants; (a) dried key lime waste-infused oil and (b) fresh key lime waste-infused oil

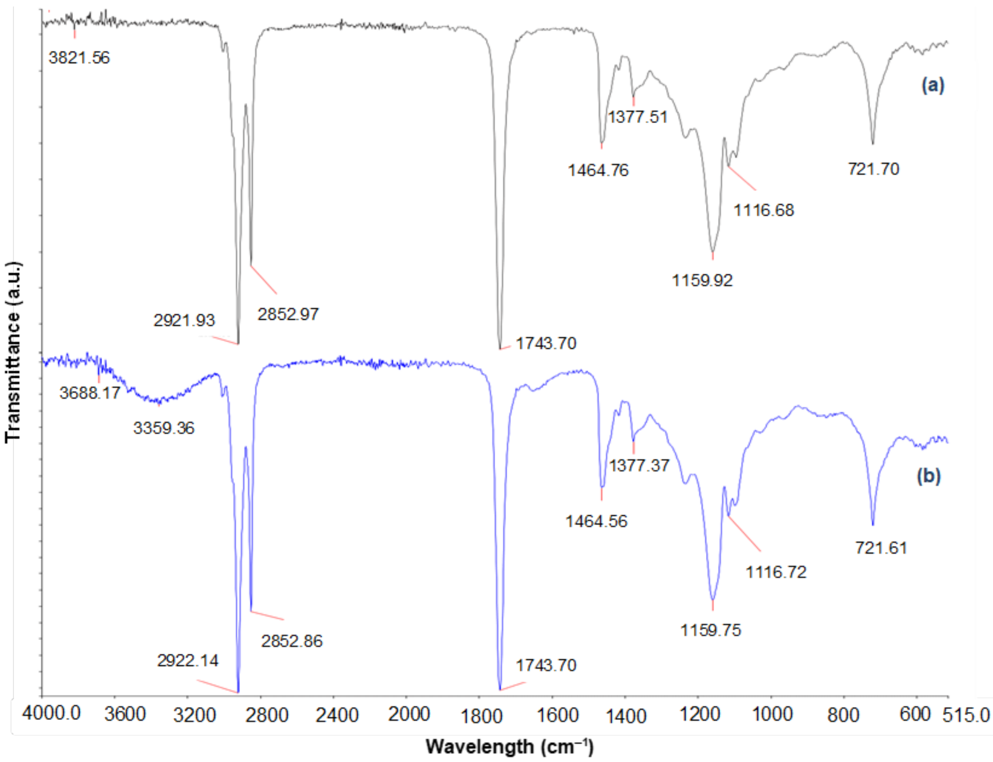

Fig 3. FTIR spectrum of lemon waste - infused UCO disinfectants; (a) dried lemon waste-infused oil and (b) fresh lemon waste-infused oil

Table 1. Classification of Functional Groups in Citrus Waste-Infused UCO

Wavenumber (cm^{-1})*	Functional group	Wavenumber (cm^{-1})					
		Orange		Lime		Lemon	
		Dried	Fresh	Dried	Fresh	Dried	Fresh
3000–3800	Coincide with OH and NH_2 (amino)	3749.88	3750.19	-	-	-	-
3200–3700	Hydroxyl (O–H)	-	-	-	-	3688.17	-
		-	-	-	-	3359.36	-
2935–2915	Methylene (alkane)	2922.02	2922.26	2922.09	2922.03	2922.14	2921.93
2880–2840	Methyl (alkyl)	2852.91	2852.93	2852.77	2852.89	2852.86	2852.97
1750–1725	Ester (triglyceride)	1743.66	1743.73	1743.67	1743.60	1743.70	1743.70
1510–1450	Aromatic ring	1464.61	1464.82	1464.72	1464.72	1464.5	1464.76
1380–1370	Alkane	1377.38	1377.23	1377.25	1377.52	1377.37	1377.51
1210–1150	Amine	1159.94	1160.39	1160.39	1159.76	1159.75	1159.92
1140–1070	Cyclic ether	1116.78	1116.76	1116.33	1116.72	1116.72	1116.72
750–720	Alkane	721.77	721.64	721.5	721.98	721.61	721.61

*Source from [17]

the previously performed research that focuses on the extraction of lemongrass-infused cooking oil [19].

Antimicrobial Compound in the Citrus Waste Infused UCO Using GC-MS Analysis

The composition of infused UCO for orange, key lime, and lemon was determined using GC-MS. Fig. 4–6 shows the chromatogram of GC-MS for all the samples of infused UCO with the dried orange, fresh orange, dried lime, fresh lime, dried lemon, and fresh lemon. The peak detects compounds in the citrus waste-infused used cooking oil disinfectants. Intense peaks were observed in the average range of 30 to 45 min where compounds such as fatty acids and phenolic were determined. Through the analysis, a total of 86 compounds were determined for both dried and fresh citrus waste infused UCO. The major phytochemical compounds in dried and fresh citrus waste infused UCO with its percentage of amount are shown in Table 2–4. These phytochemicals contributed to the properties of disinfectants and inhibited the growth of microorganisms.

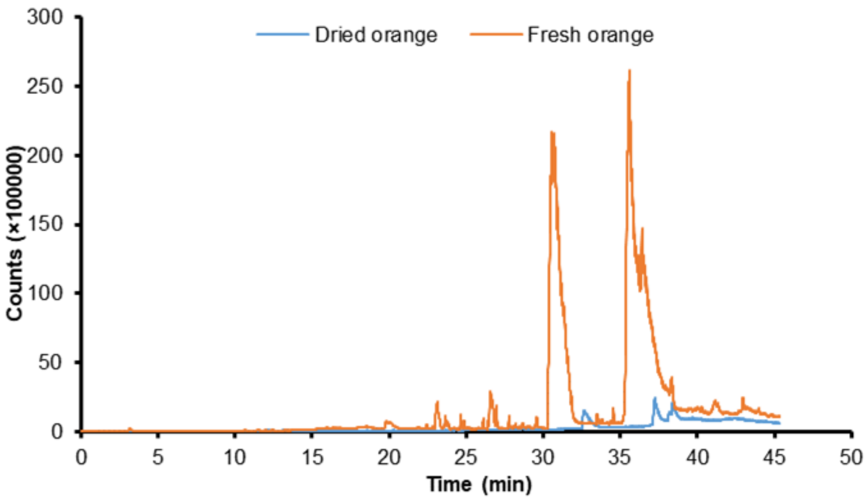

Fig 4. Chromatogram plot of orange waste-infused used cooking oil disinfectants obtained from GC-MS analysis

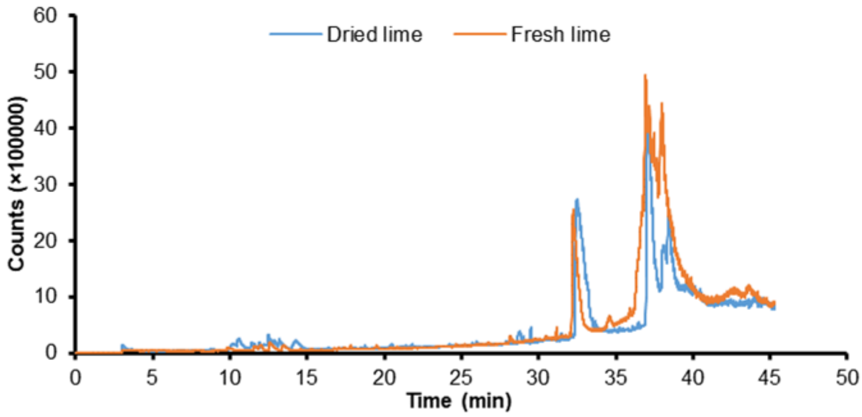

Fig 5. Chromatogram plot of key lime waste-infused used cooking oil disinfectants obtained from GC-MS analysis

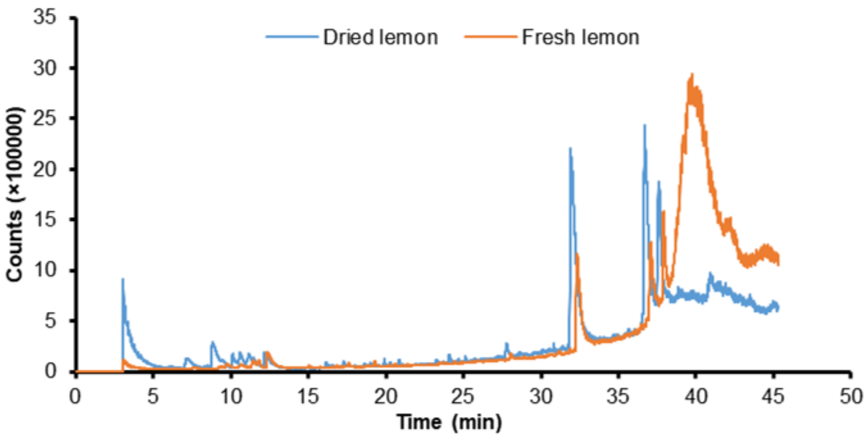

Fig 6. Chromatogram plot of lemon waste-infused used cooking oil disinfectants obtained from GC-MS analysis

Table 2. Phytochemical components of orange waste-infused used cooking oil disinfectants

Dried		Fresh	
Name of compounds	Amount (%)	Name of compounds	Amount (%)
3,6-Dimethyl-3-heptanol	0.14	3,7-Dimethyl-6-octen-1-ol propanoate	0.19
Ethyl 2-Oxo-1-(3-oxo-butyl)-cyclohexanecarboxylate	0.12	4-Methyl-1-(1-methylethyl)-3-cyclohexen-1-ol	0.16
2-Butyl-5-hexyloctahydro-1H-indene	0.11		
Z-10-Tetradecen-1-ol acetate	0.57		
1-methyl-3-(2-methylpropyl)cyclopentane	0.36		
(2,4,6-Trimethylcyclohexyl)methanol	0.21		
2-Methylene-cholestan-3-ol	0.10		
2-Dimethyl-3-(3,7,16,20-tetramethyl-heneicosa-3,7,11,15,19-pentaenyl)-oxirane	0.12		
3,7,11-Trimethyl-2,6,10-dodecatrien-1-ol	0.31		

Varies percentage yield of compounds was identified for each sample. Fatty acids such as tetradecanoic acid and oleic acid, also known as emulsifying agents in aerosol products, were detected in the greatest concentration in the samples [20]. Fig. 7 shows the percentage content of fatty acid and other compounds determined by GC-MS analysis. Other compounds such as alkaloids, phenolic, and esters were

Table 3. Phytochemical components of lime waste-infused used cooking oil disinfectants

Dried		Fresh	
Name of compounds	Amount (%)	Name of compounds	Amount (%)
n-Hexadecanoic acid	0.29	Undecanoic acid	0.16
5-Thio-d-glucopyranose	0.14	Dodecanoic acid	0.17
2,6,6-Trimethyl-bicyclo[3.1.1]heptan-3-one	0.18	Nonanoic acid	0.32
Oleic Acid	0.21	1*H*-Imidazole-2-methanol	0.23
Ethyl 3-methyl-4-oxo-2-phenyl-2-(1-piperidinyl)-4*H*-1-benzopyran-8-carboxylate	0.13	1-(Hydroxymethyl)-1,2-ethanediyl Hexadecanoate	0.24
8-Nonynoic acid	0.11	trans-2-Dodecenoic acid	0.11
2,6-Dimethyl-1,5-heptadiene	0.10	Dimethyl 3-oxo-Tetradecanedioate	0.15
3,7-Dimethyl-2,6-octadien-1-ol	0.19	7-Heptadecyne	0.11
		2,2,3,5-Tetramethyl-heptane	0.15

Table 4. Phytochemical components of lemon waste-infused used cooking oil disinfectants

Dried		Fresh	
Name of compounds	Amount (%)	Name of compounds	Amount (%)
5-(Methylenecyclopropyl)-1-pentanol	0.11	Tetradecanoic acid	0.13
3-Cyclohexene-1-methanol	0.21	6-nonenal	0.13
2-Methyl-cis-3,4,7,7-tetrahydroindan	0.12	Squalene	0.21
2,4-Decadienal	0.12	(2*R*)-Acetoxymethyl-1,3,5-trimethyl-4c-(3-methyl-2-buten-1-yl)-1c-cyclohexanol	0.22
Tetradecanoic acid	0.34	3-Hydroxypropyl oleate	0.12
9,9-Dimethoxybicyclo[3.3.1]nona-2,4-dione (phenolic)	0.48	2-Hydroxyethyl 9Z-octadecenoate	0.18
n-Hexadecanoic acid	0.14		
Dodecanoic acid	0.11		
1-Ethyl-1-heptanoyloxy-1-silacyclopentane	0.14		
5-Keto-2,2-dimethylheptanoic acid	0.11		
2,3-Dimethylaziridin-1-yl)-(4,4,6-trimethyl-7-oxabicyclo[4.1.0]hept-2-ylidene)imine	0.14		
6,10-Dimethyl-3,5,9-undecatrien-2-one	0.27		
6,9,12,15-Docosatetraenoic acid	0.13		

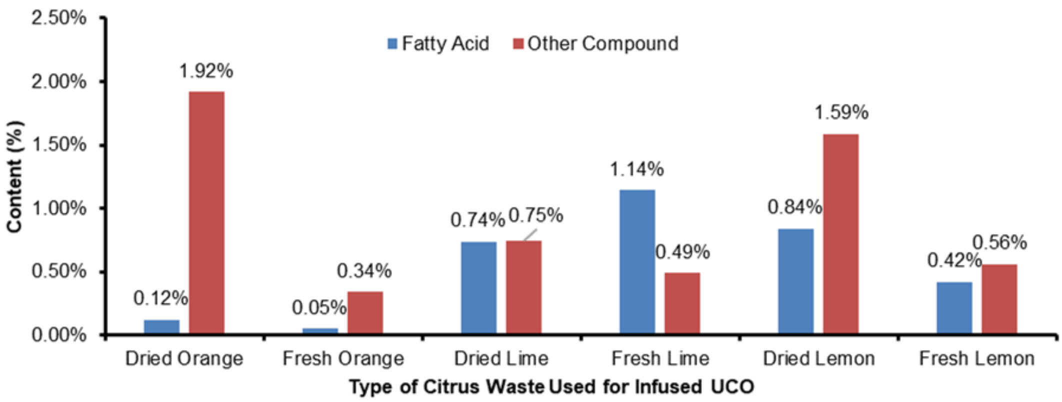

Fig 7. Content of fatty acid and other compounds found in the citrus waste infused UCO

the major compounds infused in the green disinfectants. The highest amount of major compounds determined was seen to be the one that was found in the dried orange waste-infused UCO disinfectant, which was 1.92%. However, fresh orange-infused UCO disinfectant was detected to have the least amount of major compounds, which was 0.34%. The abundance of fatty acids in the samples compared to other compounds was contributed by the ratio of UCO with the citrus waste used. Other compounds discovered in the infused used cooking oil include esters, oxygenated monoterpenoids, and triterpenes. Alkaloids such as 2,3-Dimethylaziridin-1-yl-(4,4,6-trimethyl-7-oxabicyclo[4.1.0]hept-2-ylidene)imine, which have the suffix 'ine' also found in the samples, is majorly found in the lemon samples [21].

In addition, based on a previous study, it was also found that there was a trace of alkaloids, flavonoids, tannins, and saponin compounds in their orange peels samples. These compounds play a key role in the antimicrobial properties of citrus waste [22]. However, the difference in the value compared to other studies could be influenced by the difference in the extraction process done, the environmental reasons, and the condition of the fruit waste itself [23-24].

Antimicrobial Analysis of Citrus Waste Infused UCO

Based on Fig. 8, the control agar plates for both samples taken from the doorknob and the table show that after 24 h, microbes and fungi started to appear. It was revealed that bacteria, fungi, or other microorganisms start to grow after 24 to 48 h in its preferable condition, so in the present study, the sample was observed after 24 and 48 h [12,22,25-26]. However, in the current study, the microbe colonies were clearly visible after 48 h.

Among the citrus waste infused UCO prepared, the formulation of infused UCO using fresh lemon inhibited

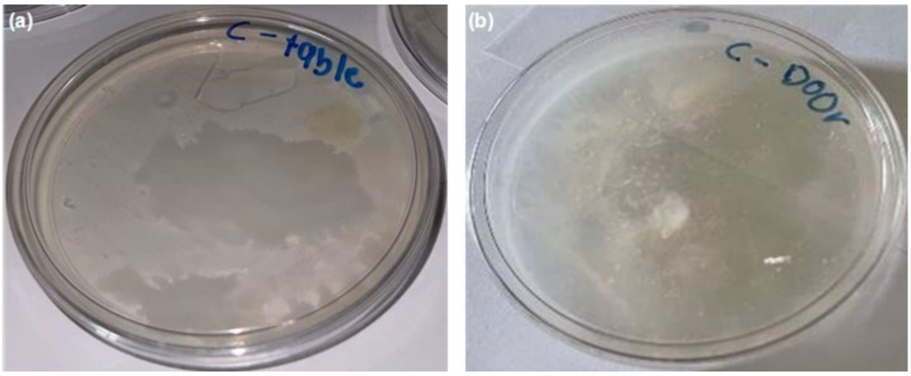

Fig 8. The colony formed on controlled agar plates for samples taken from (a) the table and (b) doorknob surfaces

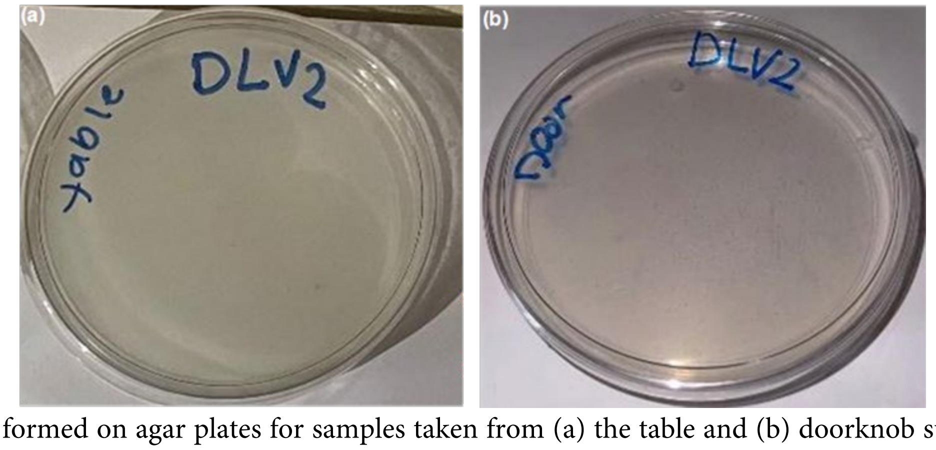

Fig 9. The colony formed on agar plates for samples taken from (a) the table and (b) doorknob surfaces which were swabbed with fresh lemon waste infused UCO disinfectants

bacteria to a higher extent than the UCO infused with orange and key lime wastes. Therefore, this paper shows the microbial activity obtained with the fresh lemon-infused UCO disinfectants on both samples taken from the doorknob and table in Fig. 9. In addition, it shows that the colonies formed on the control agar plates shown in Fig. 8 for the samples taken from the table and doorknob surfaces were found to be 20 and 49 colonies formed, respectively. However, the agar plates that were swabbed with fresh lemon waste-infused UCO were seen to have almost zero colony-forming for the agar plate of the sample taken from the table surface, while the sample from the surface of the doorknob was found to have approximately 3 colonies formed on the agar plate.

The antimicrobial study performed by other researchers using the yellow lemon, orange, and banana shows similar results with the current study. The agar plate colony counting proved that the lemon extract was the most effective material in inhibiting the growth of microorganisms [27]. However, this contradicts with the preceding research done, wherein it was mentioned that lemon has the least antimicrobial effect against the microorganisms compared to orange and lime [26]. The difference in the results of antimicrobial activity could be attributed to the type of solvent used in their study, which was n-hexane, compared to the current study, which used the UCO.

■ CONCLUSION

Compounds including esters, oxygenated monoterpenoids, triterpenes, fatty acids, and alkaloids were found in the citrus waste infused UCO. Similar to other compounds found in the infused UCO, the inclusion of fatty acids in the citrus waste infused UCO also contributes to antibacterial action while suppressing bacterial growth. Based on the agar plate colony counting method, the infused UCO formulated by employing fresh lemon was seen to be suppressing bacteria more effectively, as it was given that the number of colonies formed was decreased by more than 94% and able to reach up to 100% when swabbed with the fresh lemon infused UCO. Furthermore, the acidification of the citrus waste using vinegar was observed to increase the citrus' antibacterial capabilities while also extending the shelf life of the infused UCO, where the use of vinegar contributed to the inhibition of the bacteria growth. Findings from this study indicate that the waste from citrus fruits investigated contains antibacterial components, which justify the significance of using it as the ingredients in formulating the green-based disinfectants. Therefore, the formulation of disinfectants from citrus waste and used cooking oil were viewed to have the potential to reduce the wastes and be one of the possible ingredients in creating safer disinfectants in the future.

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■ AUTHOR CONTRIBUTIONS

Miradatul Najwa Muhd Rodhi and Nur Affaaidil Amani Mohd Zaki did the experiment, data validation for the results, and manuscript writing, while Harumi Veny and Fazlena Hamzah revised and verified the manuscript and the results obtained from the experiment. As a result, all authors agreed to the final version of this manuscript.

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