



Original article

The antioxidant properties and microbial load of *Moringa oleifera* leaves dried using a prototype convective air-dryerKivaandra Dayaa Rao Ramarao^a, Chandran Somasundram^{a,*}, Zuliana Razali^a, Wijenthiran Kunasekaran^b, Tan Li Jin^b^aInstitute of Biological Sciences, Faculty of Science, and Centre for Research in Biotechnology for Agriculture (CEBAR) Universiti Malaya, 50603 Kuala Lumpur, Malaysia^bCytonex Sdn. Bhd, Menara Uoa Bangsar, A-16-17, No. 5, Jalan Bangsar Utama 1, Bangsar, 59000 Kuala Lumpur, Malaysia

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ABSTRACT

The recent COVID-19 pandemic resulted in major postharvest losses because most fresh produce could not be sold. Drying is an important thermal-based food preservation method which could have prolonged the shelf-life of these produce, but most drying technologies are costly, and cannot be afforded by small-time farmers. From this context, we were interested in evaluating the drying of *Moringa oleifera* leaves (MOL) using a low-cost self-built prototype convective-air dryer (CAD), alongside conventional drying methods for its antioxidant properties, microbial load and phytoconstituents. Results showed total polyphenol content was the highest ($p < 0.05$) in our CAD samples, and it retained among the highest total flavonoid content, total antioxidant capacity, total alkaloid content and DPPH radical scavenging activity. Furthermore, methanolic CAD extract presented lower coliform and yeast and mold count than the aqueous CAD extract. We also briefly explored MOL as a sanitizer where the microbial load of the methanolic extract was comparable ($p > 0.05$) with several commercial non-alcoholic sanitizers, indicating its commercialization potential as a bio-friendly sanitizer. Finally, using GC-MS, we are the first to report (best of our knowledge) on the presence of caprolactam, an important bio-medical field compound, in the CAD sample's aqueous extract.

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1. Introduction

Moringa oleifera is a commonly cultivated plant species classified under the monogeneric family called Moringaceae (Fahey, 2005). It is a perennial softwood with low-quality timber originating from the sub-Himalayan regions of India and Pakistan but is now cultivated worldwide (Biswas et al., 2019). It plays a vital role in traditional Asian and African medicine, where various parts of the plant are utilised for therapeutic purposes such as rheumatism, venomous bites, and various ailments, as discussed in ancient Sanskrit texts (Roloff et al., 2009). *M. oleifera* leaves are among the most utilised parts of the plant and commonly processed fresh or dried for storage purposes. They have been traditionally used in

soups and stews (Ademiluyi et al., 2018). It is increasingly being used as a food supplement (Fahal et al., 2018). Its high antioxidant activity is touted as an important mechanism of action that confers medicinal properties. The leaves are usually prepared via drying, then powdered and packed into capsules to be marketed as supplements.

Drying is a process where heat is applied to a product or substance to remove moisture to prevent microbial contamination or decomposition of phytoconstituents and nutritious compounds (Pin et al., 2009). A low moisture content stops enzymatic reactions and oxidation in the leaf, which helps to retain its phytoconstituents (Babu et al., 2018; Ademiluyi et al., 2018). Different drying methods can result in significantly different antioxidant content in MOL. Generally, antioxidants tend to decrease with increasing drying temperature (>70 °C) due to degradation of the compounds (Katsube et al., 2009). Premi et al. (2010) reported that 60 °C was the most optimum temperature to ensure quality attributes like colour and sensory when the leaves were dried in a convective-air dryer system.

This study evaluates the effects of different drying methods, including using a self-built prototype convective-air dryer on the

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antioxidant properties, specifically, the total antioxidant capacity, total polyphenol content, 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity, total flavonoid content and total alkaloid content, of local Malaysian *M. oleifera* leaves and conducting GC-MS analysis on aqueous extracts of the dried leaves to determine its phytoconstituent compounds. Furthermore, considering the recent pandemic, which caused the demand for sanitisers to increase, we also briefly explored the potential of our leaf extract as a sanitising agent. There are two major types of sanitiser preparations: alcoholic-based (involving 70% ethanol) and non-alcoholic-based sanitisers, where the absence of alcohol is replaced with skin-friendly compounds like plant extracts. We compared *M. oleifera* leaves with several commercially available non-alcoholic sanitisers to determine its potential to compete in the existing market.

2. Materials and methods

2.1. Drying methods

M. oleifera leaves were purchased from a local market in Petaling Jaya, located approximately 6 km away from the Postharvest Laboratory, University of Malaya, Malaysia. *M. oleifera* leaflets were individually separated from the stem by hand, and 10 g were weighed and were spread as a single layer for each drying method. The four drying methods include a) convective air-drying (pre-optimized conditions: air velocity of 1.7 ms^{-1} and temperature at $60 \text{ }^\circ\text{C}$), b) oven-drying ($60 \text{ }^\circ\text{C}$), c) sun-drying, and d) freeze-drying. The samples were freeze-dried using SCANVAC 110-4 Freeze dryer (LaboGene, Denmark) at $-110 \text{ }^\circ\text{C}$ under vacuum for 72 h. Fresh leaves were used as the control in this experiment. Leaves were considered dry once they reached a constant weight. The dried leaves were then ground into powder under liquid nitrogen using pestle and mortar.

A prototype benchtop dryer designed and built in the Postharvest Biotechnology Laboratory, University of Malaya, Malaysia, was used for convective drying (Ramarao et al., 2021). The schematic layout of the benchtop dryer and its specifications are shown in Fig. 1 and Table 1, respectively.

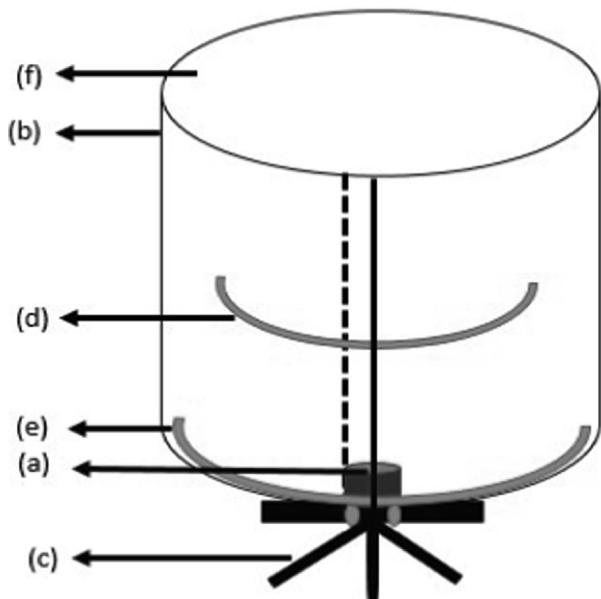


Fig. 1. A schematic layout of the bench top dryer, (a) motor, (b) cover, (c) three-legged stand, (d) drying tray, (e) water retention tray and (f) air inlet.

2.2. Preparation of leaf extract

The leaf extract for antioxidants analyses was prepared based on the method by Uribe et al. (2015). Briefly, a mixture of solid/liquid with a ratio of 1:4 comprising of powdered sample and 80% methanol was prepared. The mixture was agitated at 200 rpm for 30 min at room temperature using an orbital shaker (Shellab, USA), then centrifuged (Beckman, USA) at 6500 rpm for 10 min at $4 \text{ }^\circ\text{C}$. The supernatant was collected for subsequent analysis.

2.3. Total antioxidant capacity

The phosphomolybdenum method by Prieto et al. (1999) was used for total antioxidant capacity (TAC) determination. In brief, 28 mM sodium phosphate, 4 mM ammonium molybdate and 0.6 M sulphuric acid of equal volumes were prepared to make up a 1 mL reagent solution. A sample volume of 0.01 mL (methanol was used to replace the sample for blank) was added to the reagent mixture and incubated for 90 min at $95 \text{ }^\circ\text{C}$. Once the mixture reached room temperature, absorbance was measured using a spectrophotometer (Shimadzu UV-200, Japan) at 695 nm. The ascorbic acid standard curve had an equation of $y = 0.001x$, with an R^2 value of 0.998 and results were expressed in milligrams of ascorbic acid equivalent (AAE) per gram of plant material.

2.4. Total polyphenol content

The samples' total polyphenol content (TPC) was determined using Folin-Ciocalteu assay modified to a microscale (Bae and Suh, 2007). A sample volume of 0.02 mL was mixed with 0.78 mL of distilled water (standard solution of gallic acid with known concentrations were used to replace sample for standard curve construction) followed by the addition of 0.05 mL Folin-Ciocalteu reagent and mixed. After 1 min, 0.15 mL of sodium carbonate was added, and the mixture was incubated at room temperature ($25 \text{ }^\circ\text{C}$) for 2 h. The absorbance reading was taken at 750 nm. The standard curve prepared had an equation of $y = 0.006x$, with an R^2 value of 0.998. Results were expressed in mg of gallic acid equivalent (GAE) per gram of plant material.

2.5. 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay

DPPH assay was carried out based on the method by Bae and Suh (2007). Briefly, 1 mL of the sample + 2 mL of 80% methanolic 0.1 mM DPPH solution was prepared and immediately vortexed for 15 s. The mixture was left in the dark for 30 min at $37 \text{ }^\circ\text{C}$. An ascorbic acid standard was prepared with the equation $y = 10.145x$, $R^2 = 0.996$. Absorbance was measured at 517 nm with 80% methanol as blank and DPPH solution as a control. The results were expressed as % inhibition based on the formula:

$$\% \text{DPPH Inhibition} = (\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}} / \text{Abs}_{\text{control}}) \times 100$$

where $\text{Abs}_{\text{control}}$ = Absorbance of control and $\text{Abs}_{\text{sample}}$ = Absorbance of sample.

2.6. Total flavonoid content

Total flavonoid content (TFC) was determined using the colourimetric method with slight modifications (Sakanaka, et al., 2005). In brief, 1.25 mL of distilled water was added to 0.25 mL of the sample extract. Next, 150 μL of a 5% sodium nitrite solution was added, and the mixture was incubated for 6 min at room temperature. This was followed by adding 150 μL of a 10% aluminium chloride solution into the mixture. After 5 min, 0.5 mL of 1 M sodium hydroxide was introduced into the mixture and brought to

Table 1
Specifications of the dryer.

Description of materials used for the prototype convective-air dryer				
	Outer Material	Inner Material	Drying Tray	Retention Tray
Material	PET (Polyethylene terephthalate)	Thermo stable aluminum foil	High density polyethylene	Polypropylene
Pore size	150 nm	200 nm	–	–
Thermo Stability	100	150	95	100
Number of outlets	9	–	2378	1
Size of outlet	15 mm	–	3 × 7 mm	10 mm
Material of outlet	Aluminum alloy	–	–	PVC
Comparison of specifications between the drying methods				
Specification	Convective-air dryer	Oven	Freeze-dryer	
Brand	–	Memmert (Germany)	Scanvac (Denmark)	
Model	–	ULM500	CoolSafe 110	
Drying capacity (volume, L)	160	108	4	
Power consumption, W	1600	2000	600	
Weight, kg	7	50	55	
Cost, RM	680.00	19,855.00	30,400.00	

2.5 mL with distilled water. The absorbance reading was taken at 510 nm. A catechin standard curve was prepared and had an equation of $y = 0.0104x$, $R^2 = 0.996$, and the results obtained were expressed in milligrams of catechin equivalent (CE) per gram of plant material.

2.7. Total alkaloid content

The total alkaloid content was determined based on the method by Debnath et al. (2015). Briefly, 5 g of powdered leaves were mixed and stirred vigorously with 20 mL of *n*-butanol, then kept overnight at room temperature. The mixture was centrifuged at 6000 rpm for 10 min, and the collected supernatant was made up to 50 mL with *n*-butanol. Then, 10 mL of the supernatant was added into a separating funnel followed by 10 mL of 0.1 (N) HCl. The funnel was shaken for 2–3 min after which the bottom layer was collected in a conical flask. After that, 2–3 drops of methyl red was added to make the solution turn slightly red and titrated against 0.1 (N) NaOH until a color change of red to pale yellow was observed. The neutralisation point was determined, and the following equivalent was considered:

1 mL 0.1N HCl \equiv 0.0162 g alkaloid

2.8. Microbial inactivation analysis

The Petrifilm plates (3M, USA) for aerobic bacteria, yeast and mould, and coliform were used to determine microbial inactivation of *M. oleifera* leaf extracts. The powdered *M. oleifera* leaf sample was extracted in methanol as described in Section 2.2, and an aqueous solution based on Starzak et al. (2019) with slight modifications. The powdered leaves were suspended in sterile distilled water in a 1:10 ratio and were vigorously shaken and left at room temperature for 15 min. The extract was then filtered using Whatman No. 1 filter paper. The methanolic extract (ME) and aqueous extract (AE) were concentrated in a rotary evaporator and were re-suspended in distilled water with a final concentration of 1 g/mL. Due to the recent pandemic, we also briefly extended this study to evaluate the potential of *M. oleifera* leaf extract as a sanitiser and thus prepared extracts combined with 70% ethanol. The list of prepared solutions was a) ME only, b) AE only, c) 70% ethanol solution (containing 30% ME), and d) 70% ethanol solution (containing 30% AE). Controls were 70% ethanol solution only and 0.1% peptone water, respectively. The Petrifilm test was conducted based on the manufacturer's instructions. Briefly, 1 mL from each solution was diluted in 9 mL of 0.1% peptone water. Then, 1 mL

of the diluted sample was placed on the Petrifilm plates. The aerobic and coliform Petrifilms were incubated at 35 ± 1 °C for 24 h, while the yeast and mould Petrifilms were incubated at 25 ± 1 °C for 72 h. The microbes were calculated as colony-forming units (CFU)/mL of sample extract according to the equation:

$$\text{CFU/mL} = (\text{number of colonies} \times \text{dilution factor}) / \text{volume of aliquot plated}$$

Results were expressed as log (CFU/mL).

2.9. Gas chromatography-mass spectroscopy (GC-MS) analysis

2.9.1. Sample preparation

The powdered leaf samples were subjected to methanolic and aqueous extraction, as mentioned in sections 2.2 and 2.8, respectively. The resulting extracts were concentrated in a rotary evaporator. Then, the dried extracts were each suspended in methanol at a concentration of 1 mg/mL before GC-MS analysis.

2.9.2. Screening of compounds

The characterisation of the phytoconstituent compounds in *M. oleifera* was done using GC-MS QP2010 Plus (Shimadzu, Japan) with Rtx-5MS column (0.25 mm, 30 m). Helium acted as the carrier gas at a constant flow rate of 1.78 mL/min. The initial column temperature was 40 °C for 1 min. The injection temperature was 300 °C and was done in splitless mode. The oven temperature was programmed from 40 °C (held for 5 min) to 160 °C at a rate of 4 °C/min, then to 280 °C at a rate of 5 °C/min and held for 15 min. Identifications were based on mass spectral matching with standard compounds in the NIST library with a similarity index of at least 80%. The relative amounts of individual components were determined by the GCMSolution software (Shimadzu, Japan) and expressed as per cent peak areas relative to the total peak area.

3. Results

The results on the effects of different drying methods on TAC, TFC, TPC, DPPH radical scavenging activity and alkaloid content in *M. oleifera* leaves are shown in Table 2.

As shown in Table 2, convective-air dried leaves exhibited significantly ($p < 0.05$) higher TPC (157.20 ± 1.94 mg/g GAE) compared to other drying methods in this experiment. On the other hand, there was no significant difference between convective air-dried and oven-dried samples for TFC and TAC in the samples. However, among the four drying methods, the least amount of

Table 2
Effects of drying methods on antioxidant activity in *Moringa oleifera* leaves.

Drying method	Total antioxidant capacity (mg/g AAE)	Total flavonoid content (mg/g, CE)	Total polyphenol content (mg/g GAE)	DPPH radical scavenging activity (% inhibition)	Alkaloid content (mg/g)
Fresh	1.30 ± 0.22 ^d	6.91 ± 0.47 ^d	26.56 ± 1.59 ^e	36.15 ± 2.27 ^c	36.72 ± 0.61 ^d
Convective-air dried	38.75 ± 0.30 ^a	94.63 ± 1.95 ^a	157.20 ± 1.94 ^a	67.79 ± 1.54 ^a	58.94 ± 0.53 ^a
Freeze-dried	27.61 ± 0.13 ^b	61.79 ± 2.19 ^b	101.94 ± 1.16 ^c	64.61 ± 1.33 ^a	57.24 ± 0.81 ^b
Oven-dried	38.70 ± 0.09 ^a	91.98 ± 1.41 ^a	150.43 ± 1.30 ^b	66.36 ± 1.10 ^a	59.38 ± 0.31 ^a
Sun-dried	21.79 ± 0.03 ^c	30.93 ± 1.47 ^c	66.77 ± 1.41 ^d	41.88 ± 2.63 ^b	49.00 ± 1.10 ^c

Results are in mean ± SD (n = 3). 1-way ANOVA was carried out the treatment (p < 0.05), and where the results were significant, a posthoc analysis (Tukey HSD) was carried out to identify which treatments was/were statistically different in each column. The same letters denote mean values that are not significantly different (p > 0.05).

TFC and TAC was found in sun-dried samples at 21.79 ± 0.03 mg/g (catechin equivalent) and 30.93 ± 1.47 mg/g (ascorbic acid equivalent) respectively. From Table 2, we also noticed that the convective air-dried, freeze-dried and oven-dried samples exhibited the highest DPPH radical scavenging activity with no significant differences (p > 0.05) between them at 67.79 ± 1.54%, 64.61 ± 1.33%, and 66.36 ± 1.10% respectively. Furthermore, the highest alkaloid content was recorded in oven-dried and convective-air dried samples with no significant differences (p > 0.05) among them, at 59.38 ± 0.31 mg/g and 58.94 ± 0.53 mg/g respectively.

The Petrifilm plate method was used to enumerate microbes (yeast and mould, aerobic bacteria, and coliforms) after treatment with different solutions of *M. oleifera* leaf extract as shown in Table 3.

Based on the results (Table 3), the AE preparation presented the highest (p < 0.05) yeast and mould count, aerobic plate count and coliform count at 1.894 ± 0.091, 2.348 ± 0.024 and 2.349 ± 0.009 log CFU/mL, respectively. We also tested *M. oleifera* leaves' potential as a sanitiser by including 70% ethanol in the preparation. In all extract preparations involving 70% ethanol, no microbes formed on the Petrifilms. Furthermore, our results showed that the ME preparation of *M. oleifera* leaves presented no significant difference (p > 0.05) with 2 out of 3 (C1 and C2) commercial non-alcohol-based sanitisers for aerobic plate count and coliform count.

The phytochemicals determined using GC-MS in our convective air-dried *M. oleifera* leaves subjected to methanolic and aqueous extractions are presented in Table 4.

Table 3
Effects of convective air drying and extraction method on microbial inactivation analysis of *Moringa oleifera* leaves.

Treatment	Yeast and mould count (log CFU/mL)	Aerobic plate count (log CFU/mL)	Coliform count (log CFU/mL)
Control (peptone water)	1.800 ± 0.032 ^a	1.935 ± 0.046 ^c	1.901 ± 0.045 ^c
ME	1.519 ± 0.059 ^b	2.229 ± 0.037 ^b	1.949 ± 0.065 ^c
ME + 70% ethanol	ND ^d	ND ^d	ND ^d
AE	1.894 ± 0.091 ^a	2.348 ± 0.024 ^a	2.349 ± 0.009 ^b
AE + 70% ethanol	ND ^d	ND ^d	ND ^d
70% ethanol	ND ^d	ND ^d	ND ^d
C1	1.401 ± 0.142 ^c	2.143 ± 0.051 ^b	1.901 ± 0.045 ^c
C2	1.560 ± 0.059 ^b	2.239 ± 0.012 ^b	1.925 ± 0.109 ^c
C3	ND ^d	ND ^d	ND ^d

The results were expressed as mean ± SD (n = 3). 1-way ANOVA was carried out for each treatment (p < 0.05). Since the results from one-way ANOVA analysis was significant, a post-hoc analysis namely Tukey HSD Test was carried out to identify which pair(s) in each column was/were statistically different. The same letters denote mean values that are not significantly different (p > 0.05). ND = Not detected; CFU = colony forming unit.

Control (0.1% peptone water); ME (methanol extract); AE (Aqueous extract).

Various types of compounds were identified, where the majority were esters, heterocyclic compounds, carboxylic acids and phenolic compounds. Other compounds include ethers, alcohols, ketones, lactones, thiolanes, alkanes, amide, sulphur compounds and pyrrolidines. In both the aqueous and methanolic extracts, acetic acid and glycerin were identified as the major phytochemicals. To the best of our knowledge, this study is the first to report on the presence of caprolactam in the aqueous extract of *M. oleifera* leaves. Several phenolic compounds like 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-; 2-Methoxy-4-vinyl phenol and phenol were also found in the extract. Phenolic compounds are strong antioxidants due to their redox properties, which allow them to neutralise free radicals or prevent hydroperoxide decomposition into free radicals (Vergara-Jimenez et al., 2017). Besides that, Vitamin E (DL-alpha-Tocopherol and Gamma-Tocopherol) was identified in the methanolic extracts. They are potent antioxidants responsible for inhibiting lipid peroxidation. Various other compounds and their known biological activities are further discussed below.

4. Discussion

Various compounds such as phenolics confer antioxidant properties to a plant. Therefore, plants that have high total phenolic and flavonoid contents are regarded as good antioxidant agents. The lowest TPC found in fresh leaf samples (26.56 ± 1.59 mg/g GAE) when compared with dried leaves. Similar observations have been reported in previous studies on dried *M. oleifera* leaves compared to fresh leaves (Zullaikah et al., 2019; Saini et al., 2014) and in several types of leaves (Martinho et al., 2019; Roshanak et al., 2016; Rabeta and Lin, 2015). It is also worth noting that Hossain et al. (2010) even reported that the lower total phenolics and antioxidant capacity of fresh samples were not due to the dilution effect caused by its high moisture content, even after they corrected for its moisture content values. This was probably caused by the degradation of the phenolic compounds by polyphenol oxidase, where this enzyme could have been deactivated in the leaves upon drying, leading to a higher (p < 0.05) TPC in dried samples compared to fresh leaves (Li et al., 2018; Ghasemzadeh et al., 2016; Roshanak et al., 2016). There was no significant difference between convective air-dried and oven-dried samples for TFC and TAC in the samples. However, among the four drying methods, the least amount of TFC and TAC was found in sun-dried samples at 21.79 ± 0.03 mg/g (catechin equivalent) and 30.93 ± 1.47 mg/g (ascorbic acid equivalent) respectively. This could be due to the compromise of cell wall integrity under prolonged sun-drying, leading to leakage of flavonoids from the leaves (Rababah et al., 2015). From Table 2, we noticed that the convective air-dried, freeze-dried and oven-dried samples exhibited the highest DPPH radical scavenging activity with no significant differences (p > 0.05) between them at 67.79 ± 1.54%, 64.61 ± 1.33%, and 66.36 ± 1.10% respectively. This finding however was contrary to a report by Rababah

Table 4List of phytocompounds identified by GC-MS in the methanolic and aqueous extracts of convective air-dried *Moringa oleifera* leaves.

No.	Compound name	Nature of compound	Treatment	
			1	2
1	1,3,5,7-Cyclooctatetraene	Heterocyclic compound	✓ RT: 8.078 PA: 2.08	✓ RT: 8.078 PA: 2.08
2	1-Hexanol, 2-ethyl-	Alcohol	✓ RT: 13.943 PA: 0.91	
3	2,2'-Bioxirane	Ether	✓ RT: 4.330 PA: 5.82	
4	2,3-Dihydro-2,5-dimethyl-5H-1,4-dioxepin	Heterocyclic compound		✓ RT: 14.216 PA: 0.346
5	2,4,5,6,7-Pentamethoxyheptanoic acid, methyl ester	Ester	✓ RT: 19.713 PA: 6.70	
6	2,5-Dimethyl-4-hydroxy-3(2H)-furanone	Ketone	✓ RT: 15.894 PA: 0.75	
7	2-Cyclopenten-1-one	Ketone	✓ RT: 6.300 PA: 2.44	
8	2-Methoxy-4-vinylphenol	Phenolic compound	✓ RT: 25.088 PA: 0.78	
9	2-Oxepanone, 7-methyl-	Lactone	✓ RT: 9.187 PA: 0.95	
10	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	Ester		✓ RT: 40.686 PA: 0.149
11	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	Ester		✓ RT: 41.089 PA: 0.294
12	4-Cyclopentene-1,3-dione	Ketone	✓ RT: 7.984 PA: 2.41	
13	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	Phenolic compound	✓ RT: 18.771 PA: 6.58	
14	5-Pyrrolidino-2-pyrrolidone	Pyrrolidine	✓ RT: 7.119 PA: 0.89	
15	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	Carboxylic acid		✓ RT: 45.740 PA: 3.250
16	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	Carboxylic acid		✓ RT: 46.683 PA: 10.429
17	Acetic acid	Carboxylic acid	✓ RT: 3.068 PA: 16.08	✓ RT: 3.233 PA: 18.854
18	Benzofuran, 2,3-dihydro-	Heterocyclic compound	✓ RT: 21.942 PA: 1.80	
19	Benzophenone	Heterocyclic compound	✓ RT: 35.203 PA: 1.28	
20	Benzyl 2-chloroethyl sulfone	Sulfur compound	✓ RT: 3.331 PA: 2.14	
21	Butyrolactone	Lactone	✓ RT: 9.065 PA: 3.43	
22	Caprolactam	Amide	✓ RT: 23.216 PA: 10.63	
23	Dihydro-2(3H)-thiophenone	Thiolanes	✓ RT: 5.150 PA: 2.90	
24	Alpha-Tocopherol	Heterocyclic compound		✓ RT: 61.264 PA: 1.090

(continued on next page)

Table 4 (continued)

No.	Compound name	Nature of compound	Treatment	
			1	2
25	Gamma.-Tocopherol	Heterocyclic compound		✓ RT: 62.788 PA: 12.495
26	Glycerin	Alcohol	✓ RT: 3.973 PA: 14.84	✓ RT: 4.151 PA: 39.649
27	Hexadecanoic acid, methyl ester	Ester		✓ RT: 42.115 PA: 2.094
28	Isopropyl myristate	Ester	✓ RT: 40.276 PA: 1.64	
29	n-Hexadecanoic acid	Carboxylic acid		✓ RT: 43.049 PA: 2.951
30	Octane, 4,5-dimethyl-	Alkane	✓ RT: 11.264 PA: 0.90	
31	Octadecanoic acid	Carboxylic acid		✓ RT: 47.003 PA: 0.523
32	Oxirane, [[(2-ethylhexyl)oxy]methyl]-	Ether	✓ RT: 12.663 PA: 5.48	
33	p-Cresol	Phenolic compound	✓ RT: 16.352 PA: 2.54	
34	Phytol, acetate	Ester		✓ RT: 40.102 PA: 0.960
35	Phytol	Ester		✓ RT: 45.966 PA: 3.055
36	Pentanedioic acid, ethyl methyl ester	Ester		✓ RT: 19.229 PA: 0.294
37	Performic acid, trimethylsilyl derivative	Carboxylic acid		✓ RT: 3.563 PA: 3.567
38	Phenol	Phenolic compound	✓ RT: 12.545 PA: 5.39	
39	Propanoic acid	Carboxylic acid	✓ RT: 3.259 PA: 5.71	
40	Propanoic acid, 1-methylpropyl ester	Ester	✓ RT: 4.631 PA: 0.60	
41	Propanoic acid, 2-hydroxy-, methyl ester, (±)-	Carboxylic acid	✓ RT: 4.123 PA: 0.99	
42	Pyrazine, methyl-	Heterocyclic compound	✓ RT: 5.990 PA: 1.96	
43	Pyridine	Heterocyclic compound	✓ RT: 4.233 PA: 5.50	
44	Silane, [(11-fluoroundecyl)oxy]trimethyl-	Ether	✓ RT: 44.890 PA: 1.96	

*RT = Retention time PA = Peak area (%).

*Treatment 1 = Drying method: Convective air-dried, Extraction method: Aqueous.
Treatment 2 = Drying method: Convective air-dried, Extraction method: Methanolic.

et al. (2015), who observed fresh herbs exhibited higher DPPH radical scavenging ability compared to herbs subjected to drying. Alkaloids are among the largest groups of secondary compounds found in plants (Ademiluyi et al., 2018). They have cardio-protective, antimicrobial, and anti-fungal activities properties (Fahal et al., 2018). As shown in Table 2, the highest alkaloid content was recorded in oven-dried and convective-air dried samples with no significant differences ($p > 0.05$) among them, at 59.38 ± 0.31 mg/g and 58.94 ± 0.53 mg/g respectively. The alkaloid content

in our samples were noted to be higher compared to those reported by Ademiluyi et al. (2018), where the source of their Moringa leaves were from Nigeria. Therefore, the difference in results could be attributed to factors such as sample source, which might have influenced the growth and chemical compositions of the plant (Lin et al., 2019; Ademiluyi et al., 2018).

From a food safety standpoint, the yeast and mould count, aerobic plate count and coliform counts for our CAD samples were below the limit set by ASEAN on guidelines for contaminant limit

for food/health supplements of plant origins. This indicates that the CAD MOL is safe for consumption. Besides, our test on MOL's sanitiser potential revealed that no microbes were present on the Petrifilms upon combination with 70% ethanol. This was probably due to ethanol's ability to freely enter microbes' membranes and inhibit cross-linking of peptidoglycans (Seo et al., 2020). More importantly, these results showed that the combination of MOL with ME and AE preparations in ethanol solutions does not compromise the efficacy of ethanol in microbial reduction. The ME preparation of our MOL was comparable to commercial non-alcohol-based sanitisers for aerobic plate count and coliform count. This could be attributed to the phytochemical ingredients in those sanitisers which could have conferred strong anti-microbial activity. These results show that our *M. oleifera* leaf extract has the potential to be developed and commercialised as a sanitiser product. However, it is worth noting that the Petrifilm method relies on non-specific measurement and is most suitable for quantification of microbial load (Meador et al., 2012). Although our results revealed that the different preparations of MOL resulted in microbial counts below the ASEAN limit for foods, further isolation is recommended for future studies on species-specific efficacy of our MOL extract as a potential sanitiser product.

The major compounds identified by GC-MS in our CAD samples are glycerin and acetic acid. Glycerin has been shown to possess anti-inflammatory, anti-irritant and antioxidant properties (Ong et al., 2016; Szél et al., 2015). It can also reduce brain inflammation by scavenging oxygen free radicals, and can improve brain perfusion (Guhadasan and Carrol, 2013). Besides that, glycerin has various uses in pharmaceuticals, cosmetics and food industries (Konwar et al., 2018). Acetic acid on the other hand has been shown to protect lipid accumulation in the liver and abdominal fat, thus becoming an important contributor against obesity (Yamashita, 2016). This could be one of the main reasons as to why people consume *M. oleifera* for weight-loss purposes. We also noticed the presence of caprolactam in this study, which was never identified before. Caprolactam is a cyclic amide that is used to synthesise *N*-vinyl caprolactam, which is important in the biomedical field as a component for drug delivery systems due to its properties of being water-soluble, has high absorption ability and film-forming properties (Rao et al., 2016). The phenolic compounds like phenol, 2-Methoxy-4-vinylphenol, and vitamins (alpha-Tocopherol and gamma-Tocopherol) identified in our extracts have been shown to possess antimicrobial, antioxidant, antidiabetic, and anti-inflammatory properties (Alghamdi et al., 2018; Jeong et al., 2011; Fukai et al., 2009). The ketone-based compound, 2-Cyclopenten-1-one, was only found in the aqueous extract where it is commonly used as an anti-ulcer and gastrointestinal agent and has cryoprotective and antiviral properties (Rossi et al., 1996). Carboxylic acids like acetic acid were found in both aqueous and methanolic extracts, where they have various applications, such as in the production of adhesives, biopolymers, and pharmaceutical drugs (Badea and Radu, 2018). Phytol is an ester with powerful antioxidant properties, and is well-known for its ability to remove nitric oxide, hydroxyl radicals and prevent the formation of thiobarbituric acid reactive substances (Santos et al., 2013). Overall, we noticed various antioxidant compounds in our extracts, similar to a study conducted by Barhoi et al. (2020), and Igwe et al. (2015) on aqueous and methanolic extracts of *M. oleifera* leaves, respectively. Some major phytoconstituents identified by Bhalla et al. (2021) and Barhoi et al. (2020), such as octadecanoic acid and hexadecanoic acid, were also observed in our samples. However, hexadecenoic acid, observed in the methanolic extract from Igwe et al. (2015), was not observed in our methanolic extract samples. These differences could be attributed to the difference in plant source where environmental conditions, genetic factors, and cultivation vary (Förster et al., 2015; Leone et al., 2015). We

propose that the phytoconstituents identified in the methanolic and aqueous extracts of convective-air dried *M. oleifera* leaves using our prototype convective-air dryer such as glycerine; phenol; 2-cyclopentene-1-one; alpha-Tocopherol, gamma-Tocopherol, 2-Methoxy-4-vinylphenol, and 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-, hexadecanoic acid and phytol are compounds which possess strong antioxidant and anti-inflammatory properties, suggesting it has good potential to be employed in the preparation of nutraceuticals.

5. Conclusion

The results showed that convective air-dried shared the highest TFC and TAC with oven-dried samples (no significant difference among the treatments, $p > 0.05$). The highest TPC was also observed in the oven-dried samples method, while fresh samples exhibited the highest DPPH radical scavenging activity and contained the highest alkaloid content. The aqueous extract of convective air-dried samples contained the highest coliform, aerobic plate, yeast, and mould count. Extract preparations that had 70% ethanol prevented microbe formation on the tested Petrifilms. Various bioactive compounds with medicinal properties were also identified using GC-MS in the leaf sample, including caprolactam which has not been reported before. We propose that *M. oleifera* leaves dried using our prototype convective-air dryer exhibited suitable antioxidant activities and contained various biologically active phytoconstituents that allows for its use as a food supplement or in the nutraceuticals industry. The microbial load results also showed its potential to be developed as a sanitising product. Our future recommendations include extending this study to evaluate the effects of our drying preparation on microRNAs of *M. oleifera*. These compounds play a pivotal role in conferring the plant's bioactive properties where it has the potential for the bioproduction of active compounds (Pirrò et al., 2019). Furthermore, they possess cross-kingdom interaction introduced by the diet to control gene expression in human cells (Potestà et al., 2019; Zhang et al., 2012). It would be interesting to see whether there are significant changes to the microRNA composition after drying treatments and if these changes are beneficial to humans.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Further Reading

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