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Phytochemical Characterization and Antifungal Efficacy of Camphor (*Cinnamomum camphora* L.) Extract against Phytopathogenic Fungi

Sherien Sobhy [1], Abdulaziz A. Al-Askar [2], Elsayed K. Bakhiet [3], Mohsen M. Elsharkawy [4], Amr A. Arishi [5], Said I. Behiry [6,*] and Ahmed Abdelkhalek [1,*]

1 Plant Protection and Biomolecular Diagnosis Department, ALCRI—Arid Lands Cultivation Research Institute, City of Scientific Research and Technological Applications, New Borg El Arab City, Alexandria 21934, Egypt
2 Department of Botany and Microbiology, College of Science, King Saud University, P.O. Box 2455, Riyadh 11451, Saudi Arabia
3 Department of Botany and Microbiology, Faculty of Science, Al-Azhar University, Assiut 71524, Egypt
4 Department of Agricultural Botany, Faculty of Agriculture, Kafrelsheikh University, Kafrelsheikh 33516, Egypt
5 School of Molecular Sciences, The University of Western Australia, Perth, WA 6009, Australia
6 Agricultural Botany Department, Faculty of Agriculture (Saba Basha), Alexandria University, Alexandria 21531, Egypt
* Correspondence: said.behiry@alexu.edu.eg (S.I.B.); aabdelkhalek@srtacity.sci.eg (A.A.); Tel.: +20-1007556883 (A.A.)

Abstract: *Cinnamomum camphora* methanolic extract was tested for antifungal activity against three common, isolated, widespread phytopathogens: *Alternaria alternata*, *Fusarium solani*, and *Fusarium oxysporum*, which were molecularly identified and assigned accession numbers ON795987, ON795988, and ON795989, respectively. At 4000 µg/mL, the highest concentration of *C. camphora* methanolic extract inhibits the fungal mycelia weight of *F. oxysporum*, *A. alternata*, and *F. solani* by 60, 49, and 24%, respectively. The presence of several bioactive metabolites in the *C. camphora* extract could explain its antifungal activity. The presence of numerous phenolic and flavonoid compounds in the extract was revealed by HPLC analysis, including catechin and gallic acid, which had the highest concentrations of 6.21 and 6.98 µg/mL, respectively. Furthermore, osmoprotectants, total amino acids, and glycine betaine were abundant. Furthermore, total antioxidant activities, as measured by PMA and DPPH, were significant. The most abundant compound in the extract, according to GC-MS analysis, was mono(2-ethylhexyl) ester of 1,2-benzene dicarboxylic acid. Based on its in vitro efficacy in inhibiting mycelial growth weight, the tested extract could be recommended as a safe fungicide instead of a chemical treatment.

Keywords: *Cinnamomum camphora*; HPLC; GC-MS; phenolic compounds; flavonoid compounds; plant extract; antifungal activity

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1. Introduction

Global crop yields suffer from many pathogens that affect their productivity and quality. The major pathogens causing these diseases comprise fungi, viruses, nematodes, and bacteria [1]. The intensity of underground fungi is significantly higher than that of aboveground fungi. These soil-borne fungi can endure for extended periods within the soil due to the production of structures that help them survive, such as sclerotia, melanized hyphae, oospores, and chlamydospores [2]. A prevalent soil-borne fungus, the Fusarium species, is responsible for a variety of plant diseases, including Fusarium head blight, Fusarium root rot, Fusarium crown rot, and others. This fungus can impact a diverse range of crops, including small grain cereals, maize, various vegetables, bananas, lilies, trees, and many more, each with its own unique disease pattern, leading to substantial reductions

in yield [3]. In addition to Fusarium, *Alternaria* sp. is responsible for severe pre- and post-harvest crop losses. *Alternaria alternata* has previously been reported to cause post-harvest losses at high frequency in tomatoes [4] and produce leaf blight symptoms. Moreover, *Alternaria* invades not only vegetables, but is also responsible for the late blight symptom of nuts, which affects both foliage and fruit and is characterized by the development of large necrotic spots or lesions that eventually coalesce and consume the entire leaf and defoliate the tree [5].

There are many ways to control and deal with the rise of plant fungal diseases around the world. The most common and useful method in agriculture for getting rid of pests and diseases is to use pesticides [6]. Unfortunately, the widespread use of pesticides in agriculture to deal with problems before and after harvest has led to dangerous outbreaks and fungal infections that are resistant to fungicides [7]. In addition, once the pesticide has been applied, the chemical residue makes its way into groundwater, lakes, and marine water through various environmental processes, negatively impacting the species living in those bodies of water [8,9]. Therefore, research is now focused on finding new ways to reduce the pesticide levels left on the soil. Among these, natural extracts are safer and cheaper alternatives to replace the widespread use of synthetic chemical antifungal substances to create a chemical-free environment.

Plant extracts are characterized by diverse active compounds, minerals, secondary metabolites, and antioxidants, which give the extracts their efficacy [10,11]. Natural compounds extracted from various plants have been shown to have broad biological applications as antifungal, antibacterial, and antiviral agents [11–14]. *Cinnamomum camphora* (L.) is a huge tree commonly planted for landscaping and forestation. This tree synthesizes plenty of compounds known as terpenoids, which prevent herbivore attacks and repel fungal infections [15]. Due to its antioxidant constituents and antimicrobial activities, *C. camphora* has been widely used in the cosmetic and medical industries [16]. Moreover, it is a rich source of alcohols, ketones, terpenoids, and esters, which may increase the algaecidal functions. The *C. camphora* leaf extract has antifungal activity against *Choanephora cucurbitarum* [8] and *Aspergillus niger* [17], antibacterial activity against *Pasturella multocida* [18], and insecticidal properties against *Drosophila melanogaster*, *Chaoborus plumicornis*, and *Pieris rapae* [17]. Therefore, our research endeavors to assess the antifungal properties of a methanolic extract of *C. camphora* against three molecularly identified soil-borne phytopathogenic fungi (*F. oxysporum*, *A. alternata*, and *F. solani*) isolated from tomato plants. Additionally, total flavonoid, phenolic, ascorbic acid, amino acids, saponin, tannins, proline, glycine betaine content, total antioxidant, and DPPH scavenging activities were estimated. Additionally, the chemical composition of the extract was determined through HPLC and GC-MS analysis.

2. Materials and Methods

2.1. Plant Sampling and Extract Preparation

The leaves of *Cinnamomum camphora* plants grown in Tanta, Egypt, were picked for experimental research. The selected leaves were healthy and did not suffer from any morphological aberrations. Initially, the leaves underwent thorough washing with tap water to remove any surface dust or contaminants and were left to dry at room temperature for 10 days or until completely dry. The dried leaves were then ground into a fine powder. Subsequently, 50 g of the powder was combined with 500 mL of 80% methanol in an Erlenmeyer flask and agitated on a rotary shaker (100 rpm) at room temperature for a period of one night. The resulting methanolic leaf extract was filtered through Whatman No. 1 filter paper. To remove any remaining methanol in the extract, vacuum-assisted drying was employed. By applying a temperature range of 25–30 °C, the residual solvent can be evaporated. The dried extract was stored in the fridge at 4 °C. To use the extract in the antifungal activity test, it was dissolved in 10% dimethyl sulfoxide (DMSO). We weighed 0.1, 0.2, 0.3, and 0.4 g of the extract and dissolved each in 1 mL of 10% DMSO. We then used these stock solutions to prepare the final concentrations in 100 mL of broth

media in a flask as 1000, 2000, 3000, and 4000 µg/mL of media. The control flask received 1 mL of 10% DMSO (free of extract).

2.2. Fungal Isolates, Culture Media, and Growth Conditions

Three phytopathogenic fungi were obtained from the rhizosphere of squash plants. The purity of these fungi was verified by growing them on potato dextrose agar (PDA) medium in 9-cm Petri dishes at 27 °C for a week in an incubator. Afterward, the three fungi were preserved on slants in the refrigerator at 4 °C for further investigation.

2.3. Morphological and Molecular Characterization

The three fungal isolates were re-grown on PDA media at 27 °C for 7 days, and then the mycelia and spores were morphologically examined under the light microscope according to the identification protocols [19,20]. Afterward, the mycelia were harvested for DNA extraction using the CTAB method described by Wang et al. [21]. The DNA concentration was measured using a Nanodrop 2000 spectrophotometry device (Thermo Fisher Scientific, Wilmington, DE, USA) and stored at −20 °C in a dilution of 100 ng/µL for use in PCR amplification. The complete rDNA-ITS (ITS) region was amplified using specific PCR primers, such as ITS1/ITS4 [22], from all fungal isolates. The PCR amplification conditions were initial denaturing at 94 °C for 5 min; 35 cycles of denaturing (94 °C for 30 s each cycle); annealing at 55 °C for 30 s; extension at 72 °C for 1 min; and a final extension at 72 °C for 10 min. The PCR amplicons were visualized, purified, and sent to the Macrogen company for Sanger sequencing after being electrophoresed on a 1% agarose gel.

2.4. Antifungal Activity

The mycelial weight of the fungal mat was used to evaluate the antifungal activity of *C. camphora* extract. The final concentrations of *C. camphora* extract (1000, 2000, 3000, and 4000 µg/mL) in potato broth media were made as illustrated in Section 2.1. Each flask of broth medium was inoculated with 0.5 cm of the fungal disc and shaken for a week on an orbital shaker (100 rpm) at 27 ± 2 °C before being filtered. The fungal mats were weighed and compared to the control to find out the reduction in growth, thereby estimating the most effective concentration for decreasing fungal growth. The inhibition of fungal growth in the experiment was calculated as the percentage of inhibition of mycelial growth compared to the control. All the tests were repeated three times.

$$\text{Mycelial growth inhibition (MGI \%)} = [(D0 - Dt)/D0] \times 100$$

where D0 and Dt are the control and treatment fungi growth weights, respectively.

2.5. Phytochemical Analysis of Camphor Extracts

The number of active compounds and antioxidants in camphor methanolic extract was determined. Flavonoids, phenolics, and ascorbate were assayed as previously described [23,24]. Saponin was estimated quantitatively by the method described by Hiai et al. [25]. Tannin content was determined by Broadhurst et al. [26]. Proline, glycine betaine, and total amino acids were also investigated [27–29]. The camphor methanolic extract's antioxidant capacity was determined using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) and phosphomolybdate assay (PMA) methods described previously [30,31].

2.6. HPLC Conditions for Phenolic and Flavonoid Compounds

The presence of phenolic and flavonoid compounds in the methanolic extract of *C. camphora* was determined by using a set of standard compounds, including gallic acid, catechol, vanillic acid, *p*-hydroxybenzoic acid, syringic acid, vanillin, *p*-coumaric acid, ellagic acid, ferulic acid, caffeic acid, benzoic acid, salicylic acid, cinnamic acid, o-coumaric acid, rutin, quercetin, myricetin, apigenin, naringenin, and kaempferol. To identify the phenolic compounds, an Agilent 1260 Infinity HPLC Series was utilized, which included a Quaternary pump and a Zorbax Eclipse Plus C18 column (100 mm × 4.6 mm i.d.).

The extract was injected into a volume of 25 µL, and the separation was performed at a temperature of 30 °C with a gradient elution consisting of (A) HPLC-grade water containing 0.2% H_3PO_4 (*v*/*v*), (B) methanol, and (C) acetonitrile at a flow rate of 1 mL/min. A variable-wavelength detector was used to detect the compounds at 284 nm.

To quantify flavonoid compounds, a Knauer HPLC Smart Line equipped with a binary pump and a Zorbax Eclipse Plus C18 column (150 mm × 4.6 mm i.d.) was used. The separation process involved an eluent containing methanol and water with 0.5% H_3PO_4 in a 50:50 ratio and a flow rate of 0.7 mL/min. The extract was injected into a volume of 25 µL, and a UV detector was set at 273 nm. Data integration was carried out using ClarityChrom® Version 7.2.0 (Knauer Wissenschaftliche Geräte GmbH, Berlin, Germany) [32,33].

2.7. Gas Chromatography–Mass Spectrometry (GC-MS) Analysis

The chemical composition of the methanolic extract of *C. camphora* was studied using a Trace GC Ultra ISQ mass spectrometer from Thermo Scientific in Waltham, Massachusetts, USA. The analysis was performed using a direct capillary column, TraceGOLD TG–5MS, with specifications of 30 m × 0.25 mm × 0.25 µm film thickness. The extract was dissolved in high-quality methanol suitable for spectroscopy. The temperature of the column oven was initially set to 50 °C and increased at a rate of 5 °C/min to reach 230 °C, where it was maintained for 2 min before being increased to the final temperature of 290 °C and held for another 2 min. The injector and MS transfer line temperatures were kept at 250 and 260 °C, respectively. Using helium as a carrier gas, a 1 µL sample was injected at 250 °C, split at a 1:30 ratio. The mass spectrometer was operated in the electron ionization (EI) mode at 200 °C and 70 eV, with a scan range of 40–1000 m/z [34]. The components were identified by comparing the mass spectra and retention times to the data in the Wiley and NIST MS library databases [35,36].

2.8. Statistical Tests

A randomized design was used in the experiments, and the results were analyzed using analysis of variance with the aid of "CoSTAT" software. The results are presented as mean ± SD and are considered statistically significant if $p \leq 0.05$.

3. Results

3.1. Isolation Trails and Identification

From the rhizosphere samples, the isolation trails led to three isolates of fungi, which were found to be *Alternaria alternata*, *Fusarium solani*, and *F. oxysporum*. Their sequences were deposited into the GenBank database under the accession numbers (ON795987, ON795988, and ON795989), respectively.

3.2. Effect of Plant Extracts on the Fungal Mat Weight

The obtained results in Table 1 revealed a gradual decrease in the fungal mat weight by increasing the concentration of methanolic camphor extract; the most effective concentration giving the least fungal mat weight was (4000 µg/mL), where the mycelia growth inhibition (MGI) reached 24, 49, and 60% in *F. solani*, *A. alternata*, and *F. oxysporum*, respectively, compared to the untreated control.

Table 1. The effect of different concentrations of *Cinnamomum camphora* methanolic extract on fungal growth weight in broth media.

Concentration (µg/mL)	Weight of Fungal Mat (g)					
	F. solani	**Inhibition %**	***A. alternata***	**Inhibition %**	***F. oxysporum***	**Inhibition %**
Control (10% DMSO)	3.38 ± 0.2 a	0.00	4.12 ± 0.12 a	0.00	1.37 ± 0.1 a	0.00
1000	3.27 ± 0.1 ab	3.25	3.65 ± 0.14 b	11.41	1.20 ± 0.1 a	12.41
2000	3.22 ± 0.2 ab	4.73	2.95 ± 0.3 c	28.40	0.81 ± 0.1 b	40.88
3000	3.05 ± 0.2 b	9.76	2.33 ± 0.1 d	43.45	0.66 ± 0.1 bc	51.82
4000	2.56 ± 0.1 c	24.26	2.09 ± 0.2 d	49.27	0.55 ± 0.1 c	59.85
LSD 0.05	0.24 **		0.33 **		0.18 **	

** The values with the same letters in each column are not significantly different from each other.

3.3. Phytochemical Screening of C. camphora Methanolic Extract

The results presented in Table 2 revealed that *C. camphora* methanolic extract comprises high levels of antioxidant compounds such as ascorbate and flavonoids. In addition to its antioxidant content, *C. camphora* is considered a rich source of saponin and tannins as secondary metabolites. Moreover, osmoprotectants, total amino acids, and glycine betaine were present in a noteworthy amount. Additionally, total antioxidant activities, estimated on the basis of PMA and DPPH, were also present to a considerable extent.

Table 2. Screening of some active components of methanolic *Cinnamomum camphora* extract.

Classification	Tested Parameters	Concentration (mg/g DM)
Antioxidants	Flavonoids	48.0 ± 2.3
	Phenols	22.7 ± 1.1
	Ascorbic acid	46.6 ± 1.9
Secondary metabolites	Saponin	12.0 ± 0.9
	Tannins	16.5 ± 0.6
Osmo-regulatory molecules	Proline	2.6 ± 0.4
	Glycine betaine (GB)	16.0 ± 1.2
	Total amino acids	37.4 ± 3.4
Antioxidant activity	Total antioxidant activity (PMA)	22.0 ± 2.1
	DPPH scavenging activity %	13.7 ± 1.6

3.4. HPLC Analysis

The HPLC fingerprints of detected polyphenolic (phenolic and flavonoid) compounds in *C. camphora* are presented in Table 3 and Figure 1. The identified phenolic compounds were syringic acid, *p*-coumaric acid, caffeic acid, ferulic acid, gallic acid, benzoic acid, ellagic acid, iso-ferulic acid, and catechol (Table 3). On the other hand, the detected flavonoid compounds were naringin, quercetin, hesperidin, catechin, 7-OH flavone, and apigenin (Table 3). Among the fifteen polyphenolic compounds, ferulic acid was the main compound detected at a retention time (RT) of 7 min with a concentration of 7.22 µg/mL. The gallic acid and catechin compounds showed relatively high concentrations of 6.98 and 6.21 µg/mL, respectively (Table 3). The other compounds, such as 7-OH flavone, apigenin, naringin, and quercetin, were detected with moderated concentrations of 4.36, 3.98, 3.78, and 2.46, respectively. The three compounds (ellagic acid, catechol, and benzoic acid) were identified at low concentrations (2.11, 1.65, and 1.55 µg/mL, respectively), while the two compounds (*p*-coumaric acid and iso-ferulic acid) showed the lowest concentrations (0.98 and 0.87 µg/mL, respectively).

Table 3. The level of phenolic and flavonoid compounds (concentration, µg/mL) present in the methanolic extract of *Cinnamomum camphora* was determined using high-performance liquid chromatography (HPLC).

Phenolic Compounds			Flavonoid Compounds		
Compound	*** RT**	**µg/mL**	**Compound**	**RT**	**µg/mL**
Syringic acid	2.8	1.08	Naringin	3.8	3.78
p-coumaric acid	3.5	0.98	Quercetin	5.0	2.46
Caffeic acid	4.8	1.11	Hesperidin	7.0	1.25
Ferulic acid	7.0	7.22	Catechin	7.8	6.21
Gallic acid	8.9	6.98	7-OH flavone	8.9	4.36
Benzoic acid	10.0	1.55	Apigenin	10.0	3.98
Ellagic acid	11.0	2.11			
Iso-Ferulic acid	12.0	0.87			
Catechol	13.0	1.65			

* RT = retention time (minutes).

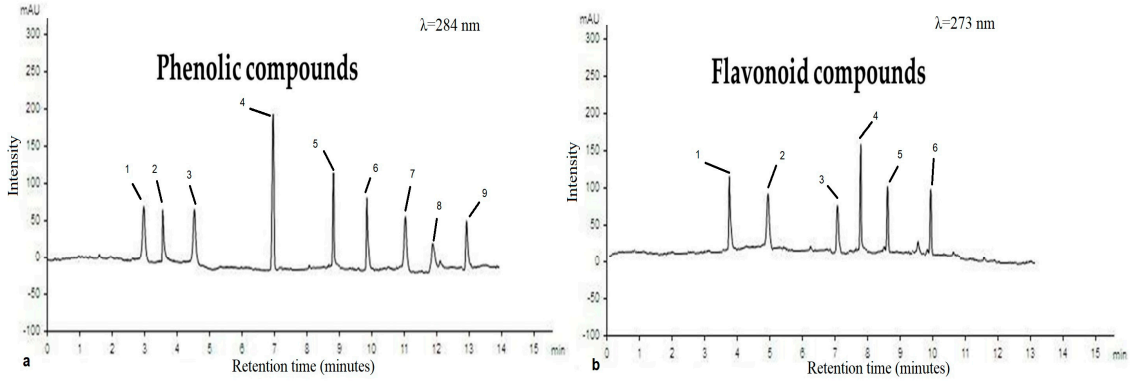


Figure 1. HPLC chromatograms of polyphenolic compounds identified in *Cinnamomum camphora* extract at (**a**) λ = 284 nm, the peaks represent the following phenolic compounds: 1 = syringic acid, 2 = *p*-coumaric acid, 3 = caffeic acid, 4 = ferulic acid, 5 = gallic acid, 6 = benzoic acid, 7 = ellagic acid, 8 = iso-ferulic acid, and 9 = catechol acid; (**b**) λ = 273 nm, the peaks represent the following flavonoid compounds: 1 = naringenin, 2 = quercetin, 3 = hesperidin, 4 = catechin, 5 = 7-OH flavone and 6 = apigenin.

3.5. GC-MS Analysis of the C. camphora Methanolic Extract

A GC-MS analysis was performed to identify the main secondary metabolites in the extract. Table 4 lists the 14 chemical components that have been found with their retention time (RT), molecular formula, and chemical structure. The most abundant compound detected at an RT of 23.267 min was 1,2-benzenedicarboxylic acid, mono(2-ethylhexyl) ester, with the molecular formula of $C_{16}H_{22}O_4$. The second highest compound was 2-Butenamide, 2-ethyl-3-methyl-N-phenyl, detected at a RT of 10.595 min with a molecular weight of $C_{13}H_{17}NO$. The third abundant compound was 1H-Cycloprop[e]azulen-7-ol, decahydro-1,1,7-trimethyl-4-methylene- have a molecular formula of $C_{15}H_{24}O$ and was observed at a RT of 12.971 min. On the other hand, 9,19-Cyclolanostan-3-ol, acetate, (3.beta.)-; Bicyclo(3.1.1)heptane-2,3-diol, 2,6,6-trimethyl, and 7-Methyl-Z-tetradecen-1-ol acetate were detected at RT of 14.115, 10.547, and 12.211 min, respectively, with a very similar moderate ratio (Table 4). The other compounds, such as 2-Cyclohexen-1-one,4-hydroxy-3-methyl-6-(1-methylethyl)-;7-Oxabicyclo[4.1.0]heptan-2-one,3-methyl-6-(1-methylethyl); trans-Z-alpha-Bisaboleneepoxide;2 Oxabicyclo[2.2.2]octan-6-ol, 1,3,3-trimethyl-; 5-Hexenal, 4-(acetyloxy)-4-methyl-; Ledol and Caryophyllene oxide, were detected at different RTs with low-level ratios (Table 4). The andrographolide compound, detected at a RT of 14.859 min with a molecular formula of $C_{20}H_{30}O_5$, showed the lowest ratio among the identified compounds (Table 4).

Table 4. Phytochemical components identified in the *Cinnamomum camphora* extract by GC-MS.

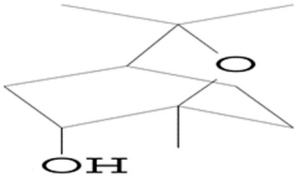
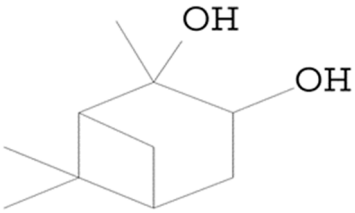
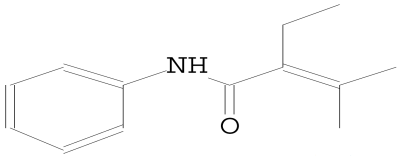
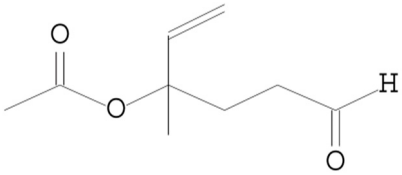
Retention Time	m/z	Area	Height	Name	Molecular Formula	Class	Chemical Structure
10.475	43.00	18,660	8351	2-Oxabicyclo[2.2.2]octan-6-ol, 1,3,3-trimethyl- (1,8-Cineole)	$C_{10}H_{18}O_2$	monoterpene	
10.547	43.00	40,620	22,112	Bicyclo(3.1.1)heptane-2,3-diol, 2,6,6-trimethyl- (Pinanediol)	$C_{10}H_{18}O_2$	terpene	
10.595	111.00	113,130	34,818	2-Butenamide, 2-ethyl-3-methyl-N-phenyl (Crotamiton)	$C_{13}H_{17}NO$	anilides	
11.253	43.00	16,397	7665	5-Hexenal, 4-(acetyloxy)-4-methyl-,	$C_9H_{14}O_3$	acetate ester	

Table 4. *Cont.*

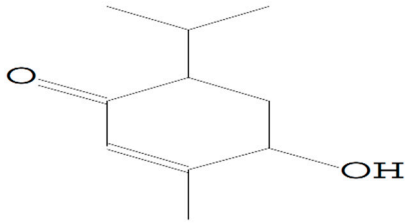
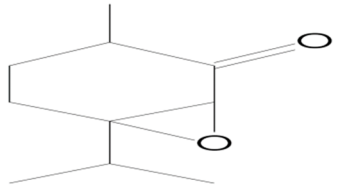
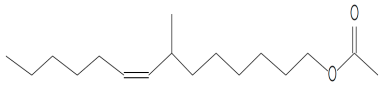
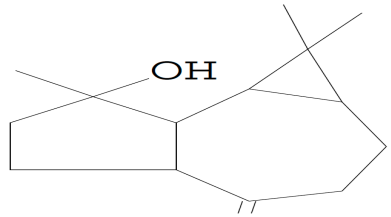
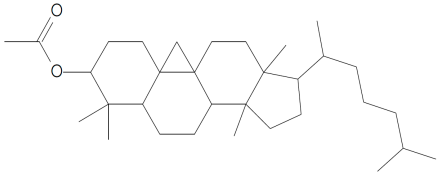
Retention Time	m/z	Area	Height	Name	Molecular Formula	Class	Chemical Structure
11.460	98.00	30,566	14,037	2-Cyclohexen-1-one, 4-hydroxy-3-methyl-6-(1-methylethyl)-, (Barosma camphor)	$C_{10}H_{16}O_2$	cyclic monoterpene ketone	
11.712	41.00	22,150	12,307	7-Oxabicyclo[4.1.0]heptan-2-one, 3-methyl-6-(1-methylethyl)	$C_{10}H_{16}O_2$	ketone	
12.211	43.00	40,594	22,972	7-Methyl-Z-tetradecen-1-ol acetate	$C_{17}H_{32}O_2$	fatty acid esters	
12.971	43.00	87,247	55,041	1H-Cycloprop[e]azulen-7-ol, decahydro-1,1,7-trimethyl-4-methylene- (Spathulenol)	$C_{15}H_{24}O$	tricyclic sesquiterpene	
14.115	43.00	45,451	32,086	9,19-Cyclolanostan-3-ol, acetate, (3.beta)-	$C_{32}H_{54}O_2$	triterpene	

Table 4. *Cont.*

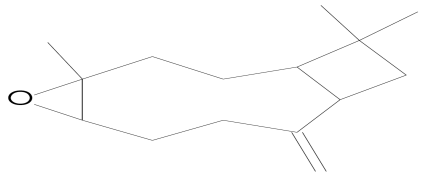
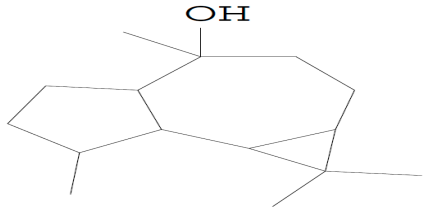
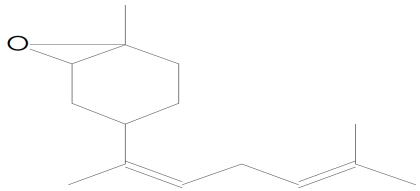
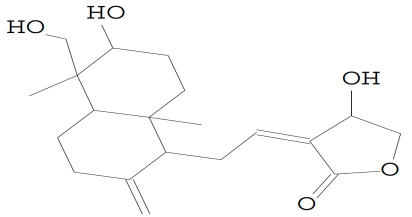
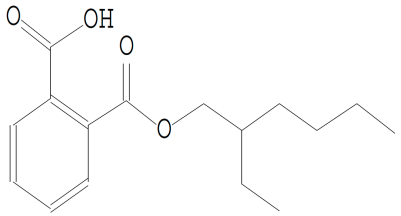
Retention Time	m/z	Area	Height	Name	Molecular Formula	Class	Chemical Structure
14.150	43.00	14,824	9135	Caryophyllene oxide	$C_{15}H_{24}O$	cyclic sesquiterpene	
14.258	43.00	15,666	10,273	Ledol	$C_{15}H_{26}O$	sesquiterpene	
14.317	43.00	20,595	12,396	trans-Z-alpha.-Bisabolene epoxide	$C_{32}H_{54}O_2$	sesquiterpene	
14.859	41.00	9363	7179	Andrographolide	$C_{20}H_{30}O_5$	diterpenoids	

Table 4. *Cont.*

Retention Time	m/z	Area	Height	Name	Molecular Formula	Class	Chemical Structure
23.267	149.00	437,984	83,389	1,2-Benzenedicarboxylic acid, mono(2-ethylhexyl) ester	$C_{16}H_{22}O_4$	phthalate esters	

4. Discussion

Cinnamomum camphora (L.) is an evergreen species widely distributed and cultivated in many tropical and subtropical areas, including Southeast and East Asia. It plays a crucial role in protecting the environment and has a volatile essential oil, camphor, which has been reported to have antibacterial properties [37,38]. The extracts from *C. camphora* have been traditionally used for herbal medicine and furniture in China. They are more commonly used as essential oils, obtained from the trunk, leaves, and twigs by steam distillation or various solvents [39]. The *C. camphora* extracts have been shown to kill pathogenic bacteria and can be used as a flavoring or preservative for food. It can also stop bacteria from growing and kill insects [40,41]. *C. camphora* extracts contain several active ingredients, including camphor, α-terpineol, linalool, eucalyptol, and safrole [42]. Additionally, the *C. camphora* tree's essential oils derived from its different parts have been reported by several authors to be effective against pathogenic fungi, bacteria, algae, and insects [17,43,44]. Our work concluded that the methanolic extract of *C. camphora* showed potential biofungicide against the growth of *F. solani*, *A. alternata*, and *F. oxysporum*, which could be considered a prospective antifungal extract with a good number of antioxidants and secondary metabolites. It is believed that several types of compounds, including terpenoids, alkaloids, flavones, glycosides, saponins, quinines, coumarins, stilbenes, esters, phenols, aldehydes, alcohols, steroids, and organic acids, contribute to antimicrobial activity.

HPLC analysis results revealed the presence of antimicrobial compounds—naringenin, catechin, 7-OH flavone, apigenin, ferulic, benzoic acid, gallic acid, and catechol. Several researchers noticed that *C. camphora* extracts contained several phenolics that exhibited antimicrobial, antifungal, anticandidal, and antioxidant activities, such as gallic acid, naringenin, and its derivatives, and ferulic acid, offering protection against oxidative stress [14,45–49]. Research has shown that phenolic compounds play a role in the durability of natural wood, and resin acids inhibit fungi growth [50]. In the same regard, the results obtained in this work are in accord with those of Al-Huqail et al. [14], who found several active compounds in acacia extract, such as benzoic acid, o-coumaric acid, naringenin, quercetin, and kaempferol that showed significant inhibition of *P. chrysogenum* mycelial growth. Additionally, our results align with those in previous work by Yakefu et al. [51], who affirmed the potential values for *C. camphora* developing as an algaecide. Additionally, Tomazoni et al. [52] discovered that treatment with the extract of *Eucalyptus staigeriana*, *E. globulus*, and *C. camphora* resulted in inhibition of *Alternaria* mycelial growth and spore germination in vitro and a decrease in early blight symptoms in plants in vivo. Furthermore, our study aligns with that of Manilal and Idhayadhulla [53], who found natural plant extracts like *Prosopis juliflora*, *Ricinus communis*, and *Carica papaya*, respectively, and *Polyalthia longifolia* is effective in controlling *Alternaria solani* [54].

The potent antimicrobial activity of *C. camphora* extract is believed to be related to its secondary metabolites and phytochemicals, which can impact the membrane integrity, hyphae initiation, and cell cycle of microbes causing structural and functional alterations, swelling, and increased permeability [55,56].

GC-MS analysis of the methanolic leaf extract of *C. camphora* identified various compounds. The most abundant compound found in the extract was 1,2-benzenedicarboxylic acid, mono (2-ethylhexyl) ester, belonging to the class of phthalate esters, has been previously reported for its biological properties, including cytotoxicity, antioxidant, anti-inflammatory, antimicrobial, and antiviral activities [57–59]. The same compound was noticed to be the major component (58.05%) in a study conducted by Ali et al. [60], who noticed that it may potentially contribute to the antifungal activity of the methanolic root extract of *Chenopodium album*. On the other hand, different compounds detected in our GC-MS analysis had potential activities, such as the detected compound 1H-Cycloprop[e]azulen-7-ol, decahydro-1,1,7-trimethyl-4-methylene-, which is called spathulenol and is used as a scent in foods. According to Sousa et al. [61], the primary component of *Eugenia calycina* essential leaf oil, spathulenol (21.36%), has demonstrated antimicrobial activity against the anaerobic bacteria *Prevotella nigrescens* and *Porphyromonas gingivalis*, with a minimum

inhibitory concentration (MIC) of 100 µg/mL. Similarly, Tan et al. [62] reported that spathulenol (23.8%) and caryophyllene (14.9%), the primary components of *Salvia cilicica* essential oil, exhibited antimicrobial activity against *Mycobacterium tuberculosis*, *Microsporum gypseum*, *Trichophyton mentagrophytes*, and *Candida* spp.

In our study, the detected compound, 7-methyl-Z-tetradecen-1-ol acetate, has previously been reported in GC-MS analysis of *Mentha viridis* and *Urospermum picroides* methanolic leaf extracts, and proved to possess anticancer, anti-inflammatory, and hepatoprotective activities [63,64]. According to Arora and Kumar [65], a compound known as 9.19-cyclolanostan-3-ol,24-methylene-(3.beta) can be used as an anti-HIV agent for the prevention of HIV. Additionally, the GC-MS results confirmed the existence of the compound 2-Oxabicyclo[2.2.2]octan-6-ol, 1,3,3-trimethyl-, also known as eucalyptol or 1,8-cineole. Many researchers have shown that 1,8-cineole has strong antimicrobial properties against various pathogens and spoilage organisms, as 1,8-cineole exhibited high inhibitory activity against *C. albicans* and *Proetus vulgaris*, with MIC values of 31.3 and 62.5 µg/mL, respectively [66–68]. The synergistic effect of 1,8-cineole combined with other molecules in essential oils, such as limonene, α-pinene, *P*-cymene, and terpineol-4-ol, could reveal strong antimicrobial activities [69,70].

Beta-caryophyllene (BCP) is a natural plant compound that belongs to the bicyclic sesquiterpene family. In its natural state, BCP is primarily found as a trans-caryophyllene ((E)-BCP), along with small quantities of its isomers such as iso-caryophyllene (Z)-β-caryophyllene, α-humulene (α-caryophyllene), and its oxidation derivative known as β-caryophyllene oxide (BCPO). Our results showed that the extract possessed BCPO, which has been studied in recent years because it has potent biological activities. BCPO, whether used in its pure form or as a component of plant essential oils, has been shown to have anti-inflammatory, antioxidant, antiviral, anticarcinogenic, and analgesic properties [71–74]. While various plant species' antibacterial and antifungal properties have been widely studied, the specific mechanisms behind these properties have not been thoroughly explored. This study highlights the need for more trials to support the in vitro research studies of the camphor extract. Further research is required to determine the extract's chemical nature and mechanisms of action, which will provide accurate and reproducible discoveries that could be used as eco-friendly alternative molecules to treat plant diseases caused by microbial pathogens.

5. Conclusions

Cinnamomum camphora methanolic extract was investigated for antifungal efficacy against three common, isolated, extensive phytopathogens: *Alternaria alternata*, *Fusarium solani*, and *Fusarium oxysporum*. *C. camphora* methanolic extract suppresses *F. oxysporum*, *A. alternata*, and *F. solani* fungal mycelia weight by 60, 49, and 24% at 4000 µg/mL. *C. camphora* extract may be antifungal due to its bioactive metabolites. HPLC examination indicated several phenolic and flavonoid components in the extract, including catechin and gallic acid, which had the highest amounts of 6.21 and 6.98 µg/mL, respectively. Glycine betaine, total amino acids, and osmoprotectants were plentiful. PMA and DPPH showed considerable total antioxidant activity. Mono(2-ethylhexyl) ester of 1,2-benzene dicarboxylic acid was the most abundant chemical in the extract's GC-MS study. The tested extract may be a safe alternative to chemical fungicides because it inhibits mycelial growth weight in vitro.

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