

Antifungal activity and phytochemical profile of *kayu manis hutan* (*Cinnamomum iners* Reinw. Ex Blume Laureceae) leaf extract against spoilage microorganisms

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Article history:

Received: 21 September 2024

Received in revised form: 17 October 2024

Accepted: 14 January 2025

Available Online: 22 April 2025

Keywords:

Antifungal,
Cinnamomum iners,
Germination conidial
inhibition,
Kayu manis hutan,
Phytol

DOI:

[https://doi.org/10.26656/fr.2017.9\(2\).132](https://doi.org/10.26656/fr.2017.9(2).132)

Abstract

Food spoilage, facilitated by fungal growth, poses significant risks to food safety and quality during storage. Fungal species like *Aspergillus* and *Rhizopus* contribute to food deterioration and produce harmful mycotoxins, highlighting the need for natural antimicrobial alternatives rather than or to chemical preservatives. Consumers increasingly prefer fresher, additive-free food products. Research is exploring plant extracts as natural preservatives for their antimicrobial and antioxidant properties. *Cinnamomum iners*, known for its aromatic and medicinal attributes, contains bioactive compounds like cinnamaldehyde and quercetin-3-rutinoside. Unfortunately, reports on the antifungal and phytochemical properties of *C. iners* leaf extracts are scarce. The study aimed to assess the antifungal efficacy of *C. iners* extracts using the disc diffusion assay, minimum inhibitory concentration (MIC), minimum fungicidal concentration (MFC), and inhibition of conidial germination assay against *Aspergillus flavus*, *A. niger*, *Rhizopus oligosporus*, and *R. oryzae*. Phytochemical profiles were analyzed using GC-MS and LC-MS. The extract exhibited antifungal properties with inhibition zones ranging from 6.75±0.00 to 7.25±0.35 mm, while MIC and MFC values ranged from 0.63 to 2.50 mg/mL and 1.25 to 5.00 mg/mL, respectively. The conidial germination assay showed that all tested spoilage microorganisms were susceptible to *C. iners* leaf extracts at 4× MIC for up to 14 days. Inhibition effects increased significantly starting at 0.5× MIC, with 84% of *A. flavus*, 83% of *A. niger*, 55% of *R. oligosporus* and 80% of *R. oryzae* inhibited, indicating strong potential. GC-MS analysis identified three major compounds, dihydro-neoclovene-(II) (18.35%), phytol (13.75%), and 2-hexadecenal (12.60%). Meanwhile, LC-MS analysis found coniferyl alcohol at an intensity of 20.53×10⁴, cinnassiol C2 at 13.62×10⁴, and gerberinol at 12.73×10⁴, which might have contributed to the antifungal activity of *C. iners* leaf extract. These findings suggest that *C. iners* leaf extract can be a viable natural alternative for controlling food spoilage.

1. Introduction

There is a greater need for food as a result of the growing world population. High-quality and safe food is required for healthy living and can be achieved through proper storage. However, food is susceptible to physical, chemical, and microbial alterations while being stored.

These alterations are connected to both internal and external factors, including water activity, pH, moisture content, microbiological activity, and storage temperature, all of which can lead to food spoilage (Mutlu-Ingok *et al.*, 2020). Food deterioration is often linked to fungi because fungal development on both raw

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and processed meals degrades their sensory qualities, causing rotting, off-flavours, and odour emissions. Eating spoiled food is a common cause of food poisoning, a disease brought on by eating food tainted with toxins produced by chemicals, plants, fungi or bacteria (Aljamali *et al.*, 2021).

Common fungal species from genera like *Aspergillus* and *Rhizopus* are accountable for food degradation. Food contamination by *Aspergillus* species is a critical concern in the food industry due to the fungi's pervasive nature and their ability to produce mycotoxins, such as aflatoxins, which pose serious health risks (Navale *et al.*, 2021). The *Rhizopus* genus causes fruit and vegetable degradation and produces dense grey moulds that are greenish, whereas the *Aspergillus* genus is linked to postharvest agricultural goods and grows on food surfaces that are coloured green, blue, black, or yellow (Hipólito *et al.*, 2020).

For many years, food manufacturing has used chemical antimicrobial agents to prevent food spoilage from fungus. However, these preservatives have drawbacks, such as potential health risks for humans, food-borne chemical residues and microbiological resistance to extend the shelf life of food goods (Chibane *et al.*, 2019). Due to that, customers like food items that are fresher and more naturally occurring, and are devoid of chemical additives. The need for natural antibacterial substances is therefore rising.

The potential use of various plant extracts as efficient natural preservatives has drawn the attention of several researchers lately (Burenjargal *et al.*, 2023). Numerous phytochemicals with antimicrobial and antioxidant qualities, including flavonoids, alkaloids, tannins, and terpenoids, are found in natural plant products (Prakash *et al.*, 2020). *Cinnamomum iners*, known as *kayu manis hutan*, are commonly used for their aromatic properties in food and the pharmaceutical industry because they possess many medicinal properties (Wahab and Hussain, 2021). The leaves of *C. iners* have been found to exhibit high antibacterial activity against methicillin-resistant *S. aureus* (MRSA) and *E. coli* and the compound responsible for its activity was found as xanthorrhizol (Mustaffa *et al.*, 2020). Other than that, the extracts of *C. iners* also exhibited potent antioxidant activity against DPPH free radicals (IC₅₀ = 0.2 mg/mL) and the total phenolic content was found to be 248.6 mg GAE/g (gallic acid equivalent). As for the flavonoid content, it was calculated that *C. iners* leaves methanolic extract contained 13.38 mg CE/g (catechin equivalent) plant material (Udayaprakash *et al.*, 2015)

The plant of *C. iners* has various medicinal properties useful for curing and treating various diseases

because of bioactive constituents such as eugenol, quercetin-3-rutinoside, kaempferol-3-glucopyranoside, cinnamaldehyde (Mustaffa *et al.*, 2020), α -caryophyllene, stigmasterol, 2-hydroxycinnamaldehyde, linalool, cineole and β -sitosterol (Arifullah *et al.*, 2014). Prior research on phytochemicals has demonstrated that the plant possesses various pharmacological activities, such as analgesic, antimicrobial, and antioxidant properties, as well as toxicity (Blaszczyk *et al.*, 2021). However, studies on its antifungal activity and phytochemical analysis of the leaves are lacking. This research aimed to provide an understanding of the antifungal effects of *C. iners* leaf extracts on spoilage microorganisms and the phytochemicals responsible for the activity.

2. Materials and methods

2.1 Collection and extraction of the sample

The *C. iners* leaves were gathered from Putra Agriculture Centre, Universiti Putra Malaysia, Serdang, Malaysia. After cleaning under running tap water, the sample undergoes oven-drying at 60°C for 48 hrs and pulverization for 2 mins at 11,000 RPM using a blender (Panasonic, model MX-EX1001WSK). A 100 g of powdered leaves was submerged in 400 mL of denatured ethanol (95%) for 48 hrs. The Whatman filter paper size No. 2 was employed to vacuum filter the plant extracts and was further concentrated using a rotary vacuum evaporator at a speed of 125 rpm for 3 to 4 hrs at 40°C to obtain the crude extracts. The crude extracts were subjected to freeze drying for 48 hrs and stored in the freezer (-20°C) for use in further studies. The stock solution was prepared in percentage of 1% by diluted 10 mg of crude extracts with 10% DMSO.

2.2 Fungal strains

The *Aspergillus flavus* ATCC22546, *A. niger* ATCC9029, *Rhizopus oligosporus* ATCC22959 and *R. oryzae* ATCC22580 utilized in this investigation came from the American Type Culture Collection (ATCC). For seven days, each strain was kept viable by subculturing it on potato dextrose agar (PDA) at 35°C. Then, the PDA was chopped into bits using a sterilized laboratory spatula and was poured into 50 mL of 1% PBS followed by vigorous shaking. The mixture was filtered using sterile cotton wool allowing the finer conidial suspension to pass through while retaining larger particulate matter and obtaining the stock conidial suspension. Then it was stored at -18°C until further use. For *Aspergillus* species, an optical density (OD₅₉₆) of 80% to 82% transmittance and for *Rhizopus* species, an OD₅₉₆ of 68% to 70% were achieved by adjusting the conidial suspension density. A 1:50 dilution of these

suspensions was made using sterile distilled water. The suspension of inoculum contained around 10^4 CFU/mL.

2.3 Disc diffusion assay

The method suggested by Hipólito *et al.* (2020) was employed to carry out the disc diffusion assay against selected spoilage microorganisms, with slight modifications. The prepared conidia suspension was used to inoculate the PDA plate using a sterilized hockey stick. A sterile self-punched disc paper with a 6 mm diameter was set into inoculated PDA. The paper disc was imbued with 10 mg/mL of *C. iners* extracts in the amount of 10 μ L. The positive control was 1 mg/mL Amphotericin B (AmpB) while the negative control was 10% DMSO. Every plate was incubated for 48 hrs at 35°C and the inhibitory zone's diameter was recorded.

2.4 Determination of minimum inhibitory concentration and minimum fungicidal concentration

The MIC and MFC of the extract against spoilage microorganisms were established as suggested by Alexander *et al.* (2017) via employing a two-step broth microdilution technique of the prepared conidia suspension. The positive growth control was filled with 100 μ L conidia suspension while the negative growth control was filled with 100 μ L of potato dextrose broth (PDB). The MIC of the extract was established with varying concentrations that ranged from 5 mg/mL to 0.019 mg/mL, and the microtiter plates were incubated at 35°C for up to 48 h. Meanwhile, the MIC concentration for AmpB ranged from 0.5 mg/mL to 0.00097 mg/mL. The MFC was determined by sub-culturing 10 μ L from each well onto the PDA and incubated at 35°C up to 48 hrs.

2.5 Qualitative inhibition conidial germination assay

The prepared inoculum suspension was diluted in a 1:10 ratio in PDB to provide 10^3 CFU/mL as the final inoculum concentration. Mixed extracts were used in the cultures, and the final extract concentrations were 0 \times , 0.5 \times , 1 \times , 2 \times and 4 \times MIC. The mixture was incubated at 35°C for 14 days. This assay was performed based on daily visual observation. The observations that indicate inhibition include reduced germination, cloudiness, colour changes, and a lack of visible spore germination.

2.6 Quantitative inhibition conidial germination assay

The adjusted inoculum suspension was mixed with extracts and the final concentrations were 0 \times , 0.5 \times , 1 \times , 2 \times and 4 \times MIC in a final volume of 1 mL. The mixture was incubated at 35°C for 36-48 hrs. After that, 100 μ L of the incubated mixture was spread on PDA plates and the plate was incubated for 36-48 h to count the CFU/

mL. Using the following formula (Eq. 1), the percentage of germination inhibition was determined as follows:

$$\text{Germination inhibition (\%)} = \frac{\bar{x}_{\text{control}} - \bar{x}_{\text{treatment}}}{\bar{x}_{\text{control}}} \times 100 \quad (1)$$

Where \bar{x} = Average conidial germination

2.7 Gas chromatography-mass spectrometry

The purpose of the study was to determine which volatile chemicals were present in 5 mg/mL of *C. iners* leaf extract. The extract was diluted in 1 mL of HPLC-grade methanol prior to injection. GC-MS analysis of *C. iners* crude leaf extract was performed using the QP2010 Ultra GC-MS (Shimadzu Corporation, Kyoto, Japan) with specific parameters: electron multiplier detector, BP5MS column (30 m length, 0.25 mm diameter, 0.25 μ m film thickness), helium carrier gas at 0.8 mL/min flow rate. The oven temperature ranged from 50°C to 300°C at a rate of 3°C/min, held for 10 mins, with ion source and injection temperatures set at 200°C. Peaks in the *C. iners* extract were determined by contrasting the patterns of mass fragments with standard spectra from previous studies and the Shimadzu GC-MS NIST/Wiley library.

2.8 Liquid chromatography-mass spectrometry

The analysis was carried out to identify the non-volatile compounds present in 5 mg/mL of *C. iners* leaf extract. The extract was diluted in 1 mL of HPLC grade methanol, filtered through a 0.45 μ M Nylon syringe filter, and further diluted 10 \times with methanol. Separation occurred on a Thermo Scientific C18 column (AcclaimTM Polar Advantage II, 3 \times 150 mm, 3 μ m particle size) using an UltiMate 3000 UHPLC system (Dionex). Gradient elution utilized H₂O + 0.1% Formic Acid (A) and 100% Acetonitrile (B) over 22 mins at a flow rate of 0.4 mL/min and a column temperature of 40°C. The sample injection volume was 10 μ L. High-resolution mass spectrometry was conducted on a MicroTOF QIII Bruker Daltonik using ESI positive ionization. Data analysis employed Compass Data Analysis software (Bruker Daltonik GmbH) and open-access libraries like Metabolomic Workbench, Metfrag, Knapsack, and FoodB for compound identification based on exact mass.

2.9 Statistical analysis

To determine the significance of the mean difference, the data were subjected to a two-way analysis of variance (ANOVA) using Tukey's multiple range test, all performed in Minitab 19. Various letters indicate values with a significant difference ($p < 0.05$). The data ($n = 3$) are reported as mean \pm SD using Microsoft Office Excel.

3. Results and discussion

3.1 Diffusion assay, minimum inhibitory concentration, minimum fungicidal concentration of *Cinnamomum iners* leaf extract

Table 1 summarizes the antifungal activity of *C. iners* leaf extracts against tested spoilage microorganisms. The inhibition zone ranges between 6.75 to 7.25 mm. *Aspergillus flavus* and *R. oryzae* were the most susceptible to *C. iners* leaf extract compared to other strains. AmpB was displayed as the highest inhibition zone with 9.50 mm. A consistent concentration of AmpB (1 mg/mL) was used as a positive control across all strains in the disc diffusion assay to provide a standard reference for comparing the antifungal activity of *C. iners* extracts, despite variations in the MIC and MFC values among the strains. Meanwhile, the MIC ranges between 0.63 to 2.50 mg/mL and MFC for all tested strains were displayed between 1.25 to 5.00 mg/mL. *Aspergillus flavus* and *R. oryzae* were the most susceptible to *C. iners* leaf extract compared to other strains. It is found that *C. iners* exhibit fungistatic properties toward all tested filamentous fungi. This might be due to the presence of bioactive compounds that exhibited antifungal activity in the extract. The MIC indicates the threshold at which the extract can effectively suppress the growth of the food spoilage fungi. MFC signifies the concentration at which the *C. iners* leaf extract exhibits fungicidal activity, eradicating the fungi rather than just inhibiting their growth (Peixoto et al., 2017). According to Wong and Ramli (2021), *Centella asiatica* exhibited antifungal activity against *A. niger* with MIC of 125 mg/mL and MFC of 250 mg/mL. Similar to the current study, *A. niger* showed susceptibility toward *C. iners* with MIC of 2.50 and MFC of 5.00 mg/mL which represents a lower concentration. Plant bioactive compounds can interfere with various fungal cellular processes, leading to growth inhibition or even fungal cell death such as alkaloids, flavonoids, terpenoids, glycosides, saponin, peptides, steroids, phenols and quinones (Costa et al., 2019).

3.2 Qualitative inhibition conidial germination assay

Figure 1 shows the qualitative inhibition of germination conidia assay of food spoilage fungi

incubated for 14 days during exposure to *C. iners* leaf extract at 0× MIC, 0.5× MIC, 1× MIC, 2× MIC and 4× MIC. Based on observation, as the day of incubation increases, the broth becomes clearer at the lowest concentration (0× MIC) due to nutrient depletion, while cloudiness begins to appear as the MIC increases. However, all the tested spoilage microorganisms were susceptible to the 2× and 4× MIC of *C. iners* leaf extracts, which effectively inhibited the germination and growth of conidia, resulting in no visible hyphae up to 14 days, as shown in Figure 2 (red box). Interestingly, Figure 1 shows that 2× MIC of *A. flavus* exhibited fungistatic effects compared to AmpB at 14 days, indicating its effectiveness compared to the commercial antifungal. Initially, the fungi undergo various growth phases and the cloudiness indicates active fungal growth, sporulation or metabolic activity. As the fungal life cycle progresses and the resources and nutrients in the broth are consumed, the culture may reach a stationary phase leading to a reduction in cloudiness (Vrabl et al., 2019). The antifungal properties of *C. iners* cause cell death and this is observable in the fungal biomass such as mycelium, which might sediment to the bottom of the culture over time (Navale et al., 2021). Thus, the qualitative result is when the *C. iners* effectively inhibits fungal growth and visually shows a clearer broth.

3.3 Quantitative inhibition conidial germination assay

Based on the results shown in Figure 2, the inhibition germination conidia endpoint was achieved at 4× MIC concentration for *A. flavus* (2.50 mg/mL), *A. niger* (10.00 mg/mL), *R. oligosporus* (5.00 mg/mL) and *R. oryzae* (2.50 mg/mL). The inhibition effects were increased drastically starting at a concentration 0.5× MIC which almost 84% of *A. flavus*, 83% of *A. niger*, 55% of *R. oligosporus* and 80% of *R. oryzae* inhibited by *C. iners* leaf extract. Generally, conidia germination of filamentous fungi was fully inhibited as the concentration and time of exposure to the extract were increased. As far as we are aware, no research has been done on the prevention of filamentous fungus conidia germination by *C. iners* leaf extract. Despite many fungal species requiring more than 48 hrs for growth, the currently study focuses on 48 hrs as the early stages of

Table 1. Antifungal activity of *C. iners* crude leaf extracts against spoilage fungi.

Strains	Zone of inhibition (mm)		MIC (mg/mL)		MFC (mg/mL)	
	Samples	AmpB	Samples	AmpB	Samples	AmpB
<i>A. flavus</i>	7.25±0.35 ^B	9.50±0.00 ^A	0.63	0.06	1.25	0.13
<i>A. niger</i>	7.00±0.00 ^B	9.50±0.00 ^A	2.50	0.06	5.00	0.13
<i>R. oligosporus</i>	6.75±0.00 ^B	9.50±0.00 ^A	1.25	0.03	2.50	0.06
<i>R. oryzae</i>	7.25±0.35 ^B	9.50±0.00 ^A	0.63	0.13	1.25	0.25

Values are presented as mean±SD of triplicate. Values with different superscripts within the same row are statistically significantly different (p<0.05).

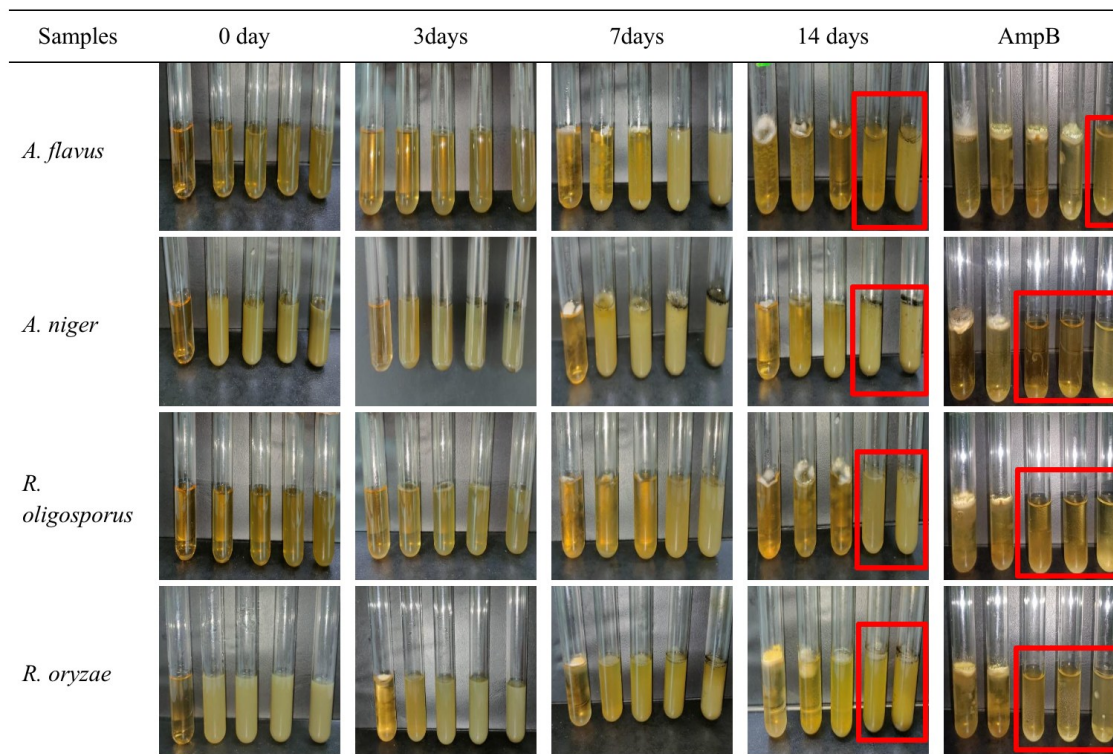


Figure 1. Qualitative inhibition germination conidia assay of food spoilage fungi incubated for 14 days at 0× MIC, 0.5× MIC, 1× MIC, 2× MIC and 4× MIC of *C. iners* leaf extracts. The red boxes indicate the susceptibility of the tested fungi, showing no visible hyphae (white growth formed on top of the broth) in both the extract and Amphotericin B (Amp B).

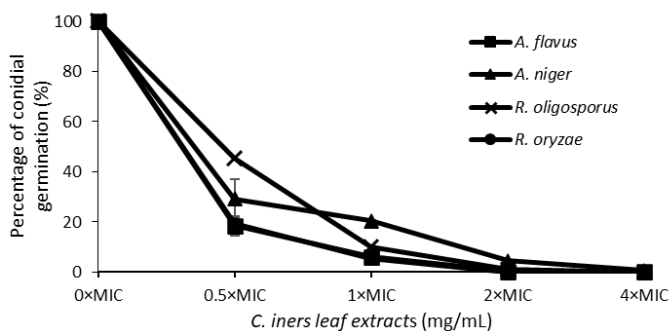


Figure 2. Effect of *C. iners* leaf extract against conidial germination of spoilage fungi at 0× MIC, 0.5× MIC, 1× MIC, 2× MIC and 4× MIC.

fungal growth as it demonstrated changes in fungal morphology, metabolic activity, and secondary metabolite production (Costa *et al.*, 2019). According to Mutlu-Ingok *et al.* (2020), *C. zeylanicum* L. inhibited 63% of the conidial germination of *Rhizopus* spp. at 0.25 mg/mL concentration and completely retarded the growth at 0.5 mg/mL compared to the current study, *R. oryzae* achieved a higher inhibition percentage as the concentration increased. Prakash *et al.* (2020) mentioned that significant lipid, protein, DNA, and glucose deterioration as well as plasma membrane damage have been documented as the potential mechanisms of inhibition. In addition, Silva-Beltran *et al.* (2023) reported similar findings, demonstrating a reduction in conidial germination against *A. flavus* with MIC values of 0.16% (v/v), indicating high antifungal activity due to bioactive compounds. Cinnamaldehyde, reported as one of the major bioactive compounds found in

Cinnamomum species, is claimed to have potential as an antifungal agent against spoilage microorganisms. This compound exerts inhibitory effects by inhibiting ATPase activity, modifying the integrity and shape of membranes and affecting the production of cell walls (Mustaffa *et al.*, 2020).

3.4 Gas chromatography-mass spectrometry analysis

The *C. iners* leaf GC-MS chromatogram, which has 35 peaks, is shown in Figure 3. The bioactive compounds were identified through comparison with known compounds listed in various libraries, such as NIST and Wiley. GC-MS analysis revealed three major compounds that might be responsible for the antifungal activity in *C. iners* leaf extract, which are dihydro-neoclovene-(II) (18.35%), phytol (13.75%), and 2-hexadecenal (12.60%). The GC-MS detected phytochemical compounds in *C. iners* leaf extracts that are shown in Table 2. Dihydro-neoclovene-(II) is a natural sesquiterpene that exhibits antimicrobial and antioxidant properties. Microbial cell membranes are thought to represent a major factor in the mechanism of action of sesquiterpenoids against bacteria, despite the fact that this process has not been thoroughly documented. Antifungal actions are primarily mediated by disrupting chemical transport, blocking the transition from yeast to hypha, altering host immunity, and redox activities (Li *et al.*, 2022). Furthermore, its antioxidant capabilities are observed, possibly because of functional groups like hydroxyl groups that enable the neutralization of free radicals and prevent oxidative

Table 2. Detected phytochemical compounds in *C. iners* leaf extracts by using GC-MS.

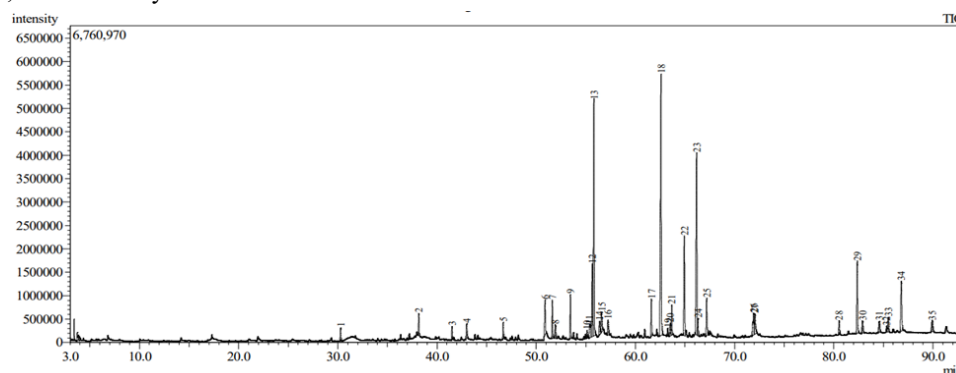
Compound name	RT (min)	MW	Mass fragments <i>m/z</i>	Area (%)	SI	Type
(E)-Caryophyllene	30.26	204.35	40.25, 41.20, 55.15, 69.15, 93.05, 105.10, 133.15	0.58	96	Sesquiterpene
Myristaldehyde	38.16	212.37	40.25, 41.15, 43.20, 57.10, 82.10, 96.10, 110.10	1.10	96	Fatty aldehyde
2-Pentadecanone	41.50	226.40	41.20, 43.15, 58.15, 71.10, 85.10, 110.20	0.61	96	Ketone
(E)-4-(3-Hydroxyprop-1-en-1-yl)-2-methoxyphenol	42.98	180.20	55.10, 77.10, 91.05, 103.05, 124.05, 137.10, 180.05	0.85	91	Phenolic
Neophytadiene	46.66	278.15	41.15, 55.15, 68.10, 95.10, 109.15, 123.15, 137.15	0.83	95	Diterpene
Hexadecanoic acid	50.89	256.42	40.25, 41.15, 43.20, 73.10, 87.05, 98.10, 129.10	2.54	93	Lipid
Palmitic acid vinyl ester	51.62	282.46	43.15, 57.15, 71.15, 109.10, 137.15, 239.20	2.00	92	Fatty acid
trans-Sinapyl alcohol	51.92	210.22	41.15, 55.15, 77.05, 107.05, 139.05, 149.10, 167.05	1.03	77	Phenolic
Heptadecane, 9-(2-cyclohexylethyl)	53.45	350.66	41.15, 55.15, 69.15, 83.10, 97.10, 111.10, 135.15, 237.20	2.39	81	No report
Methyl 2-oxohexadecanoate	55.12	284.43	43.20, 57.15, 71.15, 109.15, 137.20	0.44	86	Fatty acid
p-Methan-7-ol, (cis)-	55.36	156.26	43.20, 57.10, 95.10, 109.10	1.76	81	No report
Methyl octadecyl ether	55.64	284.52	45.15, 57.15, 83.10, 97.10, 111.15, 125.15	4.42	85	No report
Phytol	55.71	296.53	40.25, 41.15, 57.15, 71.10, 95.10, 123.10	13.75	97	Diterpene
Z,Z-8,10-Hexadecadien-1-ol	56.38	238.41	43.15, 67.10, 97.10, 114.10, 135.15	1.07	85	Fatty acid
Linoleic acid	56.60	280.45	41.15, 55.15, 79.10, 95.10, 108.10, 121.15	1.50	87	Fatty acid
2-Methyl-Z,Z-3,13-octadecadienol	57.22	280.50	41.20, 55.10, 83.10, 97.15, 111.10, 237.20	1.11	83	Terpenoid
Isolongifolol	61.60	222.36	43.15, 57.15, 82.10, 110.05, 136.05, 149.15, 177.15	2.15	75	Sesquiterpenes
Dihydro-neoclovene-(ii)	62.58	204.35	43.15, 57.15, 82.10, 110.05, 136.05, 149.10, 177.10	18.35	76	Sesquiterpenes
Bicyclo[10.1.0]trideca-4,8-diene	63.24	220.10	43.20, 67.10, 93.10, 107.05, 137.10	0.47	81	No report
Diocetyl propylphosphate	63.66	348.51	57.10, 83.110, 110.10, 125.00, 153.10	1.66	78	No report
14- β -pregnane	64.93	288.51	43.15, 70.10, 97.05, 126.00, 140.10, 163.15, 265.20	6.71	81	Steroid
2-Hexadecenal	66.17	238.40	41.15, 70.10, 97.10, 126.05, 140.10	12.60	78	Fatty aldehyde
Succinic acid, dec-2-yl dodec-9-yn-1-yl ester	66.30	366.53	43.15, 68.10, 95.10, 109.10, 121.10, 129.05, 219.25, 265.20, 311.15	0.91	73	No report
Hexadecanoic acid, 2-hydroxy	67.18	330.51	43.20, 57.15, 84.05, 98.10, 112.10, 147.05, 182.10, 239.20	2.41	91	Fatty acid
9-Octadecenoic acid (Z)-, 2,3-dihydroxypropyl ester	71.91	356.54	41.20, 55.10, 69.10, 81.10, 98.10, 112.05, 137.15, 264.25	2.11	92	Ester
Ethyl linoleate	72.04	306.50	41.15, 67.10, 79.10, 93.10, 121.10, 149.15	1.62	90	Ester
γ -tocopherol	80.53	416.66	40.25, 71.10, 95.10, 123.10, 151.10, 164.10, 191.10	0.84	92	Vitamin
α -tocopherol	82.36	436.66	40.25, 71.10, 109.10, 136.10, 165.10, 205.10, 430.45	4.66	93	Vitamin
Sesamin	82.88	354.35	65.15, 91.05, 122.05, 135.10, 149.05, 161.10, 178.10, 203.10	0.95	94	Lignan

RT: Retention time, SI: Similarity index.

Table 2 (Cont.). Detected phytochemical compounds in *C. iners* leaf extracts by using GC-MS.

Compound name	RT (min)	MW	Mass fragments <i>m/z</i>	Area (%)	SI	Type
Stigmast-5-en-3-ol, (3 β .)-	84.58	456.71	43.20, 69.10, 81.10, 95.10, 119.10, 145.15, 173.10, 185.20, 213.15, 231.15, 255.30, 315.20,	0.95	87	Triterpenes
Stigmasterol	85.31	412.69	41.15, 55.15, 83.10, 91.10, 119.05, 133.10, 145.05, 159.10, 271.20, 412.10	0.44	85	Triterpenes
Palmitone	85.47	450.84	43.20, 57.15, 85.10, 109.15, 152.15, 194.25, 239.20, 255.25	0.91	87	Lipid
Stigmast-5-en-3-ol	86.81	414.70	43.20, 81.10, 107.10, 119.10, 145.10, 163.15, 213.20, 255.20, 303.20, 329.25, 414.35	4.31	90	Triterpenes

RT: Retention time, SI: Similarity index.

Figure 3. Total ion chromatogram of phytochemical compound of *C. iners* leaf extracts.

reactions (Benedetto *et al.*, 2019). One of the chlorophyll constituents, phytol is an acyclic natural diterpene alcohol possessing several pharmacological characteristics, such as anti-inflammatory, anti-cancer, antioxidant, and antibacterial characteristics (Islam *et al.*, 2020). According to Abdel-Naime *et al.* (2019), the antifungal activity of *Melissa officinalis* and phytol was found to be the major compound responsible for it. It alters the shape and function of the fungal cell membrane by interfering with the production of ergosterol (Wei *et al.*, 2020). Other than that, Marrez *et al.* (2019) found the antimicrobial activity of 2-hexadecenal and radical scavenging activity of the compound. This is consistent with Amaegberi *et al.* (2019), who reported that 2-hexadecenal, also known as palmitolealdehyde is created when sphingolipids are destroyed by free radicals in an oxidative environment. It has biological action by preventing proliferation and triggering apoptosis. However, further spectroscopic analysis is required to confirm the presence of non-volatile compounds.

3.5 Liquid chromatography-mass spectrometry analysis

Based on LC-MS analysis, five bioactive compounds were tentatively identified in *C. iners* leaf extract. The intensities of the compounds present were coniferyl alcohol at 20.53×10^4 , cinnassiol C2 at 13.62×10^4 , gerberinol at 12.73×10^4 , α -terpineol at 3.09×10^4 , and isorhamnetin-3-O-rutinoside at 2.98×10^4 . The LC-MS phytochemical identification analysis for compounds in *C. iners* leaf extracts is shown in Table 3. Coniferyl alcohol is an important precursor in the process of producing lignin, an intricate polymer that gives plant cell walls durability and skeletal stability (Bidlack and Dashek, 2017). Langa-Lomba *et al.* (2021) reported a similar result, finding that coniferyl alcohol, present at a concentration of 47.4%, exhibited antifungal activity against *Candida albicans*. Their study highlights the potential of coniferyl alcohol as an effective natural antifungal agent. Cinnassiol belongs to the class of organic compounds known as sesquiterpenoid derived and is derived from *Cinnamomum cassia*. Cinnassiol

Table 3. Tentative identification of bioactive compounds in *C. iners* leaf extracts by LC-MS.

Compound name	RT (min)	[M+H] ⁺ <i>m/z</i>	Intensity ($\times 10^4$)	Activity	References
Coniferyl alcohol	2.0	180.10	20.53	Antibacterial	Langa-Lomba <i>et al.</i> (2021)
Gerberinol	2.2	365.10	12.73	Antimicrobial	Brahmachari <i>et al.</i> (2017)
Isorhamnetin-3-O-rutinoside	9.3	625.17	2.98	Antioxidant	Elhawary <i>et al.</i> (2018)
α -terpineol	9.6	154.14	3.09	Antimicrobial	Prakash <i>et al.</i> (2020)
Cinnassiol C2	16.2	363.18	13.62	Antioxidant	Liu <i>et al.</i> (2021)

RT: retention time, *m/z*: mass to charge ratio

and its glucoside, cinnassiol C2, from cinnamon cortex and cinnamon oil, were reported to possess antipyretic and analgesic effects (Liu *et al.*, 2021). According to Elhawary *et al.* (2018), it was found that *Ficus auriculata* L. exhibited high antifungal activity, and the presence of isorhamnetin 3-O-rutinoside as one of the major compounds was detected using LC-MS/MS. Additionally, Zhang *et al.* (2018) mentioned the compound exerted strong DPPH and ABTS radical scavenging activities.

4. Conclusion

This is the first report to find six new volatile compounds detected in ethanolic *C. iners* leaf extract by GC-MS analysis. In this research, the antifungal activity of *C. iners* leaf extracts has shown a relationship with the detected compounds. *Cinnamomum iners* leaves exert potential antifungal activity toward tested spoilage microorganisms. The major compounds responsible for the antifungal activity mentioned were dihydro-neoclovene-(II), phytol, 2-hexadecenal, coniferyl alcohol, and cinnassiol C2 based on phytochemical analysis. Therefore, this suggests that the *C. iners* leaves can be further utilized and developed as natural food preservatives.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

The author acknowledges the University Putra Malaysia for providing approval to carry out the research and evaluations and to all those who provided assistance with this study.

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