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Potential application of bioactive compounds in essential oils from selected Malaysian herbs and spices as antifungal agents in food systems

[1,5]Ramli, M., [1]Nor-Khaizura, M.A.R., [3]Nur Hanani, Z.A., [1,4]Rukayadi, Y. and [1,2,*]Samsudin, N.I.P.

[1]*Department of Food Science, Faculty of Food Science and Technology, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia*
[2]*Laboratory of Food Safety and Food Integrity, Institute of Tropical Agriculture and Food Security, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia*
[3]*Department of Food Technology, Faculty of Food Science and Technology, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia*
[4]*Laboratory of Natural Medicines and Product Research, Institute of Bioscience, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia*
[5]*National Public Health Laboratory, Lot 1853, Kampung Melayu, 47000 Sungai Buloh, Selangor, Malaysia*

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Abstract

Essential oils have a long history in their variety of applications. Although essential oils of various herbs and spices from other parts of the world have shown antimicrobial effects, those from Malaysian herbs remain underreported. Thus, can be further utilized in the search for novel bioactive compounds as natural antimicrobials to fulfil the consumers' demand for safer, healthier, and higher-quality foods with longer shelf life. In the present work, the essential oils from ten herbs and spices namely betel, cinnamon, clove, coriander, galangal, ginger, lemongrass, lime, nutmeg, and turmeric, selected based on their abundance and economic importance, were analysed by gas chromatography and mass spectrometry. A total of 120 bioactive compounds were detected. The major (>10%) bioactive compounds were anethole, 26.25% (betel), cinnamaldehyde, 63.39% (cinnamon), eugenol, 87.16% (clove), linalool, 54.79% (coriander), propenoic acid, 29.56% (galangal), α-zingiberene, 26.32% (ginger), geranial, 42.61% (lemongrass), limonene, 39.84% (lime), β-phellandrene, 27.80% (nutmeg), and ar-turmerone, 41.81% (turmeric). All essential oils also yielded minor (<10%) bioactive compounds of different classes. Some of these major and minor bioactive compounds have been reported to exert fungicidal/fungistatic effects and could be an excellent candidate in the development of efficient fungal spoilage control strategies such as an active food packaging system.

1. Introduction

The presence of spoilage and mycotoxigenic fungi in foods presents potential hazards to human health such as nutrient and quality degradation (by spoilage fungi), alimentary mycotoxicoses (acute effect of ingesting mycotoxins produced by mycotoxigenic fungi), and cancers (chronic effect upon prolonged consumption of mycotoxins) (de Saeger, 2011). Economically relevant mycotoxin-producing genera include *Aspergillus*, *Fusarium*, and *Penicillium* which can produce mycotoxins such as aflatoxins, ochratoxin A, patulin, fumonisins, trichothecenes, and zearalenone (Samson *et al*., 2004; Pitt and Hocking, 2009; Samson *et al*., 2010). Mycotoxins are naturally chemically stable and will remain in the food even after processing (Bryden, 2007). Globally, fungal colonization/spoilage leads to a huge percentage of waste pre- and postharvest. To control fungal proliferation in foods, chemically synthesized food preservatives are currently being used by the food industry. However, their massive and indiscriminate use can lead to fungal resistance (Chen *et al*., 2008). In addition, new data and evidence on the toxic effects of some of the common antifungal chemicals are also surfacing, e.g., sodium sorbate (E201; sodium salt of sorbic acid) is no longer allowed in the European Union due to potential genotoxic effects on consumers (EFSA, 2015). Furthermore, certain fungi are able to detoxify/ denature/destroy these antifungal chemicals, thus diminishing their intended protective effects (Kinderlerer

*Corresponding author.
Email: *nikiskandar@upm.edu.my*

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and Hatton, 1990). Therefore, safer and more natural antifungal agents are warranted. Having shown considerable success in inhibiting spoilage and/or pathogenic bacteria in foods, essential oils are increasingly being recognized as safe and natural substances to consumers and the environment in controlling the propagation of fungi in foods.

Essential oil is a concentrated aromatic hydrophobic/ lipophilic oily liquid containing volatile bioactive compounds of the plant. It is so named because it contains the "essence" of the plant's fragrance. The essential oil can be extracted from the bark/stem, flower, fruit/berry, leaf, peel, resin, rhizome, root, or seed of plants using different techniques such as steam distillation, hydro distillation, and solvent extraction (Calo *et al*., 2015). In food applications, even though essential oils have been used as food preservatives since ancient times (Sendra, 2016), they only attained the "Generally Recognized as Safe; GRAS" status from the Food and Drug Administration (FDA) comparatively recently. The complete list of essential oils with GRAS status as of April 2020 can be obtained from the FDA's website (CFR, 2020). Due to their natural origin, essential oils are more widely accepted by consumers than synthetic chemical agents (Edris, 2007). Presently, essential oils have found their way in wide-ranging applications; in foods, as flavouring and preservative agents, in cosmetics as fragrance agents, in pharmaceuticals as antiseptic, anti-inflammation, and anaesthetic agents, and household products as soaps, detergents, shampoos, lotions, and insect repellents (Yang *et al*., 2004; Bhargava *et al*., 2015; Rassem *et al*., 2016; Oussalah *et al*., 2017; Upadhyay, 2017).

Huge successes of essential oils against spoilage and pathogenic bacteria in foods have long been documented (Greenwood *et al*., 1982; Kivanç and Akgül, 1986; Onawunmi and Ogunlana, 1986; Deans and Ritchie, 1987), and their applications continue to recent time (Calo *et al*., 2015). Additionally, essential oils extracted from several aromatic and medicinal plants have also been found to be effective botanical fungicides in the management of several foodborne fungal pathogens in food systems (Ziedan and Farrag, 2008; Okigbo *et al*., 2009; Pandey *et al*., 2013; Shao *et al*., 2015; Tian *et al.,* 2015). Bioactive compounds such as citral and eugenol have been identified to be responsible for the fungicidal/ fungistatic effects (Ju *et al*., 2020). The bioactive compounds in essential oils are believed to inhibit fungi by disrupting the structure and functions of the cell wall and cell membranes, thus leading to their disintegration and leakage, and inhibition of sporulation and germination; by damaging/injuring the organelles such as mitochondria and efflux pumps; and by inhibiting the nuclear materials and protein synthesis, thus halting various cellular functions (Nobuyuki and Shigeru, 1983; Murray *et al*., 2003; Freiesleben and Jager, 2014; Ju *et al*., 2019).

Malaysia, being a reservoir for various herbs and spices with potent phytochemicals, provides the opportunity for the exploration of novel antifungal agents against spoilage/mycotoxigenic fungal colonization in foods and agricultural commodities pre- and postharvest. Although various herbs and spices from other parts of the world have been tested as antibacterials and antifungals, some Malaysian herbs remain underreported and therefore can be further utilized in the search for novel bioactive compounds to act as natural antimicrobials in the effort to fulfil the consumers' demand for greener (fewer chemical preservatives), safer (less microbial contamination), and higher-quality foods with longer shelf-life. In the present work, essential oils from ten selected herbs and spices namely betel, cinnamon, clove, coriander, galangal, ginger, lemongrass, lime, nutmeg, and turmeric, were selected based on their abundance and economic importance (DOA, 2018), were chromatographically analyzed to identify their major and minor bioactive compounds. Based on scientific database searches and literature review, these bioactive compounds are discussed in terms of their fungicidal and fungistatic effects in the food systems in the hope to facilitate the development of efficient fungal spoilage control strategies such as an active packaging system incorporated with essential oil containing antifungal compounds.

2. Materials and methods

2.1 Chemicals and reagents

All chemicals and reagents used in the present work were of analytical grade and purchased from Sigma-Aldrich (St. Louis, USA).

2.2 Essential oils

Essential oils were commercially obtained from Scienfield Expertise PLT, (Malaysia). A total of ten essential oils from selected Malaysian herbs and spices were selected and are listed in Table 1. Based on the certificate of analysis/purity, all essential oils were extracted by steam distillation of leaves (betel), rhizomes (galangal, ginger, turmeric), stems (lemongrass), barks (cinnamon), seeds (clove, coriander, nutmeg), and fruits (lime), and preservative-free. According to Tongnuanchan and Benjakul (2014), steam distillation is the most popular method to extract essential oils due to it being cheaper than CO_2 extraction and requires less fuel for the steam boiler; steam generated at the water boiling temperature does not degrade the volatile bioactive

Table 1. List of essential oils from selected Malaysian herbs and spices

Common name	Local name	Scientific name	Family	GRAS status*
Betel	*Sirih*	*Piper betle* L.	Piperaceae	Not yet assigned
Cinnamon	*Kayu manis*	*Cinnamomum verum* J.Presl	Lauraceae	Yes
Clove	*Cengkih*	*Syzygium aromaticum* (L.) Merr. & L.M.Perry	Myrtaceae	Not yet assigned
Coriander	*Ketumbar*	*Coriandrum sativum* L.	Apraceae	Yes
Galangal	*Lengkuas*	*Alpinia galanga* (L.) Willd	Zingiberaceae	Yes
Ginger	*Halia*	*Zingiber officinale* Roscoe	Zingiberaceae	Yes
Lemongrass	*Serai*	*Cymbopogon citratus* (DC.) Stapf	Poaceae	Yes
Lime	*Limau nipis*	*Citrus aurantiifolia* (Christm.) Swingle	Rutaceae	Yes
Nutmeg	*Pala*	*Myristica fragrans* Houtt.	Myristicaceae	Yes
Turmeric	*Kunyit*	*Curcuma longa* L.	Zingiberaceae	Yes

*GRAS status as listed by FDA, 2020 revision (CFR, 2020).

compounds; and it generates organic solvent-free products (as opposed to solvent extraction), thus avoiding subsequent separation steps. During steam distillation, plant materials were collected, distilled, and condensed by the hot steam into liquid. The essential oils came in glass amber bottles and were stored in their original container away from direct heat and light in a cool, dry, and well-ventilated place until further analysis.

2.3 Determination of bioactive compounds in essential oils using GC-MS

The determination of bioactive compounds in essential oils was performed following Armijos *et al*. (2018) with slight modification. Briefly, the analysis used an Agilent 5997 gas chromatography system (Agilent Technologies, USA) equipped with DB-5 mass spectrometry capillary column (30 m × 0.25 mm i.d., 0.25 μm), and an Agilent 7890 mass selective detector. For detection, an electron ionization system with 70 eV ionization energy was used. The carrier gas was helium at a flow rate of 1 mL/min. The injector and MS transfer line temperatures were set to 220 and 290°C, respectively. The column temperature was initially kept at 50°C for 3 min, then gradually increased to 150°C at a 3°C/min, held for 10 min, and finally raised to 250°C. The injected samples were 1.0 μL (1:10, v/v, in hexane) in the manual split mode, with a split ratio of 1:10. The identity of the essential oils' components was assigned by comparison of their relative retention time and mass spectra with the electronic library (National Institute of Standards and Technology, NIST) of the equipment (NIST 05, 2005), and literature data (Adams, 2007). The bioactive compounds detected were then grouped as major (above 10%), and minor (below 10%) constituents. Based on scientific database searches and literature review, these bioactive compounds are discussed in terms of their fungicidal/fungistatic effects in the food systems.

3. Results

From GC-MS analysis, a total of 120 bioactive compounds were detected from the ten essential oils of selected Malaysian herbs and spices (Tables 2 – 11). Although several essential oils yielded similar bioactive compounds, albeit at different percentages, the bioactive compounds with the highest percentage for each essential oil were different; betel (anethole, 26.25%), cinnamon (cinnamaldehyde, 63.39%), clove (eugenol, 87.16%), coriander (linalool, 54.79%), galangal (propenoic acid, 29.56%), ginger (α-zingiberene, 26.32%), lemongrass (geranial, 42.61%), lime (limonene, 39.84%), nutmeg (β-phellandrene, 27.80%), and turmeric (ar-turmerone, 41.81%). Similarly, several essential oils also yielded similar minor bioactive compounds, but at varying percentages.

4. Discussion

Essential oils often contain complex mixtures of volatile organic compounds in wide-ranging (from major to minor) concentrations (León-Méndez *et al*., 2019). These compositions can vary considerably between different plant species, and between similar species of different geographical origins. The major classes of essential oils' bioactive compounds are (1) terpenes and terpenoids (≈25,000 types), (2) alkaloids (≈12,000 types), and (3) phenolic compounds (≈8,000 types) (Khezerlou and Jafari, 2020). Of the various biological activities reported from essential oils or their bioactive compounds, antimicrobial activities rank second after antioxidative activities (León-Méndez *et al*., 2019). These activities vary with plant sources, chemical compositions, or extraction methods (Tongnuanchan and Benjakul, 2014).

Against microorganisms, the antibacterial activities of essential oils or their bioactive compounds have a long history and have been widely documented and reviewed (Calo *et al*., 2015). The antifungal activities of essential oils or their bioactive compounds, however, are

receiving comparatively less coverage (quantitative bibliographic search conducted in January 2021 on Scopus yielded 22,543 documents for "essential oils + antibacterial" vs. 16,795 documents for "essential oils + antifungal", while ScienceDirect yielded 20,280 documents vs. 11,909 documents, respectively); though they are gaining increasing attention in recent times. The chemical structures of essential oil bioactive component and their antifungal properties are related. The presence and position of the hydroxyl group, the presence of the aromatic nucleus, solubility in fats, and spatial orientation all affect the antifungal activities (Kohlert *et al*., 2000). Bioactive compounds with a higher alkyl group have shown stronger antifungal activities. Phenolic compounds such as carvacrol, thymol, and eugenol have exhibited the strongest antifungal and antimycotoxigenic activities, followed by alcohols, aldehydes, ketones, esters, and hydrocarbons (Nobuyuki *et al*., 1981). Cinnamaldehyde showed the strongest antifungal activity out of the aliphatic aldehydes, followed by peril-aldehyde and citral (Nobuyuki and Shigeru, 1983). Primary alcohols such as citronellol, geraniol, perilalcohol, and 1-decanol have also exhibited very high antifungal activity. α,β-saturated aliphatic aldehydes such as citronellal and decanal, secondary alcohols such as L-menthol and borneol, and tertiary alcohols such as linalool and terpinol exhibited middle antifungal activity, while hydrocarbons showed weak effects (Morcia *et al*., 2012). The antifungal activities of terpenes and terpenoids are due to their highly lipophilic nature and a low molecular weight that are capable of disrupting the cell membrane, causing cell death, or inhibiting the sporulation and germination of fungi (Nobuyuki and Shigeru, 1983). It has been suggested that the mechanism of antifungal activities of essential oils or their bioactive compounds is via binding to ergosterol, the major sterol found in the fungal cellular membrane (Murray *et al*., 2003). This binding destroys the osmotic integrity of the membrane, and is followed by leakage of intracellular potassium, magnesium, sugars, and metabolites, and finally by cellular death. Further, essential oils' bioactive compounds can also block the linking of β-glucans in the formation of the fungal cell wall (Freiesleben and Jager, 2014).

In the present work, GC-MS analysis detected a total of 120 bioactive compounds from the ten essential oils of selected Malaysian herbs and spices. In terms of composition, the highest percentage of bioactive compounds detected relatively agree with previous works; slight discrepancy might come from different extraction methods; harvesting seasons, and geographical origins (Fotsing *et al*., 2020): betel (anethole, 26.25%) (Madhumita *et al*., 2019), cinnamon (cinnamaldehyde, 63.39%) (Wang *et al*., 2005), clove (eugenol, 87.16%) (Raina *et al*., 2001), coriander (linalool, 54.79%) (Chahal *et al*., 2016), galangal (propenoic acid, 29.56%) (Kumar, 2014), ginger (α-zingiberene, 26.32%) (Aziz *et al*., 2012), lemongrass (geranial, 42.61%) (Nguefack *et al*., 2012), lime (limonene, 39.84%) (Quyen *et al*., 2020), nutmeg (β-phellandrene, 27.80%) (Gomathi *et al*., 2016), and turmeric (ar-turmerone, 41.81%) (Avanço *et al*., 2017).

In terms of antifungal activity, all essential oils yielded bioactive compounds (either as major or minor) with known action against fungi. In betel essential oil, anethole (Fujita *et al*., 2007), caryophyllene and caryophyllene oxide (Yang *et al*., 2000), eugenol (Campaniello *et al*., 2010), methyl eugenol (Tan and Nishida, 2012), *p*-cymene (Kupska *et al*., 2016), and cinnamaldehyde (Jantan *et al*., 2008) detected in the present work have been reported to exert antifungal action. In cinnamon essential oil, cinnamaldehyde and eugenol (Bullerman *et al*., 1977), caryophyllene, caryophyllene oxide, α-phellandrene (Zhang *et al*., 2017), and camphene (Marei *et al*., 2012) detected in the present work have been reported to effectively inhibit fungal growth and aflatoxin production. In clove essential oil, eugenol detected in the present work has been reported to act as an antifungal agent against yeasts and molds (Ranasinghe *et al*., 2002; Velluti *et al*., 2004; Gayoso *et al*., 2005). In coriander essential oil, limonene (Darughe *et al*., 2012), geraniol (Chahal *et al*., 2016), caryophyllene, and camphene detected in the present work have been reported to exert antifungal activities. In galangal essential oil, propenoic acid (Jantan *et al*., 2003), ethyl cinnamate and ethyl-p-methoxycinnamate (Ajay, 2014), sandaracopimara-8(14),15-diane (Demirci *et al*., 2008), camphene, cymene, and caryophyllene detected in the present work have been reported to exert antifungal activities.

In ginger essential oil, α-zingiberene (Bansod and Rai, 2008), β-bisabolene (Soares *et al*., 2015), β-sesquiphellandrene (Mahboubi, 2019), camphene, nerolidol (Chan *et al*., 2016), cymene, α-phellandrene, and caryophyllene detected in the present work have been suggested as important compounds responsible for the antifungal activities. In lemongrass essential oil, geranial and neral (Nobuyuki *et al*., 1981; Burt, 2004), caryophyllene, caryophyllene oxide, limonene, camphene, and geranyl formate (Rath *et al*., 2005) detected in the present work have been reported to exert antifungal activities. In lime essential oil, limonene, camphene, caryophyllene, methyl eugenol, α-phellandrene, β-phellandrene, and p-cymene detected in the present work had shown inhibitory activity against a wide range of fungi such as *Aspergillus niger*, *Penicillium digitatum*, *Rhizoctonia solani*, *Fusarium*

Table 2. Bioactive compounds in betel essential oil as detected by GC-MS. Major bioactive compounds are in bold.

RT (min)	Chemical formula	Compound	Composition (%)
7.035	$C_{10}H_{16}$	α-Thujene	0.37
7.338	$C_{10}H_{16}$	α-Pinene	0.59
9.536	$C_{10}H_{16}$	Sabinene	0.26
9.701	$C_{10}H_{16}$	β-Pinene	0.47
12.442	$C_{10}H_{16}$	Carene	0.15
13.232	$C_{15}H_{24}$	Cymene	3.17
13.541	$C_{10}H_{18}O$	1,8-Cineole	2.76
18.342	$C_{10}H_{18}O$	Linalool	0.59
22.713	$C_{10}H_{18}O$	Terpinen-4-ol	0.43
26.152	$C_{10}H_{12}O$	Cumin aldehyde	0.10
26.269	$C_{10}H_{16}O$	Neral	0.45
27.869	$C_{10}H_{16}O$	Geranial	0.53
28.441	$C_{12}H_{20}O_2$	Isobornyl acetate	0.16
29.190	**$C_{10}H_{12}O$**	**Anethole**	**26.25**
31.845	$C_8H_6O_3$	Piperonal	6.93
32.343	**C_5H_8O**	**1-Cyclopropyl methyl**	**21.09**
32.818	$C_{10}H_{12}O_2$	Eugenol	7.09
33.127	$C_{15}H_{24}$	α-Copaene	5.12
33.642	$C_{15}H_{24}$	β-Cubebene	0.38
34.578	$C_{11}H_{14}O_2$	Methyl eugenol	2.00
35.324	**$C_{15}H_{24}$**	**Caryophyllene**	**15.44**
36.648	$C_{15}H_{24}$	α-Humulene	0.54
37.670	$C_{15}H_{24}$	Germacrene D	0.09
39.536	$C_{15}H_{24}$	δ-Cadinene	1.20
41.630	$C_{12}H_{10}O_2$	*p*-Cinnamaldehyde-methoxy	0.21
44.002	$C_{15}H_{24}O$	Caryophyllene oxide	0.44

Table 3. Bioactive compounds in cinnamon essential oil as detected by GC-MS. Major bioactive compounds are in bold.

RT (min)	Chemical formula	Compound	Composition (%)
7.012	$C_{10}H_{16}$	α-Thujene	0.36
7.321	$C_{10}H_{16}$	α-Pinene	0.41
8.036	$C_{10}H_{16}$	Camphene	0.06
8.849	C_7H_6O	Benzaldehyde	0.10
9.524	$C_{10}H_{16}$	Sabinene	0.14
9.690	$C_{10}H_{16}$	β-Pinene	0.46
18.433	$C_{10}H_{18}O$	Linalool	1.66
23.669	$C_{10}H_{18}O$	α-Terpineol	1.09
27.176	$C_{10}H_{18}O$	Geraniol	0.67
29.025	**C_9H_8O**	**Cinnamaldehyde**	**63.39**
29.740	$C_{11}H_{18}O_2$	Geranyl formate	0.17
33.076	**$C_{10}H_{12}O_2$**	**Eugenol**	**19.86**
34.895	$C_{15}H_{24}$	Caryophyllene	0.80
36.526	$C_{11}H_{12}O_2$	Cinnamyl acetate	3.15
36.148	$C_9H_6O_2$	Coumarin	0.72
39.496	$C_{15}H_{24}$	δ-Cadinene	0.16
39.914	$C_{12}H_{14}O_3$	Eugenol acetate	1.08
42.048	$C_{15}H_{24}O$	Caryophyllene oxide	0.35
46.734	$C_{15}H_{26}O$	Farnesol	0.22
49.275	$C_{14}H_{12}O_2$	Benzyl benzoate	1.66

Table 4. Bioactive compounds in clove essential oil as detected by GC-MS. Major bioactive compounds are in bold.

RT (min)	Chemical formula	Compound	Composition (%)
33.423	**$C_{10}H_{12}O_2$**	**Eugenol**	**87.16**
35.204	$C_{15}H_{24}$	Caryophyllene	9.14
36.560	$C_{15}H_{24}$	α-Humulene	1.74
38.901	$C_{15}H_{24}$	α-Farnesene	0.12
39.519	$C_{15}H_{24}$	δ-Cadinene	0.08
39.868	$C_{12}H_{14}O_3$	Eugenol acetate	0.51

Table 5. Bioactive compounds in coriander essential oil as detected by GC-MS. Major bioactive compounds are in bold.

RT (min)	Chemical formula	Compound	Composition (%)
7.024	$C_{10}H_{16}$	α-Thujene	0.37
7.418	$C_{10}H_{16}$	α-Pinene	5.85
8.048	$C_{10}H_{16}$	Camphene	0.07
9.564	$C_{10}H_{16}$	Sabinene	1.22
9.742	$C_{10}H_{16}$	β-Pinene	1.77
10.817	$C_{10}H_{16}$	Myrcene	0.41
11.950	$C_{10}H_{16}$	δ-3-Carene	0.10
13.238	$C_{10}H_{14}$	Cymene	8.04
13.426	$C_{10}H_{16}$	Limonene	3.56
15.395	$C_{10}H_{16}$	γ-Terpinene	2.92
16.326	$C_{10}H_{18}O_2$	Linalool oxide	1.66
19.509	**$C_{10}H_{18}O$**	**Linalool**	**54.79**
20.716	$C_{10}H_{18}O_2$	Dihydro linalool	0.90
20.991	$C_{10}H_{16}O$	Camphor	3.20
22.811	$C_{10}H_{18}O$	Terpinen-4-ol	0.11
23.760	$C_{10}H_{18}O$	α-Terpineol	1.79
27.085	$C_{12}H_{20}O_2$	Linalool acetate	1.37
27.348	$C_{10}H_{18}O$	Geraniol	3.05
32.509	$C_{12}H_{20}O_2$	Neryl acetate	2.66
32.778	$C_{15}H_{24}$	α-Copaene	0.33
33.471	$C_{12}H_{20}O_2$	Geranyl acetate	4.14
34.815	$C_{15}H_{24}$	Caryophyllene	0.25
39.444	$C_{15}H_{24}$	δ-Cadinene	0.08
49.154	$C_{14}H_{12}O_2$	Benzyl benzoate	0.11
63.803	$C_{25}H_{52}$	Pentacosane	0.14

Table 6. Bioactive compounds in galangal essential oil as detected by GC-MS. Major bioactive compounds are in bold.

RT (min)	Chemical formula	Compound	Composition (%)
7.315	$C_{10}H_{16}$	α-Pinene	0.29
8.053	$C_{10}H_{16}$	Camphene	0.45
12.087	$C_{10}H_{16}$	δ-3-Carene	2.67
13.072	$C_{15}H_{24}$	Cymene	0.45
13.455	$C_{10}H_{18}O$	1,8-Cineole	1.98
22.135	$C_{15}H_{24}$	Borneol	0.93
23.257	$C_{10}H_{14}O$	*p*-Cymene-8-ol	0.20
26.953	$C_8H_8O_2$	*p*-Anis aldehyde	0.13
29.168	$C_{13}H_{28}$	Tridecane	0.11
29.810	$C_{10}H_{14}O$	Eucarvone	0.24
32.532	$C_{15}H_{24}$	α-Ylangene	0.16
33.608	$C_{15}H_{24}$	β-Elemene	0.23
33.871	$C_{15}H_{24}$	Cyperene	1.44
34.031	$C_{14}H_{30}$	Tetradecane	0.83
34.323	$C_{15}H_{24}$	α-Gurjunene	0.49
34.787	$C_{15}H_{24}$	Caryophyllene	0.16
35.368	$C_{15}H_{24}$	α-Guaiene	0.12
36.354	$C_{15}H_{24}$	α-Humulene	0.15
37.710	**$C_{11}H_{12}O_2$**	**Ethyl cinnamate**	**22.27**
38.283	$C_{15}H_{24}$	Valencene	0.28
39.175	**$C_{15}H_{32}$**	**Pentadecane**	**22.15**
39.541	$C_{15}H_{24}$	γ-Cadinene	1.40
39.793	$C_{15}H_{24}$	δ-Cadinene	0.54
41.082	$C_{15}H_{24}$	Germacrene B	0.11
45.052	$C_{15}H_{26}O$	Murrolol	0.18
46.625	$C_{17}H_{36}$	Heptadecane	3.00
49.624	**$C_3H_4O_2$**	**Propenoic acid**	**29.56**
56.072	$C_{20}H_{32}$	Sandaracopimara-8(14),15-diane	0.65

Table 7. Bioactive compounds in ginger essential oil as detected by GC-MS. Major bioactive compounds are in bold.

RT (min)	Chemical formula	Compound	Composition (%)
3.459	$C_6H_{12}O$	Hexanal	0.10
7.344	$C_{10}H_{16}$	α-Pinene	1.04
8.111	$C_{10}H_{16}$	Camphene	1.85
9.719	$C_{10}H_{16}$	β-Pinene	0.79
10.817	$C_{10}H_{16}$	Myrcene	0.24
11.595	$C_{10}H_{16}$	α-Phellandrene	0.14
13.095	$C_{15}H_{24}$	Cymene	0.23
13.421	$C_{10}H_{16}$	β-Terpinene	4.27
15.292	$C_{10}H_{16}$	γ-Terpinene	0.12
17.157	$C_{10}H_{16}$	Terpinolene	0.10
18.279	$C_{10}H_{18}O$	Linalool	0.21
20.522	$C_{10}H_{16}O$	Camphor	0.06
22.141	$C_{10}H_{18}O$	Borneol	0.96
22.633	$C_{10}H_{14}O_2$	Rosefuran epoxide	0.30
23.617	$C_{10}H_{18}O$	α-Terpineol	0.57
24.327	$C_{10}H_{20}O$	*n*-Decanal	0.08
25.780	$C_{10}H_{20}O$	Citronellol	0.16
27.131	$C_{10}H_{18}O$	Geraniol	0.14
30.936	$C_{15}H_{24}$	δ-Elemene	0.13
32.315	$C_{15}H_{24}$	Cyclosativene	0.11
32.778	$C_{15}H_{24}$	α-Copaene	0.72
33.310	$C_{12}H_{20}O_2$	Geranyl acetate	0.11
33.619	$C_{15}H_{24}$	β-Elemene	0.85
34.787	$C_{15}H_{24}$	Caryophyllene	0.11
35.553	$C_{15}H_{24}$	α-Bergamotene	0.25
36.669	$C_{15}H_{24}$	Aromadendrene	1.21
37.436	$C_{15}H_{24}$	β-Guaiene	0.76
37.676	$C_{15}H_{24}$	Germacrene D	1.58
37.997	**$C_{15}H_{22}$**	**Ar-Curcumene**	**12.04**
38.832	**$C_{15}H_{24}$**	**α-Zingiberene**	**26.32**
39.273	**$C_{15}H_{24}$**	**β-Bisabolene**	**11.34**
39.959	**$C_{15}H_{24}$**	**β-Sesquiphellandrene**	**10.61**
40.091	$C_{15}H_{24}$	γ-Bisabolene	0.43
40.829	$C_{15}H_{26}O$	Elemol	0.36
41.041	$C_{15}H_{24}$	Germacrene B	1.04
41.355	$C_{15}H_{26}O$	Nerolidol	0.70
46.402	$C_{15}H_{24}$	Iso-italicene	1.24

Table 8. Bioactive compounds in lemongrass essential oil as detected by GC-MS. Major bioactive compounds are in bold.

RT (min)	Chemical formula	Compound	Composition (%)
6.776	$C_{10}H_{16}$	Tricyclene	0.23
7.310	$C_{10}H_{16}$	α-Pinene	0.08
8.065	$C_{10}H_{16}$	Camphene	1.41
10.817	$C_{10}H_{16}$	Myrcene	0.11
10.674	$C_8H_{14}O$	6-methyl-5-hepten-2-one	0.89
13.295	$C_{10}H_{16}$	Limonene	1.68
18.365	$C_{10}H_{18}O$	Linalool	1.61
22.616	$C_{10}H_{14}O_2$	Rosefuran epoxide	0.21
23.640	$C_{10}H_{18}O$	α-Terpineol	0.99
26.976	**$C_{10}H_{16}O$**	**Neral**	**31.48**
27.234	$C_{10}H_{16}O$	Piperitone	0.17
28.813	**$C_{10}H_{16}O$**	**Geranial**	**42.61**
29.625	$C_{11}H_{18}O_2$	Geranyl formate	0.30
31.960	$C_{12}H_{22}O_2$	Citronellyl acetate	0.10
32.343	$C_{10}H_{12}O_2$	Eugenol	0.20
32.830	$C_{15}H_{24}$	α-Copaene	0.16
33.522	$C_{12}H_{20}O_2$	Geranyl acetate	3.86
34.901	$C_{15}H_{24}$	Caryophyllene	2.02
35.588	$C_{15}H_{24}$	α-Bergamotene	0.18
36.395	$C_{15}H_{24}$	α-Humulene	0.23
39.084	$C_{15}H_{24}$	γ-Cadinene	0.58
39.461	$C_{15}H_{24}$	δ-Amorphene	0.20
42.036	$C_{15}H_{24}O$	Caryophyllene oxide	1.96
43.084	$C_{15}H_{24}O$	Humulene oxide	0.12

Table 9. Bioactive compounds in lime essential oil as detected by GC-MS. Major bioactive compounds are in bold.

RT (min)	Chemical formula	Compound	Composition (%)
7.018	$C_{10}H_{16}$	α-Thujene	0.98
7.361	$C_{10}H_{16}$	α-Pinene	2.41
8.042	$C_{10}H_{16}$	Camphene	0.16
10.125	**$C_{10}H_{16}$**	**β-Pinene**	**18.95**
10.926	$C_{10}H_{16}$	β-Phellandrene	1.14
11.990	$C_{10}H_{17}$	δ-3-Carene	0.23
12.494	$C_{10}H_{16}$	δ-2-Carene	0.24
13.730	**$C_{10}H_{16}$**	**Limonene**	**39.84**
15.612	$C_{10}H_{16}$	γ-Terpinene	6.59
17.249	$C_{10}H_{16}$	Terpinolene	0.79
17.403	$C_{15}H_{24}$	*p*-Cymene	0.67
18.439	$C_{10}H_{18}O$	Linalool	1.54
22.765	$C_{10}H_{18}O$	Terpinen-4-ol	1.21
23.669	$C_{10}H_{18}O$	α-Terpineol	1.15
26.381	$C_{10}H_{16}O$	Neral	2.12
26.747	C_6H_8	1,3-Cyclohexadiene	0.44
28.017	$C_{10}H_{16}O$	Geranial	2.90
28.687	$C_{10}H_{10}O_2$	Safrole	0.13
34.397	$C_{11}H_{14}O_2$	Methyl eugenol	0.16
34.769	$C_{15}H_{24}$	Caryophyllene	0.08
39.273	$C_{15}H_{24}O$	Butylated hydroxytoluene	9.64
39.564	$C_{11}H_{12}O_3$	Myristicin	0.55
41.018	$C_{12}H_{16}O_3$	Elemicin	0.40
49.194	$C_{14}H_{12}O_2$	Benzyl benzoate	1.00

Table 10. Bioactive compounds in nutmeg essential oil as detected by GC-MS. Major bioactive compounds are in bold.

RT (min)	Chemical formula	Compound	Composition (%)
7.069	$C_{10}H_{16}$	α-Thujene	3.08
7.590	**$C_{10}H_{16}$**	**α-Pinene**	**15.35**
8.059	$C_{10}H_{16}$	Camphene	0.60
10.142	**$C_{10}H_{16}$**	**β-Phellandrene**	**27.80**
10.302	$C_{10}H_{16}$	β-Pinene	8.22
11.046	$C_{10}H_{16}$	Myrcene	1.86
12.042	$C_{10}H_{16}$	δ-3-Carene	0.39
13.329	$C_{10}H_{14}$	o-Cymene	7.42
13.569	$C_{10}H_{16}$	Limonene	5.86
15.509	$C_{10}H_{16}$	γ-Terpinene	5.33
17.209	$C_{10}H_{16}$	Terpinolene	0.45
18.832	$C_{10}H_{18}O$	Linalool	1.31
22.908	$C_{10}H_{18}O$	4-Terpineol	5.35
23.789	$C_{10}H_{18}O$	α-Terpineol	3.78
24.035	$C_{10}H_{18}O$	γ-Terpineol	0.82
27.811	$C_{10}H_{16}O$	Geranial	0.11
28.647	$C_{10}H_{10}O_2$	Safrole	0.13
32.292	$C_{10}H_{12}O_2$	Eugenol	1.84
34.397	$C_{11}H_{14}O_2$	Methyl eugenol	0.19
34.775	$C_{15}H_{24}$	Caryophyllene	0.11
35.673	$C_{15}H_{24}$	Aromadendrene	0.16
38.197	$C_{15}H_{24}$	Viridiflorene	0.18
38.666	$C_{11}H_{14}O_2$	Methyl isoeugenol	2.15
39.582	$C_{11}H_{12}O_3$	Myristicin	0.95
41.035	$C_{12}H_{16}O_3$	Elemicin	0.76
47.518	$C_{15}H_{30}O_2$	Methyl tetradecanoate	0.30

Table 11. Bioactive compounds in turmeric essential oil as detected by GC-MS. Major bioactive compounds are in bold.

RT (min)	Chemical formula	Compound	Composition (%)
7.327	$C_{10}H_{16}$	α-Pinene	0.45
9.547	$C_{10}H_{16}$	Sabinene	0.38
9.736	$C_{10}H_{16}$	β-Pinene	0.85
10.794	$C_{10}H_{16}$	Myrcene	0.06
11.619	$C_{10}H_{16}$	α-Phellandrene	0.30
12.431	$C_{10}H_{16}$	δ-2-Carene	0.06
37.956	$C_{15}H_{22}$	Ar-Curcumene	5.50
38.498	$C_{15}H_{24}$	α-Zingiberene	4.25
39.662	$C_{15}H_{24}$	β-Sesquiphellandrene	2.47
36.297	$C_{15}H_{22}$	γ-Curcumene	1.89
38.969	$C_{15}H_{24}$	β-Bisabolene	1.84
34.765	$C_{15}H_{24}$	Caryophyllene	1.45
13.346	$C_{10}H_{16}$	Limonene	1.08
17.638	$C_7H_8O_2$	*o*-Guaiacol	0.75
13.077	$C_{15}H_{24}$	*o*-Cymene	0.55
7.327	$C_{10}H_{16}$	α-Pinene	0.45
13.426	$C_{10}H_{18}O$	1,8-Cineole	0.35
15.292	$C_{10}H_{16}$	γ-Terpinene	0.16
17.157	$C_{10}H_{16}$	Terpinolene	0.12
23.074	C_8H_8O	Acetophenone	0.07
34.437	$C_{15}H_{24}$	α-Cedrene	0.15
35.588	$C_{15}H_{24}$	α-Bergamotene	0.38
36.383	$C_{15}H_{24}$	α-Humulene	0.11
36.858	$C_{15}H_{24}$	Italicene	0.20
37.304	$C_{15}H_{24}$	β-Acoradiene	0.19
39.092	$C_{15}H_{22}$	β-Curcumene	0.44
39.885	$C_{15}H_{24}$	γ-Bisabolene	0.31
42.422	$C_{15}H_{22}O$	γ-Atlantone	0.81
46.368	**$C_{15}H_{20}O$**	**Ar-Turmerone**	**41.81**
47.306	$C_{15}H_{22}O$	Curlone	8.48
49.755	$C_{15}H_{22}O$	α-Atlantone	3.41

oxysporum, and *F. verticillioides*, as well as against mycotoxins (Dambolena *et al*., 2008). In nutmeg essential oil, β-phellandrene (Knobloch *et al*., 1986), α-pinene and β-pinene (da Silva *et al*., 2012), eugenol, camphene, methyl eugenol, and caryophyllene detected in the present work have been reported to have moderate to high antifungal activities. In turmeric essential oil, other than caryophyllene, o-cymene, and α-phellandrene, ar-turmerone detected in the present work has been reported to exert broad-spectrum antifungal activities (Singh *et al.*, 2010; Avanço *et al*., 2017), as well as anti-aflatoxigenic activities (Ferreira *et al*., 2013).

Since essential oils often contain complex mixtures of bioactive compounds, it is thus important to establish either the antifungal effects exerted are due to a single bioactive compound or several of them (synergistic effect), or if the bioactive compound is present at the highest level based on the gas chromatography analysis is exerting the effect (León-Méndez *et al*., 2019). Although a wealth of information is currently available in the literature on the chemical compositions of essential oils and their related biological activities, none has specifically answered this question. Nevertheless, generally, it has been found that the major bioactive compound of the essential oil often reflects its biological activity. To a certain extent, this biological activity is also modulated by the other minor bioactive compounds present in the essential oil. Therefore, for biological purposes, it could be more beneficial and informative to study a complete essential oil instead of individual bioactive compounds since the synergistic effect might play a pivotal role in the intended biological activity (Bakkali *et al*., 2008).

Of the ten essential oils selected from Malaysian herbs and spices in the present work, only two are yet to be assigned with the GRAS status: betel and clove (CFR, 2020). It is noteworthy that clove was in fact included in the GRAS list during the 1998 revision (CFR, 1998). As to why it was removed during the 2020 revision (CFR, 2020) is unclear. Nevertheless, a more recent publication (Gooderham *et al*., 2020) (in November 2020; (CFR, 2020) was published earlier in April 2020) states that the Expert Panel of the Flavor and Extract Manufacturers Association (FEMA) has initiated the safety re-evaluation of over 250 natural flavour complexes (NFCs) used as flavour ingredients, during which seven NFCs, including clove essential oil, were affirmed as GRAS under their conditions of intended use as flavour ingredients. For betel essential oil, less than 100 scientific publications are available on Scopus (in January 2021). This is comparatively low; cinnamon (1,715), clove (1,843), coriander (560), galangal (55), ginger (975), lemongrass (772), lime (425), nutmeg (275), and turmeric (340). This might explain the lack of scientific, clinical, and safety data for GRAS affirmation. According to FDA, a food substance may attain the GRAS status if it fulfils two conditions stipulated by the regulations; (1) 21CFR170.30(b) requires scientific procedures, evidence, data, or information of such food which are usually published, and may be corroborated with unpublished scientific data, information, or methods, and (2) 21CFR170.30(c) and 21CFR 170.3(f) require a substantial history of consumption of such food by a significant number of consumers (FDA, 2021). This thus provides opportunities for the exploration and utilization of betel essential oil in the area of food safety and preservation.

To conclude, a total of 120 bioactive compounds were detected from the ten essential oils of selected Malaysian herbs and spices with clove (eugenol, 87.16%) and cinnamon (cinnamaldehyde, 63.39%) essential oils yielded the highest percentage of bioactive compounds. Based on the literature search, these bioactive compounds have been reported with antifungal activities. Since smart packaging is garnering increasing attention in recent times, these could facilitate the development of efficient fungal spoilage control strategies such as an active packaging system incorporated with essential oil containing antifungal compounds to preserve food and extend shelf life. To this end, essential oils from various herbs and spices have been widely used in conjunction with other preservation techniques (hurdle technology, green technology). Nevertheless, a strong odour of essential oils may limit their use in foods for fear of sensory alteration. Further studies to mitigate this are therefore warranted. *In vitro* analyses (Minimal Inhibitory Concentration, Minimal Fungicidal Concentration, Spore Germination Test) using the selected essential oils against spoilage and mycotoxigenic fungi are currently ongoing. These could also be extended to other underreported and underutilized Malaysian herbs and spices in the search for novel bioactive compounds to act as natural antimicrobials in the effort to fulfil the consumers' demand for greener (less chemical preservatives), safer (less microbial contamination), and higher-quality foods with longer shelf life.

Conflict of interest

The authors declare no conflict of interest.

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