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In vitro antimicrobial activity of citrus waste-infused used cooking oil

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Abstract

This study produced a natural disinfectant from used cooking oil (UCO) and citrus waste. Different formulation of citrus waste was infused in UCO and the antibacterial activity was evaluated. The formulation of waste citrus fruit infused UCO was at the ratio of 10%, 25%, and 50% of waste citrus and UCO by weight/volume (w/v) basis. The peels and seeds of orange (*Citrus sinensis*), lemon (*Citrus limon*), and key lime (*Citrus aurantifo*lia) were used. The 50% w/v key lime citrus waste-infused UCO best inhibits *Escherichia coli* (*E. coli*) growth. The bacterial growth was reduced to 60% and 68% for dried and fresh lime-infused UCO. The presence of flavonoid, monoterpenes, monoterpenoid, sesquiterpene, sesquiterpenoid, alkaloid and aromatic compounds in the citrus waste-infused UCO was detected by UV-Vis and GC-MS. This contributes to legitimising the utilisation of citrus waste as a part of green-based disinfectants, while having the capacity to lessen waste and act as one of the potential materials in producing safer disinfectants.

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1.0 Introduction

Disinfectant usage is not peculiar among us for hygiene purposes. Moreover, sanitising has become a daily human culture due to pandemics. A disinfectant is a substance that is directly administered to an inanimate object, where most harmful bacteria and some viruses, but not spores, are killed or irreversibly inactivated (Dvorak et al., 2008). There are many types of disinfectant such as chorine-based, alcohol-based, quaternary ammonium compounds, and many more (Chapman, 2003). Chemical type disinfectant is widely used. However, the usage of chemical disinfectants can be detrimental to health. It can cause bronchospasm due to inhalation, nausea, eye and skin irritation, and vomiting (Ghafoor et al., 2021). The natural-based disinfectant was formulated in this research by incorporating the UCO and citrus waste.

The wide usage of cooking oil in private households, restaurants, and established catering has contributed to an outstanding amount of 500,000 tonnes of waste cooking oil annually in Malaysia (Daud et al., 2020). Based on the study done by Mohd Rodhi et al. (2020) and Awogbemi et al. (2019), the GC-MS analysis of UCO detects the existence of capric acid (C10:0), palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1), and myristic acid (C14:0). Study mentioned that consuming UCO could harm human health as higher saturated fatty acid contents that can increase the risk of diabetes and cholesterol (Awogbemi et al., 2019). Currently, the UCO is a valuable raw material for biodiesel, detergent, and soap production. Therefore, to value-add UCO, it is recommended that the UCO is used as the ingredient in disinfectant making. Free fatty acid (FFA) in UCO has antimicrobial properties, which make it beneficial as a natural-based disinfectants (Desbois & Smith, 2010). UCO also is surfactants which can chemically coat the particle of disinfectant due to the leftover fatty acid content (Filipe et al., 2021). The fatty acid molecules in leftover cooking oil encountering the fatty outer layer of microbes, where they can be solubilised and damage the microbial structure (Desbois & Smith, 2010; Filipe et al., 2021). Simultaneously, the phytochemical content in citrus waste act as antimicrobial.

In this research, the citrus waste-infused UCO were formulated using dried and fresh citrus waste of orange (*Citrus sinensis*), lemon (*Citrus limon*), and key lime (*Citrus aurantifolia*). Previous work by Saleem & Saeed (2020) found that lemon showed the best antimicrobial activity, followed by orange and banana

Table 1: Citrus waste in inhibiting bacteria

Citrus Waste	Finding	References
Lemon, banana, and orange peel	Lemon conveys the best antimicrobial activity, followed by orange and banana.	Saleem & Saeed (2020)
Orange peel extract	Orange peel extract using alcohol gave the best antimicrobial activity.	Baba et al. (2018)
Grapefruit, lemon, mandarin, tangerine, and orange essential oil	*E. coli ATCC 25922* was effectively inhibited by grapefruit, mandarin, and lemon essential oil.	Raspo et al. (2020)
Key lime juice	Key lime citrus juice suppresses the growth of *S. aureus* in 3 hours, compared to 1 hour for *E. coli.*	Ping Ooi et al. (2019)

fruits. A study conducted by Ping Ooi et al. (2019) observed the reduction of bacteria colony by *Citrus aurontifolia* juice. Another study conducted by Raspo et al. (2020) proved that the essential oils of grapefruit, mandarin, and lemon were effective in inhibiting *E. coli ATCC 25922*. Thus, citrus wastes are an ideal ingredient for disinfectants. Table 1 summarised the ability of citrus waste in inhibiting bacteria. Hence, this research aims to produce eco-friendly disinfectants from citrus waste and UCO. Rather than merely disposing the waste, incorporating these wastes into the disinfectant should be considered as a creative innovation. Utilising the raw material from waste sources also was helpful to the environment in improving waste management.

2.0 Methodology

2.1 Material

The peels and seeds of the fresh and dried of orange (*Citrus sinensis*), lemon (*Citrus limon*), and key lime (*Citrus aurantifolia*) were utilised. The UCO was collected from various cooking activities for a week. *E. coli* suspension was obtained from the Biotechnology Laboratory at School of Chemical Engineering, UiTM.

2.2 Preparation of citrus fruit and UCO

The citrus waste was dried in the oven at 100 °C for four hours. Then, the fresh and dried citrus waste was ground. Heated vinegar (5%) was mixed with the fresh citrus waste at a ratio of 3:1 w/v. Vinegar was added as a preservation technique. The UCO was filtered to remove the foreign matter and heated to 90 °C. The citrus waste infused UCO was prepared as Table 2, based on the ratio of the citrus fruit and UCO. Then, it was strained out after 12 hours. Eq. (1) was used to calculate the weight/volume percentage:

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

Table 2: Formulations of citrus waste

Type of Citrus Waste	Ratio (% w/v)	Citrus Waste (g)	UCO (mL)	Vinegar (mL)
Fresh	10	10	100	30
	25	25	100	750
	50	50	100	150
Dried	10	10	100	-
	25	25	100	
	50	50	100	

2.3 Total plate count

One mL of *E. coli* suspension was pipetted into nine mL of autoclaved peptone water in a universal bottle, to ensure a countable colony on the plate was obtained. The bottle was shaken vigorously as the bacteria were added into it. Approximately 0.05 mL of diluted bacteria was spread on the agar plate. After a few minutes, the infused oil samples were spread on the agar plate. The plate was then sealed using parafilm and incubated for 24 hours at 37 °C. After incubation, single colonies formed were calculated using Eq. (2).

$$\frac{\text{CFU}}{\text{mL}} = \frac{\text{No. of colonies} \times \text{Total dilution factor}}{\text{Volume of culture plate (mL)}} \quad (2)$$

2.4 Ultraviolet-visible spectrophotometer (UV-Vis) Analysis

UV-Vis (Agilent Technologies/Cary60) was used to determine the content of the citrus waste-infused UCO based on the wavelengths of the spectrum. Scan mode was utilised by setting the wavelength range of 200–800 nm.

2.5 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 both the injector and the interface. 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 ionisation, the electron impact ionisation (EI) mode was used. Full scan mode (m/z range, 50–500) was used for qualitative analysis. The percent composition of compound also was recorded.

3.0 Results and discussion

3.1 UV-Vis Analysis

All spectrums obtained are shown in Fig. 1 to 4. It can be observed that there were two intense peaks at 315 nm and 325 nm for UCO infused with fresh lime at 10%, 25%, 50%, dried lemon at 25%, and dried orange at 25% w/v. Other samples also showed an intense peak along 250−350 nm. This indicates one of the oil property in the infused UCO, which is tocopherol (de Oliveira et al., 2019; Goncalves et al., 2014). According to Ghimire et al. (2017), higher amount of α-tocopherol and polyphenolic compounds in the samples contributes to greater antimicrobial activity. Only fresh lemon-infused UCO of 50% w/v did not exhibit intense peaks within those wavelengths. The oxidation, polymerisation and hydrolysis process might contribute to the significant variation from its initial properties (Mannu et al., 2020).

Most of infused UCO show an intense peak at the range of 240−295 nm and 300−400 nm, indicating the flavonoid. Three samples UCO infused with dried orange 25%, 50% and dried lime 50% w/v flaunted at 285 nm, exhibiting naringin (Cordenonsi et al., 2017). Naringin is a subclass of flavanone, which is flavonoid glycoside, which is an essential component to unleash the antimicrobial properties of infused UCO. It is also proven to inhibit *Enterococcus faecalis, Candida albicans, Lactobacillus, Staphylococcus aureus, Aggregatibacter actinomycetemcomitans, Actinomyces naeslundii,* and *Escherichia coli* (Gutiérrez-Venegas et al., 2019).

Another type of flavanone was detected, which was tangeretin, a polymethoxylated flavone, at 210, 250, 270, and 334 nm (Sharma et al., 2019). The infused UCO that exhibited the peak was dried lemon of 10% and 50% w/v, and fresh orange of 50% w/v. Tangertin is indeed inhibiting bacteria such as *Pseudomonas.*

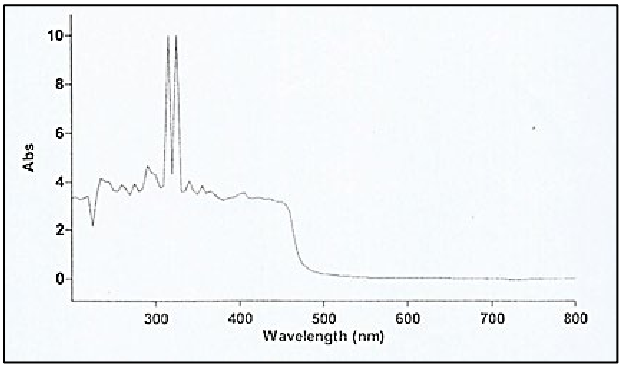


Fig. 2: UV-Vis spectrum of UCO

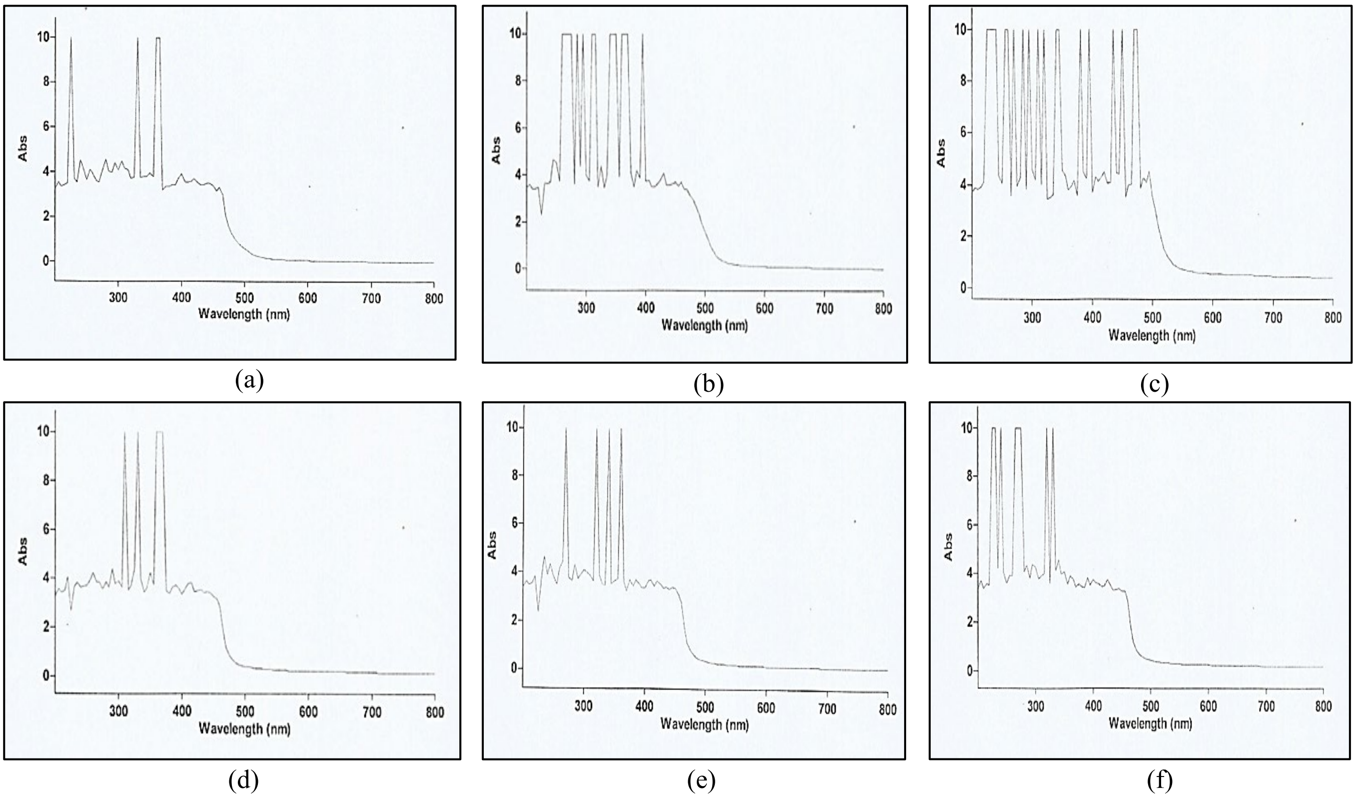


Fig. 1: UV-Vis spectrum of (a) dried orange 10% w/v, (b) dried orange 25% w/v, (c) dried orange 50% w/v, (d) fresh orange 10% w/v, (e) fresh orange 25% w/v, and (f) fresh orange 50% w/v

Fluorescens and *Pseudomonoas aeruginosa* (Yao et al., 2012). According to Shamsudin et al. (2022), flavonoids were capable of exerting antibacterial activities through various mechanisms of action.

Throughout the studies, flavonoids was found to reduce adhesion and biofilm formation, porin on the cell membrane, membrane permeability, and pathogenicity, all of which are crucial for bacterial growth (Górniak et al., 2019; Biharee et al., 2020; Donadio et al, 2021).

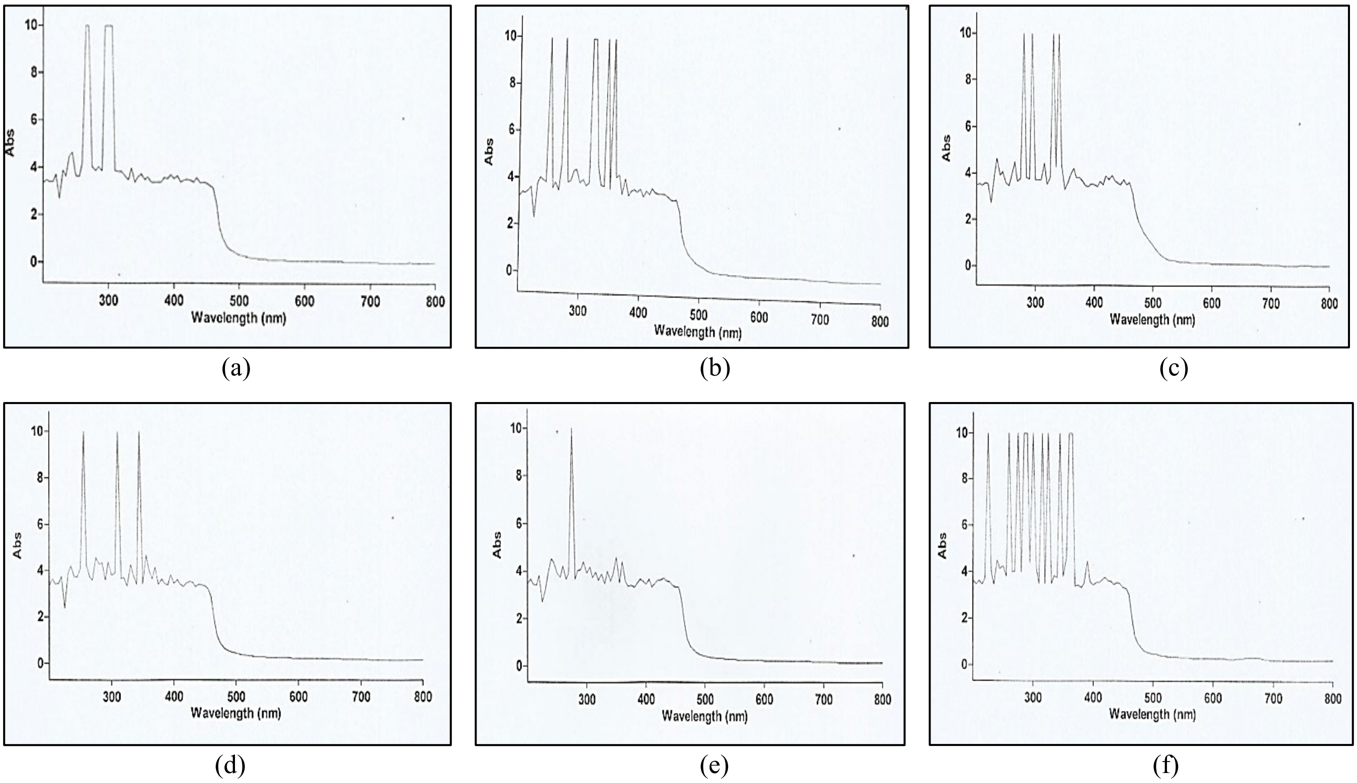


Fig. 3: UV-Vis spectrum of (a) dried lemon 10% w/v, (b) dried lemon 25% w/v, (c) dried lemon 50% w/v, (d) fresh lemon 10% w/v, (e) fresh lemon 25% w/v, and (f) fresh lemon 50% w/v

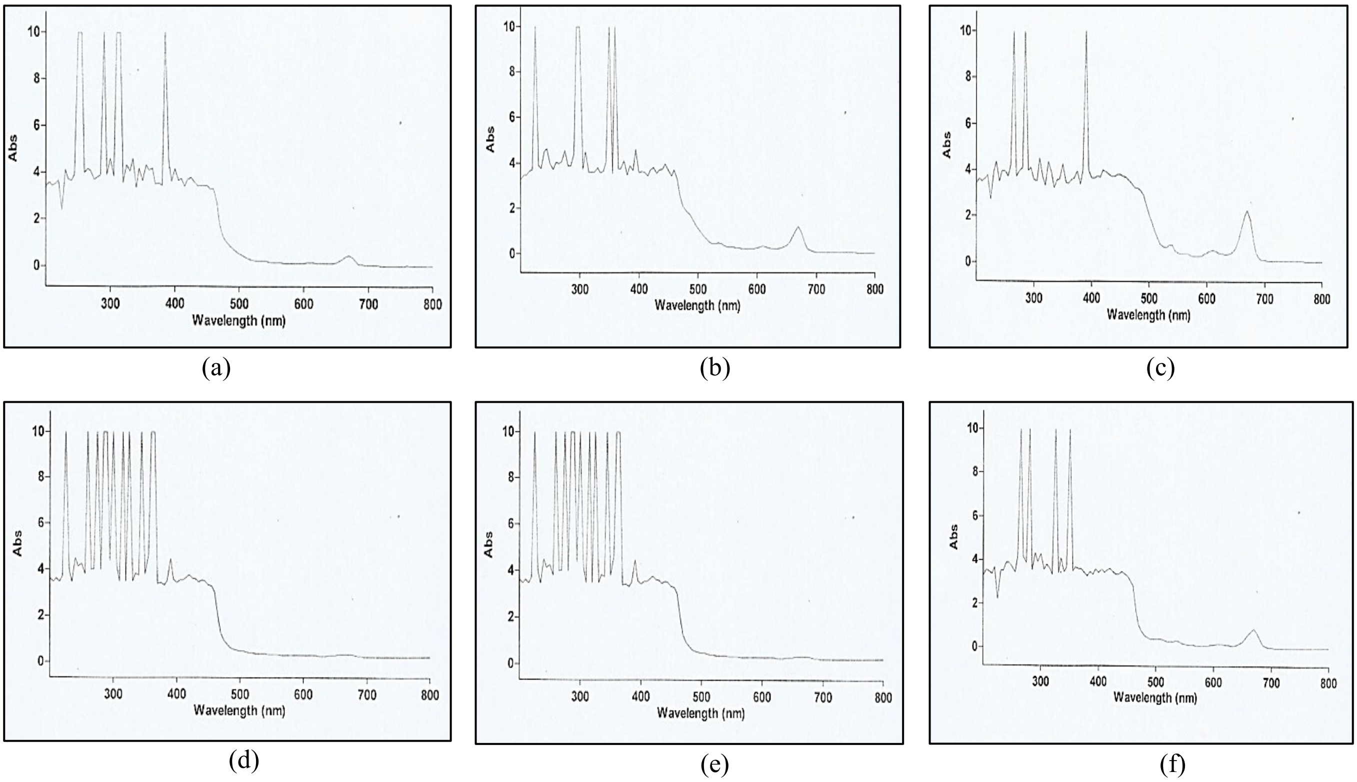


Fig. 4: UV-Vis spectrum of (a) dried lime 10% w/v (b) dried lime 25% w/v (c) dried lime 50% w/v (d) fresh lime 10% w/v (e) fresh lime 25% w/v, and (f) fresh lime 50% w/v

3.2 GC-MS Analysis

The phytochemical content from the GC-MS analysis is shown in Tables 3 to 6. Generally, compound such as terpene, alkaloid, aromatic compound, ester, sesquiterpene, farnesene, trans-bergamotene, curlone, sesquiterpenoid and fatty acids was discovered in the infused UCO. Terpene found including monoterpene, -pinene, myrcene, camphene, -phellandrene, and 4-carene, and monoterpenoids, including isomocyrene and fenchyl acetate. Fatty acids such as oleic acid, and n- hexadecenoic acid, also were primarily found which was similar to the finding by Lim et al. (2018) and Mohd Rodhi et al. (2020). It works as an emulsifying agent and the carrier oil for disinfectant purposes (Muhd Rodhi et al., 2022). The results were supported by research done by Hajlaoui et al. (2021), Kaskoos (2019), and Smith et al. (2001).

Table 3: Phytochemical compositions in cooking oil and UCO

Cooking Oil		UCO	
Compound	**Composition (%)**	**Compound**	**Composition (%)**
2,4-decadienal	0.341	Phenolic	0.18
Hexadecanoic acid	0.312	Hexadecanoic acid	0.169
Secobarbital	0.121	Ascorbyl Palmitate	0.205

Table 4: Phytochemical compositions in orange waste-infused UCO.

Type	Ratio (% w/v)	Compound	Composition (%)
Fresh	10	Terpene	0.372
		Sesquiterpene	0.105
		Fatty Acid	0.291
	25	Monoterpenoid	0.312
		Fatty acid	0.283
	50	Terpene	1.401
		Toluene-D3	0.121
		2,4-Decadienal	0.102
		Palmitic anhydride	0.103
Dried	10	Terpene	0.104
		Ester	0.641
		Alkaloid	0.334
		Sesquiterpene	0.251
	25	Terpene	0.463
		Alkaloid	0.629
		Carboxylic acid	0.173
		Sesquiterpene	0.288
		Ester	0.645
		Fatty acid	0.303
	50	Terpene	1.059
		Sesquiterpene	0.156

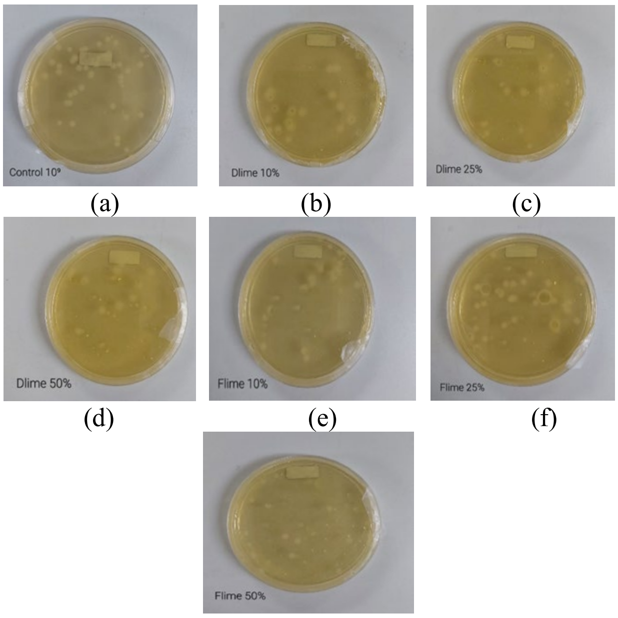
Monoterpenes have many beneficial medicinal characteristics, especially insecticidal ones (Sharma et al., 2022).

Table 5: Phytochemical compositions in lemon waste-infused UCO

Type	Ratio (% w/v)	Compound	Composition (%)
Fresh	10	Benzene	0.471
		Ester	0.172
		Fatty Acid	0.118
	25	Terpene	1.377
		Ester	0.137
	50	Benzene	0.195
		Alkaloid	0.120
		Terpene	0.118
		Alcohol	0.136
Dried	10	Terpene	1.537
		Sesquiterpene	0.321
		Alcohol	0.114
	25	Ester	0.535
		Alkaloid	1.143
		Ketone	0.176
		Alcohol	0.525
		Terpene	3.202
		Sesquiterpene	0.202
	50	Terpene	0.516
		Alkaloid	1.69
		Ester	0.953

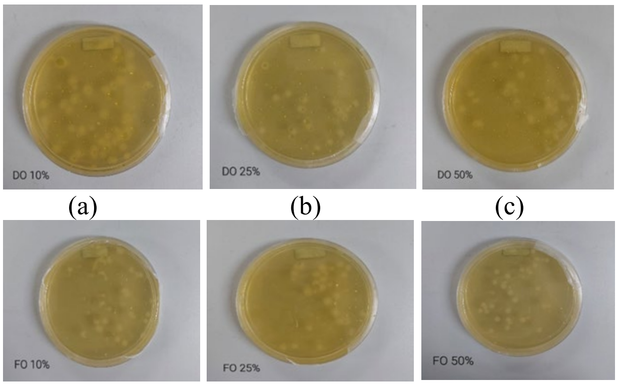
Table 6: Phytochemical compositions in key lime waste-infused UCO

Type	Ratio (% w/v)	Compound	Composition (%)
Fresh	10	Terpene	0.1
		Ester	0.129
		Fatty acid	0.210
	25	Terpene	0.192
		Alcohol	1.0
		Ester	0.225
	50	Terpene	0.336
		Alkene	0.667
		Ester	0.458
		Aromatic compound	0.16
Dried	10	Sesquiterpene	0.166
		Terpene	0.147
		Ester	0.377
	25	Ether	0.138
		Terpene	2.814
		Alkaloid	0.251
		Ester	0.122
		Sesquiterpene	1.04
		Ester	0.11
		Fatty acid	0.251
	50	Benzene	0.11
		Alkaloid	0.289
		Ester	0.157
		Terpene	1.184



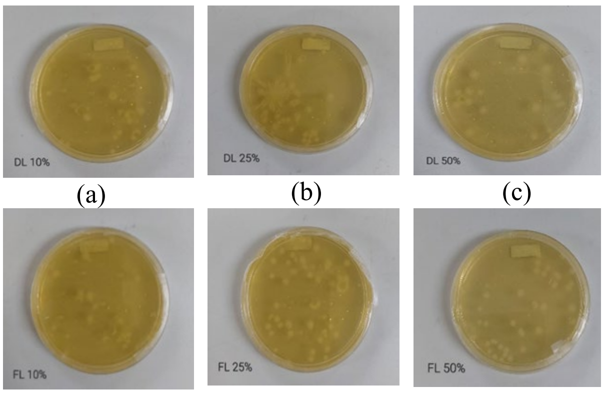
(a) (b) (c) (d) (e) (f) (g)

Fig. 5: Growth of microorganisms in 24 hours for w/v of (a) Control, (b) dried key lime 10%, (c) dried key lime 25%, (d) dried key lime 50%, (e) fresh key lime 10%, (f) fresh key lime 25%, and (g) fresh key lime 50%



(a) (b) (c) (d) (e) (f)

Fig. 6: Growth of microorganisms in 24 hours for w/v of (a) dried orange 10%, (b) dried orange 25%, (c) dried orange 50%, (d) fresh orange 10%, (e) fresh orange 25%, and (f) fresh orange 50%



(a) (b) (c) (d) (e) (f)

Fig. 7: Growth of microorganisms in 24 hours for w/v of (a) dried lemon 10%, (b) dried lemon 25%, (c) dried lemon 50%, (d) fresh lemon 10% (e) fresh Lemon 25% (f) fresh lemon 50%

Alkaloids present in the produced infused oil include 4-isobutylpyrimidine, pyridine, 2-chloro-6-(2-furanylmethoxy)-4-(trichloromethyl), and 1-(1-Cyanocyclopentyl) pyrrolidine (Rohloff, 2015). However, many studies used a standard test rather than GC-MS analysis to find alkaloids (Baba et al., 2018; Javed et al., 2014; Sharma et al., 2022). Nevertheless, the composition might be varied as the involvement of solvent, type of test implements and many other factors. The GC-MS study also shows the presence of ester, aromatic, and phenolic chemicals. Every bioactive element does contribute to the infused UCO's ability to act as a disinfectant. As a result, the production of citrus waste-infused UCO has a lot of favourable properties.

3.3 *Antimicrobial Activity Analysis*

The *E. coli* inhibition activities by citrus waste-infused UCO are shown in Table 7 and Fig. 5 to 7. The antimicrobial analysis showed that both dried and fresh key lime with 50% w/v gave the highest *E. coli* inhibition compared to other infused UCO within 24-hour of incubation. Only 19 visible colonies were counted on the agar plate spread with dried lime infused oil of 50% w/v, showing a 60% reduction compared to the control plate. 68% reduction was obtained for the agar plate spread with fresh key lime 50% w/v.

Research also corroborates Ping Ooi et al. (2019) findings, wherein 91.72% reduction in colony growing was found when *Citrus aurantifolia* juice was used as an antimicrobial agent. The 25% w/v of fresh lemon

Table 7: Colony-forming

Sample		Ratio (% w/v)	Colony formed per unit (CFU/mL)
Control		-	9.6×10^{17}
Fresh	Orange	10	6.2×10^{17}
		25	6.6×10^{17}
		50	7×10^{17}
	Lemon	10	5.4×10^{17}
		25	9.4×10^{17}
		50	8.8×10^{17}
	Key Lime	10	4×10^{17}
		25	6.2×10^{17}
		50	3.3×10^{17}
Dried	Orange	10	9×10^{17}
		25	5.4×10^{17}
		50	7.2×10^{17}
	Lemon	10	4.4×10^{17}
		25	7.2×10^{17}
		50	5.6×10^{17}
	Key Lime	10	4.8×10^{17}
		25	4.6×10^{17}
		50	3.8×10^{17}

infused oil gave poor inhibition of *E. coli* growth with 47 colonies. PRO et al. (2019) also mentioned that lemon gave the least bacteria inhibition effect. However, this finding contradicts with the previous research done by Muhd Rodhi et al. (2022) and Saleem & Saeed (2020). Their study concludes that lemon exhibited the highest inhibitory effect on microbe growth due to the higher content of antimicrobial compound.

The microbial analysis has proven that UCO infused citrus waste was able to inhibit the growth of microbes. The results supported the existence of substances identified from UV-Vis and GC-MS analysis that carries out the activity.

4.0 Conclusions

This study demonstrated that UCO infused with citrus waste is an environmentally beneficial disinfectant due to fatty acid, flavonoid, monoterpene, monoterpenoid, sesquiterpene, sesquiterpenoid, alkaloid, and aromatic chemicals found in it. The antimicrobial activity demonstrates the product's potential with the reduction of *E. coli* colony growth on the agar plate. 50% of dried and fresh citrus waste infused with UCO unleashes the best antimicrobial effect. The results of this study also show that the waste from citrus fruits contains antibacterial components, justifying its use as green-based disinfectants. As a result, the formulation of disinfectants from citrus waste and UCO was seen as having the potential to reduce waste and serve as one of the possible ingredients in the future creation of safer disinfectants.

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