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Gas chromatography profiling and antimicrobial activity of calamansi (*Citrus macrocarpa*) peels essential oil

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Abstract

Calamansi (*Citrus macrocarpa*), a citrus fruit widely used in the Philippines, produces substantial peel waste, particularly in Northern Luzon, where it is a key condiment in Filipino stir-fried noodles commonly known as "pansit". Despite the nutritional and culinary importance of calamansi, its peels, which are rich in essential oils, remain underutilized. This study aims to profile the chemical composition of calamansi peel essential oil extracted using a fabricated extractor machine for community use and to evaluate its antimicrobial properties. Gas Chromatography-Mass Spectrometry (GC-MS) identified 109 compounds in the extracted oil, with D-limonene comprising 65.59%. The antimicrobial activity was tested against various bacterial and fungal strains using the disc diffusion method. The oil showed partially active to very active inhibitory effects against *Staphylococcus aureus*, *Staphylococcus pyogenes*, and *Bacillus subtilis*, particularly at higher concentrations (up to 10%). Additionally, it demonstrated significant antifungal activity against *Aspergillus flavus*, *Aspergillus niger*, and *Aspergillus fumigatus*, with the 10% concentration yielding comparable results to positive control. These findings highlight the potential of calamansi peel essential oil as a natural antimicrobial agent with applications in food preservation, pharmaceuticals, and cosmetics. The study also emphasizes the economic and environmental benefits of repurposing calamansi peel waste, suggesting that future research may focus on optimizing extraction methods and exploring higher concentrations for enhanced efficacy.

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Introduction

Calamansi (*Citrus macrocarpa*), a citrus fruit native to the Philippines, is widely cultivated for its significant nutritional and culinary benefits. The fruit is commonly used in various food products and as a condiment in traditional Filipino dishes, making it a staple ingredient in many households and food industries. However, despite its popularity, a large portion of calamansi, particularly its peels, seeds, and pulp, is often discarded during processing. The peels, which can account for 50% to 70% of the fruit's total weight (Zema *et al.*, 2018), are typically treated as waste, resulting in missed opportunities to utilize their potential value.

Emerging studies have demonstrated that biological activities predominantly occur in peels rather than in the often-ingested fruit pulp (Czech *et al.*, 2021). Calamansi peels are rich in essential oils that have potential applications in various industries, including cosmetics, pharmaceuticals, and food preservation, due to their antibacterial and antioxidant properties (Venkatachalam *et al.*, 2023). However, despite the well-documented benefits of calamansi peels, they remain underutilized, especially in local industries where they are often discarded as waste byproducts.

Pansit, a traditional Filipino noodle dish enjoyed nationwide in diverse forms and flavors, holds a special place in Filipino cuisine. Its popularity is so widespread that various regions, particularly Northern Luzon, often referred to as the "pansit capital," have developed their own unique variations. Calamansi is a staple condiment served alongside *pansit*, adding a distinctive flavor to the dish. With more than 200 *pansiterias* (noodle restaurants) in Northern Luzon alone, high demand for calamansi generates considerable peel waste as a byproduct. While there is growing recognition of the potential uses of calamansi peels, particularly in the extraction of essential oils, a gap exists in the knowledge and availability of efficient, cost-effective extraction technologies that are accessible to local communities.

Traditional methods of essential oil extraction, such as hydrodistillation, cold pressing, and solvent extraction, are often labor-intensive, expensive, and require specialized equipment. These barriers limit the adoption of essential oil extraction at the household or small-scale level, particularly in rural areas. To address this gap, a steam distillation extractor machine has been developed in collaboration with mechanical engineers and industrial technologists (Capili, 2023). This machine is designed to be portable, affordable, and easy to use, making it suitable for community-level essential oil extraction.

The aim of this study is to profile the chemical composition of the essential oil extracted through the developed machine by Capili (2023) from calamansi peelings using Gas Chromatography-Mass Spectrometry (GC-MS) and to evaluate its antimicrobial properties. By using the newly developed steam distillation machine, this research seeks to establish a more efficient and practical approach to utilizing calamansi peel waste, thereby contributing to both waste reduction and the local economy. The findings of this study could provide valuable insights for industries and communities in the Philippines and beyond, promoting the sustainable use of agricultural byproducts.

Materials and methods

Calamansi peelings were collected in *pansiterias*. The fruit peels were washed and cut into small pieces. The cut peels were subjected to hydrodistillation using the two fabricated extractors' prototypes.

For the extraction, the dried chopped calamansi peels were weighed and introduced into the boiler chamber and closed tightly with stainless steel clips, so that oil and vapor do not leak out the system. A temperature gauge monitored the boiling chamber, while a thermal camera recorded burner

and heating element temperatures. The amount of oil collected was measured and recorded.

Gas Chromatography-Mass Spectrometry analysis was conducted at XPRT Analytical Services laboratory to identify the specific compounds present in the extract. The disc diffusion method was employed for the determination of the antibacterial and antifungal activity of the essential oil. The conduct of the study was outsourced at Department of Science and Technology [DOST] Region 02 service laboratory.

Results

Table 1 presents the chemical composition of the extracted essential oil based on GCMS analysis. A total of 109 compounds were detected in the extracted essential oil, including terpenes, terpenoids, sesquiterpenes, alcohols, aldehydes, esters, and ketones. Analysis revealed that the predominant component in calamansi peelings essential oil is D-limonene (65.59%).

This extracted oil has been tested for its antibacterial activity in which the results are summarized in Table 2. The table indicates that the essential oil has active to very active inhibitory effect against *Staphylococcus aureus* and *Staphylococcus pyogenes* at different concentrations, specifically 1.5%, 2.5%, 5%, and 10% while the extract shows partially active to active inhibitory effect against *Bacillus subtilis* at the same concentrations.

Table 1. Chemical constituents of the extracted essential oil

SL	Component	Composition (%)	Retention time
1	D-limonene	65.59	8.1176
2	beta-myrcene	3.98	6.7123
3	beta-pinene	3.50	6.434
4	alpha-pinene	1.93	5.4835
5	(1R,28,6S,7S,8S)-8-isopropyl-1-methyl-3-methylenetricyclo[4.4.0.02,7]decane-rel-	1.92	18.2871
6	gamma-terpinene	1.73	7.2079
7	p-mentha-1(7),8-dien-2-ol	1.63	11.9804
8	alpha-terpineol	1.49	12.0686
9	trans-1-methyl-4-(1-methylethenyl)-2-cyclohexen-1-ol	1.23	10.127
10	cis-2-methyl-5-(1-methylethenyl)-2-cyclohexen-1-ol	1.16	12.7882
11	cis-p-mentha-1(7)-8-dien-2-ol	1.05	13.0394
12	(-)-Carvone	1.04	13.4671
13	Geranyl acetate	0.75	17.0448
14	1-methyl-4-(1-methylethenyl)-Benzene	0.67	9.326
15	(-)-trans-sopiperitenol	0.67	12.3062
16	[4aR- (4a.alpha.,7.alpha.,8a.beta.)]-decahydro-4a-methyl-1-methylene-7-(1-methylethenyl)-naphthalene	0.63	19.7331
17	Decanal	0.56	12.3605
18	p-mentha-1,5,8-triene	0.54	7.0585
19	trans-2-methyl-5-(1-methylethenyl)-cyclohexanone	0.45	12.2044
20	4-isopropenylcyclohexanone	0.41	11.0503
21	(R)-4-methyl-1-(1-methylethyl)-3-cyclohexen-1-ol	0.38	11.6477
22	Carveol	0.37	13.1005
23	Linalool	0.36	9.55
24	(1-alpha-2-alpha-5-beta)-2-methyl-5-(1-methylethenyl) cyclohexanol	0.35	12.1433
25	[2R-(2-alpha,4a-alpha,8a-beta)]decahydro-alpha,alpha,4a- trimethyl-8-methylene-2-Naphthalenemethanol	0.34	23.5484
26	trans-limonene oxide	0.33	10.5751
27	stereoisomer 1-methyl-3-methylene-8-(1-methylethyl)-tricyclo[4.4.0.0(2,7)]decane	0.32	18.0359
28	Nonanal	0.31	9.6518
29	(2R-cis)-1,2,3,4,4a,5,6,7-octahydro-alpha,alpha-4a,8- tetramethyl-2-naphthalenemethanol	0.29	23.6027
30	2-isopropenyl-4a,8-dimethyl-1,2,3,4,4a,5,6,8a-octahydronaphthalene	0.25	19.93
31	(1R,7S,E)-7-isopropyl-4,10-dimethylenecyclodec-5-enol	0.25	22.0956
32	(3R-trans)-4-ethenyl-4-methyl-3-(1-methylethenyl)-1-(1-methylethenyl)cyclohexene	0.24	15.911
33	Dodecanal	0.24	17.6693

34	4-methylene-1-(1-methylethyl)-bicyclo[3.1.0]hexane	0.23	6.3321
35	beta-bisabolene	0.23	20.1744
36	(E)-2-decenal	0.22	13.8541
37	(Z)-3,7-dimethyl-2,6-octadien-1-ol acetate	0.22	16.5288
38	Anethole	0.18	14.5329
39	[1S-(1-alpha-2-beta-4-beta)]-1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)cyclohexane	0.18	17.3231
40	1,3,8-p-menthatriene	0.17	9.8894
41	Acetic acid octyl ester	0.15	12.5031
42	Undecanal	0.15	15.0489
43	(1S-cis)-1,2,3,5,6,8a-hexahydro-4,7-dimethyl-1-(1- methylethyl)-naphthalene	0.15	20.5613
44	(-)-beta-bourbonene	0.14	17.167
45	beta-ocimene	0.13	8.4366
46	trans-alpha-bergamotene	0.13	18.3957
47	4,11,11-trimethyl-8-methylene-[1R-(1R*,4Z,9S*)]-bicyclo[7.2.0]undec-4-ene	0.13	18.8913
48	gamma-muurolene	0.13	19.4615
49	(S)-4-(1-methylethenyl)-1-cyclohexene-1-carboxaldehyde	0.12	14.2546
50	alpha-muurolene	0.12	20.0046
51	cis-5-ethenyltetrahydro-alpha,alpha,5-trimethyl-2- furanmethanol	0.1	8.9254
52	2-Methylbutanoic anhydride	0.09	5.8434
53	(E)-5,9-dimethyl-5,8-decadien-2-one	0.09	10.9145
54	Isospathulenol	0.09	23.046
55	1-octanol	0.08	8.8032
56	Cyclododecane	0.08	14.1052
57	(S)-3-methyl-6-(1-methylethenyl)-2-cyclohexen-1-one	0.07	14.1867
58	Junenol	0.07	22.8423
59	1-methyl-2-methylene-cycloheptanol	0.06	10.8195
60	p-mentha-1(7),8(10)-dien-9-ol	0.06	14.6415
61	trans-carveyl acetate	0.06	16.3591
62	Lavandulyl propionate	0.06	19.2918
63	Ledene oxide-(II)	0.06	20.7514
64	[1R-(1-alpha-3-alpha-4-beta-)]-4-ethenyl-alpha-alpha-4-trimethyl-3-(1-methylethenyl)-cyclohexanemethanol	0.06	21.1723
65	[1ar-(1a-alpha-4a-alpha-7-beta-7a-beta-7b-alpha)]- decahydro-1,1,7-trimethyl-4-methylene-1H- cycloprop[e]azulen-7-ol	0.06	21.8783
66	Ylangenal	0.06	23.2361
67	(4-alpha)-kaur-16-en-18-al	0.06	34.2881
68	2-methyl-5-(1-methylethyl)-bicyclo[3.1.0]hex-2-ene	0.05	5.3138
69	(E)-2-octenal	0.05	8.5792
70	1-methyl-4-(1-methylethenyl)-cyclohexanol	0.05	10.7516
71	Pinocarvone	0.05	11.2608
72	(S,1Z,6Z)-8-isopropyl-1-methyl-5-methylenecyclodeca-1,6-diene	0.05	18.6605
73	(1-alpha-4a-beta-8a-alpha)-1,2,3,4,4a,5,6,8a-octahydro-7-methyl-4-methylene-1-(1-methylethyl)-naphthalene	0.05	20.3577
74	Bis(2-ethylhexyl) phthalate	0.05	39.8753
75	4-acetyl-1-methylcyclohexene	0.04	10.385
76	p-mentha-1,8-dien-7-ol	0.04	14.8792
77	2-(4a,8-dimethyl-1,2,3,4,4a,5,6,7-octahydro-naphthalen-2- yl)-prop-2-en-1-ol	0.04	23.4194
78	6,10,14-trimethyl-2-pentadecanone	0.04	27.5333
79	Octane	0.03	3.2365
80	Acetic acid nonyl ester	0.03	15.1303
81	(E,E)-2,4-decadienal	0.03	15.3001
82	Copaene	0.03	16.9226
83	(+)-epi-bicyclosesquiphellandrene	0.03	19.1289
84	Hexadecanoic acid methyl ester	0.03	30.4593
85	Hexadecanoic acid ethyl ester	0.03	30.4593
86	5-methyl-2-furancarboxaldehyde	0.02	6.0402
87	Geraniol	0.02	13.6572
88	3-methyl-6-(1-methylethylidene)-2-Cyclohexen-1-one	0.02	16.0129
89	8-methylene-dispiro[2.0.2.5]undecane	0.02	18.5519
90	[1R-(1-alpha-4a-alpha-8a-alpha]-1,2,4a,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-naphthalene	0.02	20.9075
91	(1S,3aR,4R,8R,8aS)-1-isopropyl-3a-methyl-7- methylenedecahydro-4,8-epoxyazulene	0.02	21.6272

92	(E)-9-octadecenoic acid methyl ester	0.02	32.4484
93	2,2'-methylenebis[6-(1,1-dimethylethyl)-4-methylphenol	0.02	37.9201
94	Hexanal	0.01	3.2025
95	Furfural	0.01	3.6166
96	Heptanal	0.01	4.7504
97	2,6,6-trimethyl-2-cyclohexene-1-carboxaldehyde	0.01	10.0456
98	Dodecamethylcyclohexasiloxane	0.01	15.5852
99	E-11,13-tetradecadien-1-ol	0.01	16.7393
100	Ylangene	0.01	16.7393
101	[1aR-(1a-alpha-7-alpha-7a-alpha-7b-alpha)]-1a,2,6,7,7a,7b- hexahydro-1,1,7,7a-tetramethyl-1H-cyclopropa[a]naphthalene	0.01	17.52
102	2,6,10-trimethyltridecane	0.01	18.966
103	3,7,11-trimethyl-1,6,10-dodecatrien-3-ol	0.01	21.4506
104	Hexadecanal	0.01	26.9495
105	m-camphorene	0.01	29.7125
106	(Z,Z)-9,12-octadecadienoic acid methyl ester	0.01	32.333
107	Ethyl oleate	0.01	33.6432
108	Heneicosane	0.01	35.8699
109	5,5-Dimethyl-1-vinylbicyclo[2.1.1]hexane	0.005	5.1781

Table 2. Mean comparison on the antibacterial activity of different concentrations of the calamansi essential oil against test organisms

Concentration levels	Mean zone of inhibition per test organism (mm)		
	S. aureus	*S. pyogenes*	*B. subtilis*
1.5%	14	16	13
2.5%	17	16	15
5%	18	19	16
10%	21	23	18
(+) control	37	37	37
(-) control	6	6	6

*Note: The DOST standard for interpretation of zone of inhibition are as follows: <10mm- inactive, 10-13mm- partially active, 14-19mm- active, >19mm- very active

At 5% level of significance, there is sufficient evidence to say that there is a significant difference on the zone of inhibition across the treatments and control groups in terms of the test organisms as shown in Table 3. This suggests that antibacterial activity of the extracts significantly vary across concentrations against tested organisms.

Table 3. Test of effects on the zone of inhibition of the different concentrations and control groups against bacterial organisms

Test organisms	F-value	p-value
Staphylococcus aureus	299.832	<0.00
Staphylococcus pyogenes	105.233	<0.00
Bacillus subtilis	136.276	<0.00

Furthermore, based on the post hoc analysis conducted on all types of test organisms in Table 4, higher extract concentrations yield greater zone of inhibition. Moreover, compared to the treatments prepared at lower concentrations, the antibacterial activity of the highest concentration (10%) is significantly higher.

Table 4. Comparison among means (LSD) on the zone of inhibition of the different concentrations

Test Organisms	Treatment	Means	Mean differences					
			10%	5%	2.5%	1.5%	(+) control	(-) control
Staphylococcus aureus	1.5%	14	-					
	2.5%	17	3.00*	-				
	5%	18	4.00*	1.00	-			
	10%	21	7.00*	4.00*	3.00*	-		
	(+) control	37	23.00*	20.00*	19.00*	16.00*	-	
	(-) control	6	-8.00*	-11.00*	-12.00*	-15.00*	-31.00*	-
Staphylococcus pyogenes	1.5%	16	-					
	2.5%	16	0.00	-				
	5%	19	3.00*	3.00*	-			

	10%	23	7.00*	7.00*	4.00*	-		
	(+) control	35	19.00*	19.00*	16.00*	12.00*	-	
	(-) control	6	-10.00*	-10.00*	-13.00*	-17.00*	-29.00*	-
Bacillus subtilis	1.5%	13	-					
	2.5%	15	2.00*	-				
	5%	16	3.00*	-1.00	-			
	10%	18	5.00*	3.00*	2.00*	-		
	(+) control	32	19.00*	17.00*	16.00*	14.00*	-	
	(-) control	6	-7.00*	-9.00*	-10.00*	-10.00*	-26.00*	-

*Mean differences are significant at 0.05

Meanwhile, Table 5 presents the antifungal activity of various doses of essential oils against test organisms. At 1.5%, 2.5%, 5%, and 10%, the essential oil exhibits an active to very active inhibitory effect against *Aspergillus niger* and *Aspergillus flavus* while demonstrates a partially active to active inhibitory effect against *Aspergillus fumigatus*.

Table 5. Mean comparison on the antifungal activity of different concentrations of the calamansi peel essential oil against fungal test organisms

Concentration levels	Mean zone of inhibition per test organism (mm)		
	A. niger	*A. flavus*	*A. fumigatus*
1.5%	15	14	12
2.5%	16	17	14
5%	19	18	17
10%	22	22	21
(+) control	36	36	36
(-) control	6	6	6

*Note: The DOST standard for interpretation of zone of inhibition are as follows: <10mm- inactive, 10-13mm-partially active, 14 19mm- active, >19mm- very active

Table 6. Test of effects on the zone of inhibition of the different concentrations and control groups for *Aspergillus flavus*

Test organisms	F-value	p-value
Aspergillus flavus	152.4	<0.00

In addition, the effects of extract concentration against *Aspergillus flavus* can be gleaned on Table 6. An F-value of 152.4, with associated probability of 0.00, suggests that there is enough evidence to reject the null hypothesis. This implies that there is significant difference on the antifungal activity of the extracts against *A. flavus* when grouped according to treatments.

Table 7. Comparison among means on the zone of inhibition of the different concentrations and control groups for *Aspergillus flavus*

Test organisms	Treatment	Means	Mean differences					
			1.5%	2.5%	5%	10%	(+) control	(-) control
Aspergillus flavus	1.5%	14	-					
	2.5%	17	3.00*	-				
	5%	18	4.00*	1.00	-			
	10%	22	8.00*	5.00*	4.00*	-		
	(+) control	34	20.00*	17.00*	16.00*	12.00*	-	
	(-) control	6	8.00*	-11.00*	-12.00*	-16.00*	-28.00*	-

*Mean differences are significant at 0.05

Post hoc analysis in Table 7 shows that the antifungal activity of 10% concentration is significantly higher than the other lower concentrations when tested against *A. flavus*. Moreover, the antifungal activity of the extract significantly differs with the positive control.

On the other hand, Table 8 summarizes the effects of the extracts' concentration on the zone of inhibition for *Aspergillus niger* and *Aspergillus fumigatus* based on the Kruskal-Wallis test. At 5% level of significance, there is sufficient evidence to say that there is a significant difference on the area of inhibition across the treatments and control groups of the fungi being studied.

Moreover, the pairwise comparisons for both organisms tested indicate that the negative control

treatment differed significantly from the treatments with 10%, 5%, and the positive control. Furthermore, the positive control treatment differed significantly from the treatments with 2.5% and 1.5%. It is worthy to note that the 10% concentration did not show a significant difference with the positive control.

Table 8. Test of effects on the zone of inhibition of the different concentrations and control groups for *Aspergillus niger* and *Aspergillus fumigatus*

Test organisms	Descriptive statistic			Kruskal-Wallis H				Pairwise comparison
	Treatment	n	Mean rank	X^2	df	p-value	Decision	
Aspergillus niger	1.5%	3	5.83	16.11	5	0.007	Reject H_o	(-) control with 10%, 5% & (+) control
	2.5%	3	7.17					
	5%	3	11.33					(+) control with 2.5% and 1.5%
	10%	3	13.67					
	(+) control	3	17.00					
	(-) control	3	2.00					
Aspergillus fumigatus	1.5%	3	5.50	16.33	5	0.006	Reject H_o	(-) control with 10%, 5% & (+) control
	2.5%	3	7.50					
	5%	3	11.17					(+) control with 2.5% and 1.5%
	10%	3	13.83					
	(+) control	3	17.00					
	(-) control	3	2.00					

Discussion

The major component of the extracted essential oil comprising 65.59% is D-limonene. This finding is consistent with Palma *et al.* (2019), who reported that D-limonene constitutes at least 92.67% of the total oil content in calamansi essential oil, making it as its chief constituent.

D-limonene is a monocyclic monoterpene compound characterized by a lemon-like scent and is a prominent component found in various citrus oils, including those derived from orange, lemon, mandarin, lime, and grapefruit (Sun, 2007). The use of D-limonene as a flavor and fragrance enhancer in perfumes, soaps, foods, and beverages has been steadily growing due to its excellent aromatic qualities (Anandakumar *et al.*, 2020).

Apart from its industrial applications, D-limonene has also a wide range of clinical uses such as dissolving cholesterol-containing gall stones (Igimi *et al.*, 1976), relieving heartburn and Gastroesophageal Reflux Disease because of its potential for gastric acid neutralization and its support for healthy peristalsis (Wilkins, 2002) as well as chemo preventive activity against many types of cancer (Yu *et al.*, 2018).

This result is economically interesting considering that a number of *pansiterias* or noodle houses around the locale of the study produce a large amount of calamansi fruit waste particularly its peelings which can be good sources of D-limonene. Further studies may also explore the various factors that may influence in recovering optimum amount of D-limonene from calamansi peelings essential oil.

The antibacterial activity results highlight the potential of the extracted essential oil as an effective antimicrobial agent. The results indicate a broad-spectrum effectiveness that intensifies with increased concentration suggesting that the essential oil components possess strong bacteriostatic or bactericidal properties capable of targeting these common pathogens, which are often responsible for skin and soft tissue infections. This can be attributed to the presence of the combination of terpenes and terpenoids.

Literature claimed that various terpenoids have bacteriostatic and bactericidal effect on a spectrum of bacteria (Yamaguchi, 2022). Moreover, significant monoterpene component of the extract, D-limonene, has been shown in numerous investigations to be an efficient inhibitor of gram-positive and gram-negative bacteria (Hsouna *et al.*, 2011; Han *et al.*, 2019). Furthermore, D-limonene has been shown by numerous studies to effectively prevent the formation

of spoilage microorganisms like *Escherichia coli*, *Pseudomonas aeruginosa*, *Aspergillus niger*, and *Staphylococcus aureus* (Lee *et al.*, 2007; Celaya *et al.*, 2014).

Additionally, results of this study reveal that higher essential oil extract concentration result in higher zone of inhibition where highest antibacterial activity is observed at 10% concentration. This is corroborated in one study where higher extract concentrations demonstrated a considerable antibacterial and antifungal action (Mehta *et al.*, 2020). This implies that extract's antibacterial activity is concentration dependent. Study showed that following treatment with two limonene isomers, there was a concentration-dependent decrease in the production of biofilm (Kim *et al.*, 2024). Higher extract concentrations have a greater zone of inhibition because they contain greater number of phytochemicals constituents.

Moreover, there is a significant difference between the inhibitory activity of the positive control and 10% concentration. This suggests that in terms of antibacterial activity, the greatest concentration of essential oil under study is not as effective as the positive control. This implies that future studies may consider preparing calamansi peelings essential oil concentrations that are higher than 10% and explore the concentrations that will optimize the antibacterial activity of the oil and formulate concentrations that will be comparable to the positive control.

The calamansi peelings essential oil has been assayed for antifungal activity and is noted to show partially active to very active inhibitory affects against *Aspergillus flavus, Aspergillus niger*, and *Aspergillus fumigatus*. Based on the findings of Leite-Andrade *et al.* (2022), D-limonene displayed an antifungal activity by blocking the fungi morphogenesis and adhesion to human epithelium. Another study revealed that D-limonene disrupts cell anabolism and catabolism, as well as break the potential, permeability, and integrity of the cell membrane which results to its antifungal activity (Yu *et al.*, 2022).

The post-hoc analysis of this study establishes that the antifungal activity at a 10% concentration was notably higher compared to the lower concentrations when tested against *Aspergillus flavus*. Additionally, the antifungal effect of the extract shows a significant difference compared to the positive control. This result can be attributed to concentration-dependence of extract antifungal activity. In the antifungal and inhibitory effects of DL-limonene study conducted by Ünal *et al.* (2012), it has been demonstrated that as the quantity of DL-limonene increases, the diameter of the inhibition zone also increases.

Moreover, results show that significant difference in zone of inhibition across the various treatments and control groups for both *Aspergillus niger* and *Aspergillus fumigatus*. It is important to highlight that the 10% concentration did not exhibit a significant difference compared to the positive control, indicating that the efficacy of the 10% treatment is similar to that of the positive control. This result aligns with the findings of Ünal *et al.* (2012), who reported antifungal activity associated with D-limonene.

Conclusion

This study successfully demonstrated the potential of calamansi peel essential oil, particularly its primary constituent D-limonene, as a viable antibacterial and antifungal agent. Through the use of fabricated portable extraction machine, calamansi peels, which are commonly discarded as waste, were effectively utilized to produce essential oils rich in bioactive compounds. The GC-MS analysis identified 109 compounds in the extracted oil, with D-limonene comprising 65.59%, consistent with previous studies.

The essential oil exhibited significant antimicrobial activity, showing concentration-dependent effects against both bacterial and fungal test organisms. The antibacterial tests indicated strong inhibitory effects against *Staphylococcus aureus*, *Staphylococcus pyogenes*, and *Bacillus subtilis*, particularly at higher concentrations. Similarly, the antifungal activity against *Aspergillus flavus*, *Aspergillus niger*, and *Aspergillus fumigatus* demonstrated that the oil's

effectiveness increased with higher concentrations, with the 10% concentration showing results comparable to the positive control.

These findings underscore the potential of calamansi peel essential oil as a natural antimicrobial agent with applications in various industries, including food preservation, pharmaceuticals, and cosmetics. Moreover, the study highlights the economic and environmental benefits of repurposing calamansi peel waste, particularly in regions like Northern Luzon, where large quantities of peel waste are generated from *pansiterias*. Future research should focus on optimizing extraction methods, exploring higher concentration formulations, and investigating other potential applications for calamansi essential oil to further enhance its commercial and environmental value.

Recommendation(s)

Based on the findings of the study, the following recommendations are hereby presented:

1. Consider testing the antimicrobial activity of the extracts for other pathogens with significance to both clinical and food industry.
2. Conduct antioxidant assay on the extracted essential oil.
3. Sensory evaluation for the essential oil extract may be considered.
4. For commercialization of the produced calamansi essential oil, a bigger prototype can be explored to guarantee huge volume extraction to sustain the source.

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