

Investigating the In Vitro Antifungal Activity and Phytochemical Composition of *Commiphora gileadensis* Extracts

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ABSTRACT

Pathogenic fungi employ diverse mechanisms that cause infection and promote disease progression, which can become life-threatening, especially in immunocompromised patients, highlighting the need to identify affordable natural antifungal agents. *Commiphora gileadensis* is a medicinal plant associated with diverse biological activities; however, its antifungal potential remains underexplored. This study evaluated the *in vitro* antifungal effects of *C. gileadensis* extracts prepared from different plant parts (leaves, bark, and branches) using different solvents (methanol, ethanol, and water) against four fungal strains using the agar-well diffusion method. Additionally, the phytochemical composition of these extracts was characterized using gas chromatography–mass spectrometry (GC-MS). Results showed that extraction yields ranged from 10.35% to 28% for bark extracts and from 5.9% to 6.6% for branch extracts, while the leaf extract yield was 5.1%. All extracts exhibited antifungal activity to varying degrees, with the ethanolic branch extract producing the largest inhibition zone (33.47 mm), whereas the aqueous bark extract produced the smallest zone (11.62 mm). GC-MS analysis detected variations in the number and composition of compounds among the extracts, with the greatest number (27) detected in the methanolic branch extract and the fewest (2) in the aqueous bark extract. Thunbergol was the most frequently identified constituent across extracts, while other major constituents included ursolic aldehyde, spathulenol, hexadecanoic acid-ethyl ester, and geranyl- α -terpinene, all of which may contribute to the observed antifungal activity. These findings provide further insight into the composition and antifungal potential of *C. gileadensis* and support its continued investigation as a potential source of antifungal agents.

Keywords: *Commiphora gileadensis*, antifungal activity, gas chromatography–mass spectrometry, medicinal plant extraction, phytochemical composition, *in vitro* testing

1. INTRODUCTION

Fungi are the most prevalent microorganisms, characterized by their extreme resilience to harsh conditions and rapid replication. Fungal pathogenicity refers to the ability of a certain fungal species to cause disease in a host organism. Although most fungi are nonpathogenic and play essential ecological roles, a limited number of species can infect humans, animals, or plants, particularly under favorable conditions¹. Opportunistic fungi, such as *Candida spp.*, *Aspergillus spp.*, and *Cryptococcus spp.*, primarily infect immunocompromised individuals, whereas primary pathogens can infect healthy hosts¹. Pathogenic fungi use several virulence factors to facilitate infection and disease progression. These include the production of tissue-degrading enzymes, such as proteases, lipases, and keratinases, the ability to adhere to host cells, and mechanisms for evading or suppressing the host immune response. Some fungi also produce toxins (mycotoxins) that contribute to tissue damage².

Fungal infections can be classified into superficial, cutaneous, subcutaneous, and systemic (invasive) mycoses, depending on the depth of tissue involvement³. Many antifungal medications

have been used for decades because of the dangerous nature and possible lethality of such infections. However, fungi are continuously evolving and developing resistance to most fungicidal drugs, thereby posing a greater public health risk. Therefore, investigating new antifungal compounds that can overcome resistance while exhibiting low toxicity rates and are most likely to be found in medicinal plant extracts is essential.

Researchers have reported promising antifungal properties for extracts from various plants, including citrus peel⁴, different Pakistani plants⁵, brown seaweed⁶, and *Rosa damascena* flower⁷.

One medicinal plant that has attracted attention for its biological activities is *Commiphora gileadensis* (*C. gileadensis*), which has been used for centuries to treat various common health ailments. Commonly known as Bisham or the Balm of Makkah, it grows naturally in the southwestern region of Saudi Arabia and in Yemen. *C. gileadensis* is rich in polyphenols, essential oils, and various bioactive phytochemicals^{8,9}, and its extracts have been linked to antioxidant, antidiabetic¹⁰, antibiofilm¹¹, and antiproliferative¹² activities.

To our knowledge, only a limited number of studies have examined the antifungal properties of *C. gileadensis*, and in most

cases, antifungal activity was not the primary focus of these investigations. Al-Qarni et al.¹³ evaluated the influence of location on the phytochemical composition and anticandidal activity of *C. gileadensis*, reporting significant antifungal activity that varied depending on location, solvent, and freshness. Abdel-Kader et al.¹⁴ also studied the molecular composition and antimicrobial activity of *C. gileadensis* extracts against multiple bacteria and *Candida albicans* and found that the CHCl₃ extract exhibited a promising minimum inhibitory concentration against *C. albicans*. Additionally, Al-Zoubi¹⁵ studied extracts from callus cultures of *C. gileadensis* as an alternative means of producing secondary metabolites, given its rarity. The study demonstrated that both methanolic and ethanolic extracts from callus cultures and ex vitro-grown plants exhibited considerable antifungal activity against all tested fungi, including two *Aspergillus* species. Al-Mahbashi et al.¹⁶ concluded that *C. gileadensis* methanolic extract is nontoxic and safe for human consumption and reported notable antifungal activity of the extract against *Candida* spp. that were resistant to two positive controls. Finally, Al-Sum et al.¹⁷ found that, against two tested fungi (*Aspergillus niger* and *Penicillium italicum*), the aqueous extracts of *C. gileadensis* showed dose-dependent inhibition of radial and mycelial growth, with greater significance for the latter.

Although these studies collectively indicate antifungal potential of *C. gileadensis*, they are significantly different in extraction methods, plant origins, and experimental procedures. Consequently, the antifungal activity of *C. gileadensis* remains poorly defined, and the specific phytochemical profile responsible for these effects is not well understood, highlighting the need for more research in this area.

Therefore, this study aimed to extract bioactive compounds from *C. gileadensis* and evaluate the influence of different parts

and solvents on the yield and activity. The antifungal activity of each extract was investigated using the agar-well diffusion method, and the phytochemical composition was determined using gas chromatography-mass spectrometry (GC-MS).

The novelty of this study, summarized in Table 1, lies in the systematic evaluation of the antifungal properties of *C. gileadensis* bark, branches, and leaves collected from a single source and extracted using methanol, ethanol, and water under standardized experimental conditions, together with the correlation of antifungal activity with phytochemical composition through GC-MS profiling. This enables identifying compounds potentially associated with the observed effects and providing a more comprehensive assessment of the antifungal potential of this medicinal plant.

The following sections describe the experimental procedures, including the preparation and extraction of *C. gileadensis* extracts, and the evaluation of their antifungal activity using the agar-well diffusion method. The resulting extraction yields, antifungal effects, and phytochemical profiles obtained through GC-MS analysis are then presented and discussed in relation to previous findings, followed by the main conclusions and recommendations for future research.

2. MATERIALS AND METHODS

2.1. Plant Collection. *C. gileadensis* branches, leaves, and bark were purchased from a local collector in August 2025 in a mountainous desert north of Al-Ji'ranah (21°35'32.6" N 39°56'10.3" E), Makkah region, Saudi Arabia (Figure 1). The plant had a very distinct, strong aromatic fragrance. As shown in Figure 2, the dried leaves consisted of three small, hunter-green

Table 1. Literature summary and proposed novelty

Study	Plant part(s)	Solvent(s)	Characterization	Main objective
Al-Qarni et al. ¹³	Fresh and dried stems from different locations	Multiple solvents	GC-MS	Composition and anticandidal activity variation with location, solvent, and freshness
Abdel-Kader et al. ¹⁴	Fresh stems	Chloroform (CHCl ₃) fraction	Chromatographic isolation, Nuclear Magnetic Resonance (NMR), High-Resolution Electrospray Ionization Mass Spectrometry	Isolation and identification of antimicrobial compounds
Al-Zoubi ¹⁵	Ex vitro plants and callus cultures	Methanol and ethanol	Not reported	Antimicrobial activity comparison between ex vitro plants and tissue-culture-derived callus
Al-Mahbashi et al. ¹⁶	Bark	Methanol	Preliminary phytochemical screening	Acute toxicity and antimicrobial potential assessment
Al-Sum et al. ¹⁷	Leaves	Water	Not reported	Antimicrobial activity investigation
Present study	Bark, branches, and leaves	Methanol, ethanol, and water	GC-MS	Evaluation of the influence of plant part and extraction solvent variations on chemical composition and antifungal activity



Figure 1. Map showing the geographic coordinates of the plant collection site

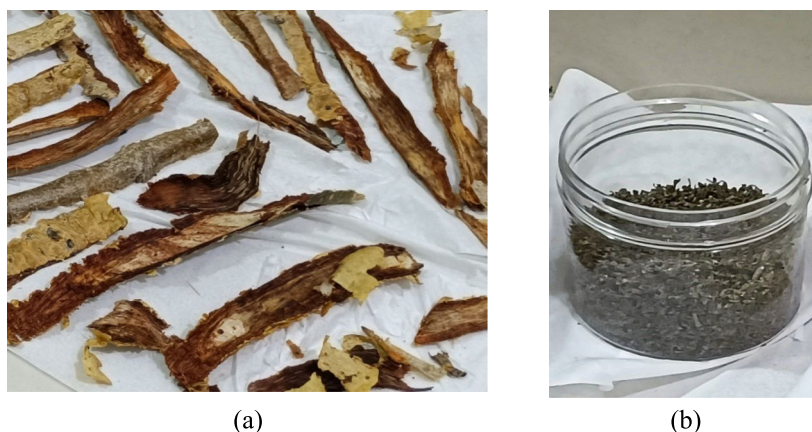


Figure 2. Dried *C. gileadensis* samples: (a) bark and (b) leaves

leaflets, whereas the bark appeared as reddish-brown woody structures with a concave inner surface.

2.2. Extraction. Following the procedure of Al-Hanbali et al.¹⁸, plant leaves were separated from the branches, and all plant parts were thoroughly washed with deionized water to remove dirt before being air-dried at ambient temperature for a week. Subsequently, they were further dried in a hot air oven at a low temperature (50–60 °C) for 2 h. The dried plant parts were separately ground into a fine powder using a herbal medicine grinder, sifted to remove large chunks, and weighed. Subsequently, the extraction was performed. Because of the small size and limited amount of plant leaves, only the ethanolic extract was possible for this part, whereas the bark and branches underwent extraction using ethanolic, methanolic, and aqueous solvents. Given the amount of plant collected and the final concentrations needed for biological assays, a ratio of 1:5 solute to solvent was chosen for extraction by soaking 20 g of each part in 100 mL of 95% ethanol, and additional similar amounts of branches and bark were soaked in pure methanol and in sterilized deionized water, yielding seven extracts. All of the extracts were then put in a shaker at 27 °C and 100 rpm for 7 days. Subsequently, the mixtures were run through qualitative filter paper to discard any plant debris and

then centrifuged at 5000 rpm for 10 min. Finally, a rotary evaporator (Figure 3) was used to concentrate and dry the extracts, which were then weighed, dissolved in 10 mL of dimethyl sulfoxide (DMSO) to form stock solutions, and frozen at -20 °C until further testing. The percentage yield of each extract was calculated using Eq. 1.

$$\text{Extraction yield (\%)} = \frac{\text{mass of dried extract (g)}}{\text{mass of raw dried plant (g)}} \times 100 \quad (1)$$

2.3. Fungal Strain Preparation.

2.3.1. Media preparation. Sabouraud dextrose agar from Schalau (Art. No. 01-089) (SDA) was used as the medium in this study. Following the manufacturer's directions, 43 g of powder was dissolved in 1 L of distilled water, and the pH was adjusted to 5.5. The solution was then autoclaved at 121 °C, poured into sterilized Petri plates, and refrigerated for further use.

2.3.2. Fungi collection, growth, and isolation. To prepare the fungal strains, 5 g of soil was collected from beneath a tree where accumulated fallen leaves provided optimal moisture and shadow conditions for fungal proliferation. The soil was



Figure 3. Sample drying in the rotary evaporator

then added to sterile deionized water to a final volume of 30 mL, vortexed, and allowed to rest for 15 min. Finally, 500 μ L of this solution was spread onto an SDA plate, incubated at 37 °C, and sub-cultured twice weekly.

The procedure for identifying and confirming the isolated fungal strains consisted of morphological identification in addition to biochemical and physiological characterization. The morphological identification was performed using standard mycological keys based on macroscopic (colony morphology, color, texture, and growth rate) and microscopic (hyphal structure, spore type, and arrangement) characteristics, whereas additional confirmatory tests, including growth on selective media and physiological assays, were incorporated to support colonial identification where applicable.

Following the aforementioned tests, four distinct fungal colonies were isolated from subcultured plates and suspended in dextrose broth, prepared by mixing 40 g dextrose with 10 g peptone in 1 L of autoclaved deionized water and adjusting the pH to 5.6. The solutions were then incubated in the shaker at 37 °C for approximately 2 h until their growth reached 0.1–0.2 O.D., measured at 600 nm wavelength using a spectrophotometer in accordance with the manufacturer's instructions

2.4. Biological Activity.

2.4.1. Agar diffusion method. To test the antifungal potential of the extracts, with insight from Ogunshe et al.¹⁹, 100 μ L of each of the four isolated fungal inocula was spread evenly on labeled SDA plates and left to dry. Subsequently, 9-mm wells were introduced, and 50 μ L of each extract was added to each well. Plain DMSO was used as a negative control, and a mixture of the methanolic extract of branches and the ethanolic extract of leaves was added to each well to test the synergistic effects. All plates were then placed in an incubator for 3 days, photographed, and inhibition diameters were measured using software²⁰. All experiments were performed once; therefore, no statistical analyses were conducted, except for the mean calculation in Microsoft Excel.

2.4.2. Phytochemical composition. The phytochemical composition of the extracts was analyzed using a Thermo Scientific TRACE 1300 gas chromatograph coupled to a Thermo Scientific ISQ 7000 single-quadrupole mass spectrometer and equipped with an AI 1310 liquid autosampler (Thermo Fisher Scientific), with helium used as the carrier gas, in line with standard laboratory procedures. Sample preparation consisted of adding 1.5 mL of DMSO to the injection vials along with three drops of each extract, after which the prepared samples were subjected to GC-MS analysis. An injection volume of 1.0 μ L was used for each sample, with each analytical run requiring approximately 75 min. Additionally, instrument control and data processing were performed using Chromeleon software (version 7.2.10, Thermo Fisher Scientific). Analyses were performed in scan mode, and data were acquired as total ion chromatograms using qualitative processing, while compound assignments were obtained by comparison of the acquired mass spectra with a mass-spectral reference library and were further evaluated using retention time and mass-spectral ion information.

3. RESULTS AND DISCUSSION

3.1. Extraction Yield. The plant parts were extracted using different solvents, as discussed in Section 2.2. The percentage yields are illustrated in Figure 4. They ranged from 5.9% to 6.6% for the branch extracts, from 10.35% to 28% for the bark extracts, and were 5.1% for the leaf extract. They were noticeably lower than those reported by Shadid et al.⁸, perhaps due to differences in the extraction method. However, these results are similar to those obtained by Ahmed et al.²¹. Additionally, water appeared to be the solvent with the highest yield, contrary to the presumed phenolic and fatty acid characteristics of *C. gileaden-sis* extracts⁹. The high yield observed in the aqueous extract may be attributed to the abundance of water-soluble compounds in plants, including carbohydrates, secondary metabolites, and non-bioactive substances such as salts and cellular debris, which contribute significantly to the total extract weight, thereby increasing the yield.

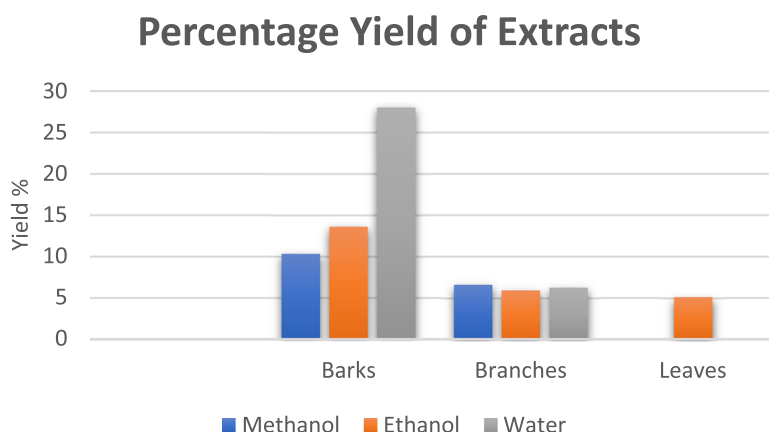


Figure 4. Yield percentages for each tested sample

3.2. Antifungal Activity. Antifungal activity was tested against four visibly distinct fungal strains, named F1–F4, using the agar-well diffusion method. The plates after 3 days of incubation are shown in Figure 5, with the inhibition zones marked by blue bars. Despite the absence of a positive control in this study owing to availability limitations, the validity of the results could be demonstrated by comparison with other studies. The inhibition zones ranged from 11.62 to 33.47 mm for the aqueous bark extract and the ethanolic branch extract, respectively, which are within the overall range of inhibition zones reported by Al-Qarni et al.¹³ and Al-Zoubi¹⁵. On average, the leaves exhibited the highest activity, followed by the branches, whereas the bark exhibited the least inhibition. Solvent-wise, aqueous extracts performed poorly in terms of antifungal activity compared to that of other solvents, with methanolic extracts showing the best performance. This confirmed the claim in Section 3.1 that, despite the high yield of the aqueous extracts, the solutions exhibited little bioactivity. Synergistic effects were also studied by combining the methanolic branch extract with the ethanolic leaf extract, where the average effect was not significantly better than that of either extract alone.

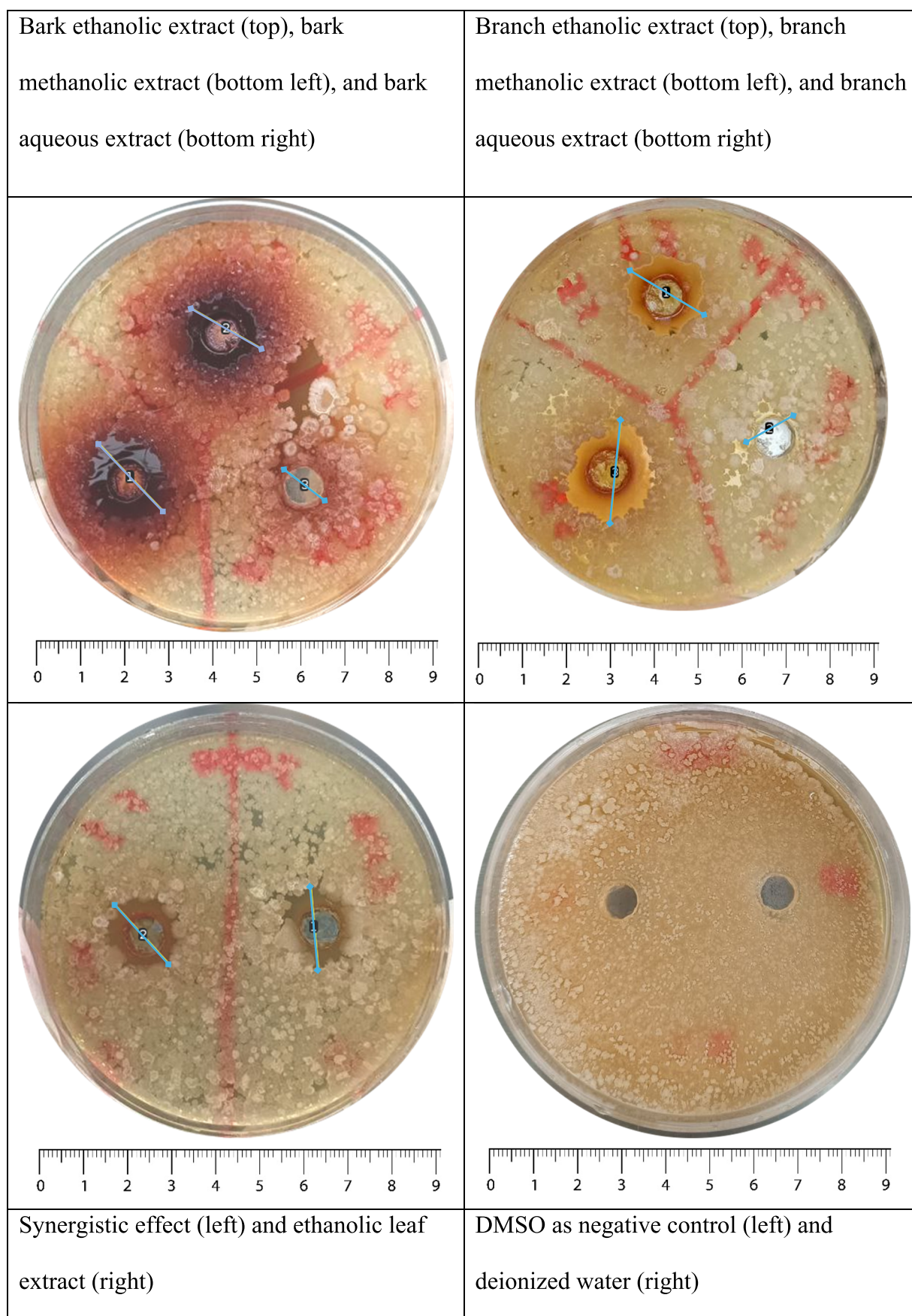
The highest activity observed in methanolic extracts can be attributed to the ability of methanol to extract a broad range of moderately polar and some less-polar secondary metabolites, which enables efficient extraction of bioactive phytochemicals. Methanol and ethanol are particularly effective in solubilizing phenolic compounds, flavonoids, and terpenoids, many of which are well documented for their antifungal properties²². Phenolic compounds and flavonoids disrupt fungal cell walls and membranes, interfere with enzyme activity, and induce oxidative stress in fungal cells²³. Terpenoids can alter membrane permeability and inhibit fungal growth²⁴. The higher concentrations and diversity of these compounds in the ethanolic and methanolic extracts likely contributed to their stronger antifungal effects. The effects of each plant part and solvent on each fungal sample are summarized in Table 2 and Figure 6.

3.3. GC-MS Analysis. To identify the fungicidal compound responsible for the previously mentioned inhibition, GC-MS analysis was performed on each sample. After excluding analytical contaminants, significant variation was observed in the number of phytochemical compounds identified among the samples. The highest number of compounds (27) was identified in

the methanolic branch extract, whereas the aqueous bark extract contained the least number (2), consistent with the observed activity. The limited number of identified bioactive compounds in aqueous extracts may be due to the fact that water essentially extracts highly polar compounds, such as sugars, salts, and other hydrophilic constituents, many of which are nonvolatile and not suitable for GC-MS detection. Figure 7 provides a qualitative analysis of the phytochemical richness of each extract.

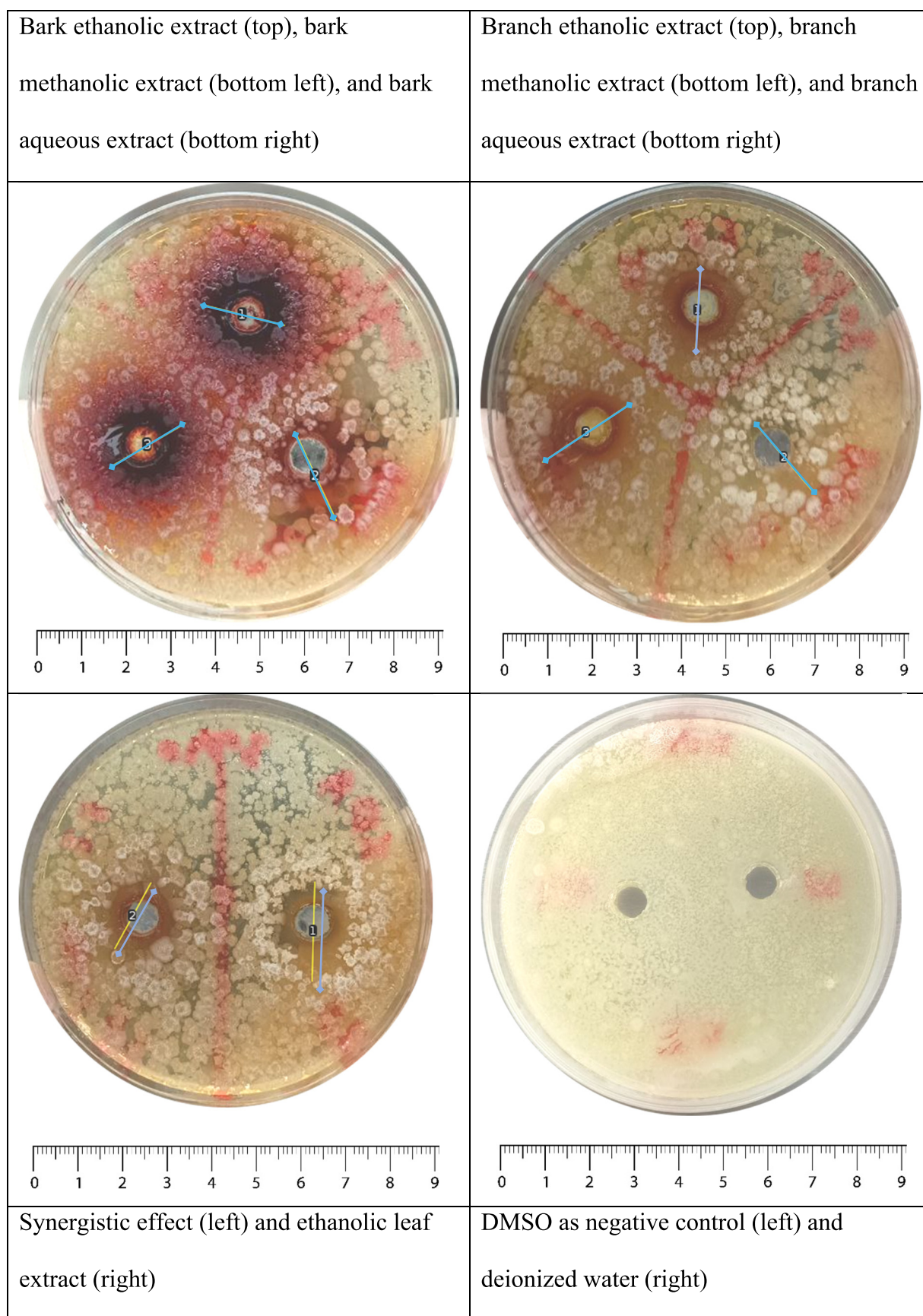
Table 3 lists all the identified contents, together with retention times and relative area percentages. Most compounds were common across multiple extracts. This list differs from that of Shadid et al.⁸, likely due to differences in extraction methods (use of essential oil extracts, which may contain more bioactive compounds) and plant origins.

Of the compounds listed in Table 3, the highlighted compounds have been previously linked to antifungal activity. For instance, globulol was separated by bioassay-guided isolation from the petroleum ether fraction of the extract of *Eucalyptus globulus* Labill. fruits, and it demonstrated antifungal activity against a range of microorganisms, including *Alternaria solani*, *Fusarium oxysporum* f. sp. *niveum*, and *F. graminearum*²⁵. Furthermore, benzoic acid 4-hydroxy-3,5-dimethoxy, better known as syringic acid²⁷, was detected and identified by Liquid Chromatography-Tandem Mass Spectrometry and NMR in the ethyl acetate extract fractions of *Asteriscus graveolens*, which displayed a powerful antifungal effect against *F. oxysporum* f. sp. *albedinis*²⁶. Additionally, T-cadinol isolated from *Taiwania cryptomerioides* with HPLC exhibited significant antifungal activity against *Coriolus versicolor* and *Laetiporus sulphureus*²⁸. Moreover, spathulenol was identified as a major compound in the essential oils of *Annonaceae* species and showed fungicidal activity against major *Candida* species²⁹. Thunbergol was also identified as a major constituent of the essential oil of *Pinus halepensis*, which displays antifungal activity against *Aspergillus flavus*, *Aspergillus niger*, *F. oxysporum*, and *Rhizopus stolonifer*³⁰. β -Terpinene (Cyclohexene, 4-methylene-1-(1-methylethyl)-³²) was also present as a major constituent in *Origanum majorana* essential oil, contributing to its significant antifungal activity against *F. oxysporum* f. sp. *melonis* and *F. solani*³¹. Furthermore, ursolic acid was found to be effective against *Alternaria alternata* by damaging the cell membrane and inhibiting efflux pumps, demonstrating its antifungal properties³³. Additionally, cedrene was identified among the constituents of *Juniperus* heartwood extracts, which



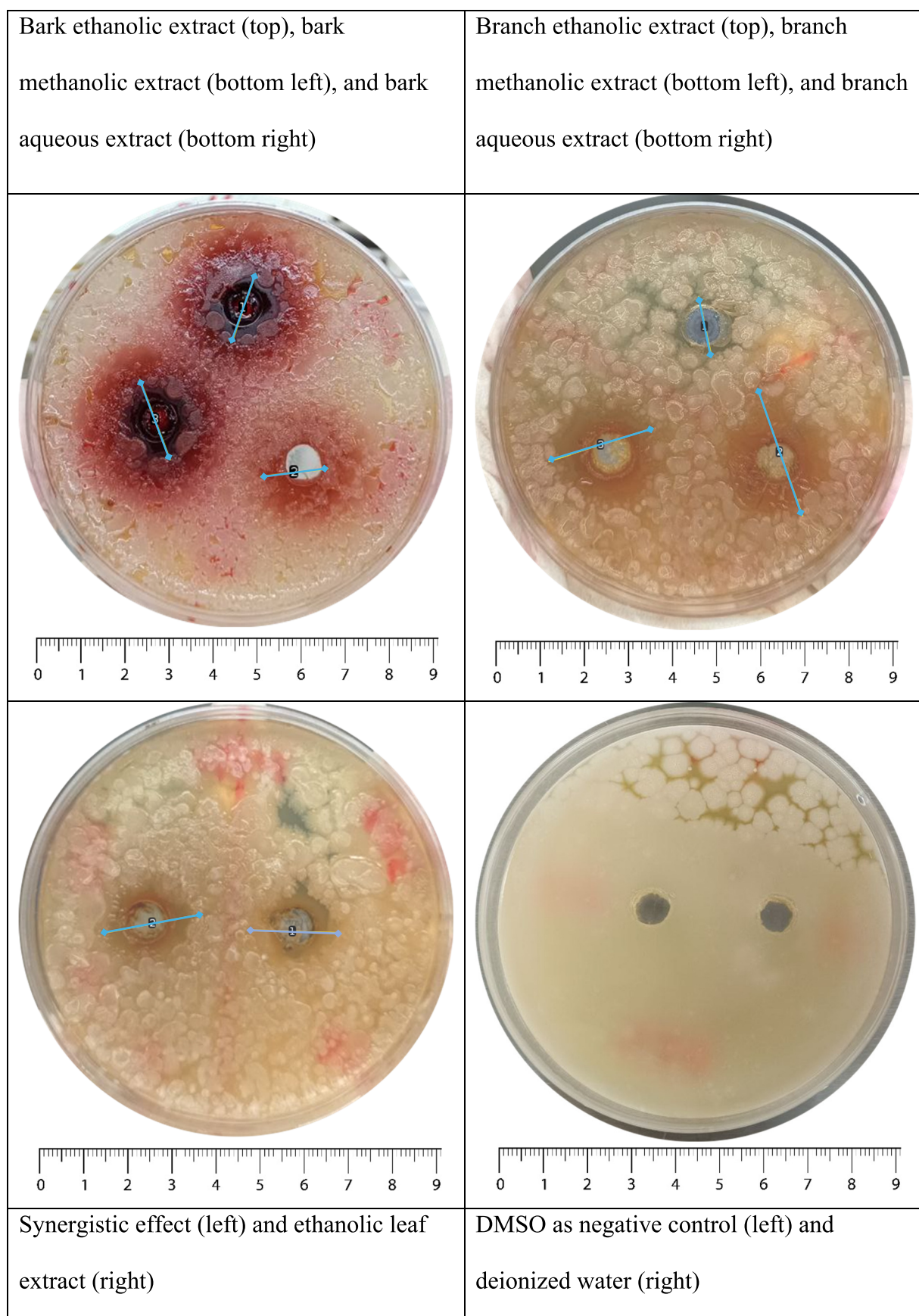
(a) Fungal strain F1

Figure 5. Agar well diffusion plates featuring fungal strains F1–F4 showing the effects of the plant extracts, the synergistic treatment, and the controls. Blue bars indicate the inhibition zones. The scale is in centimeters and corresponds to the plate diameter



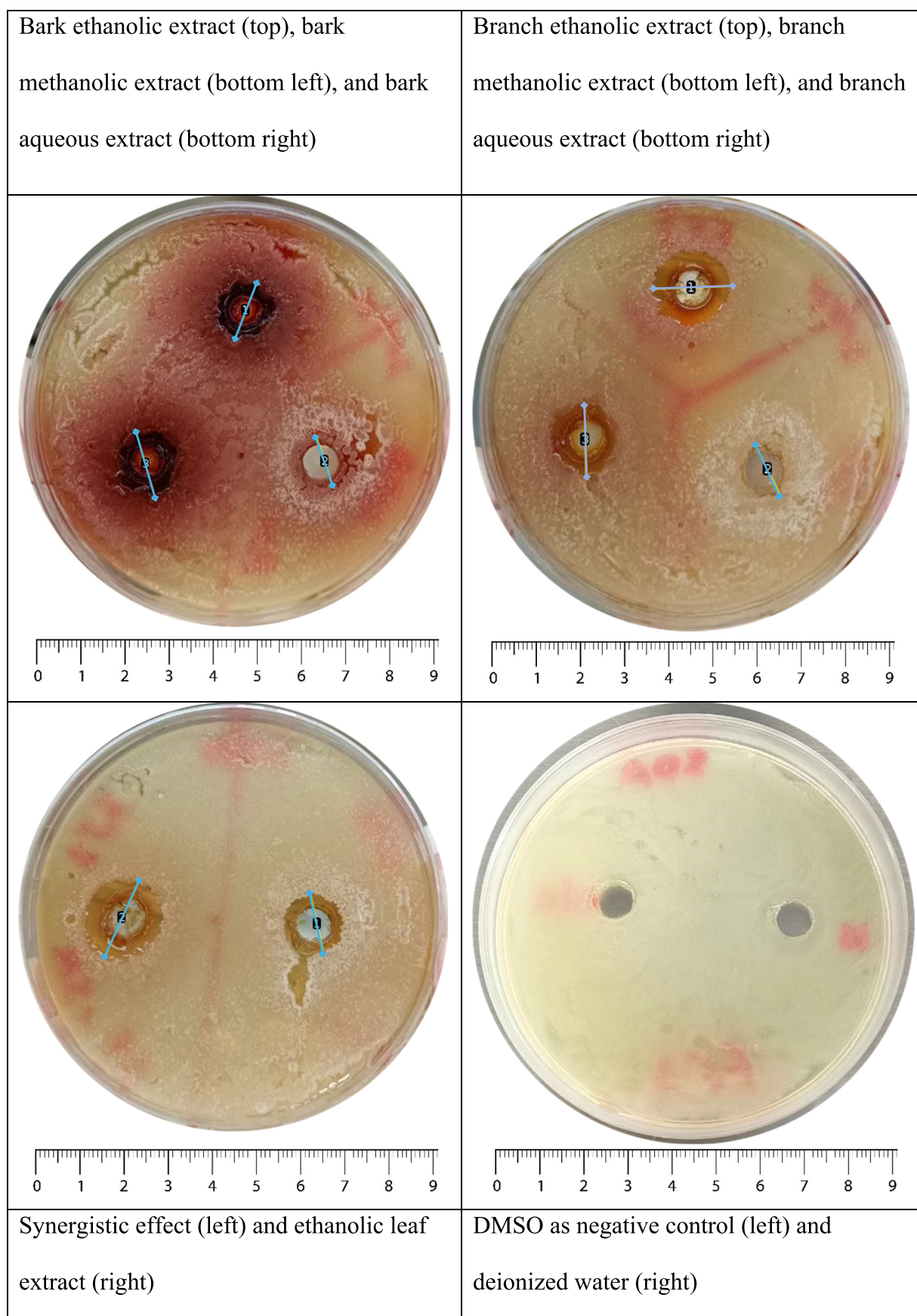
(b) *Fungal strain F2*

Figure 5. (Continued.)



(c) *Fungal strain F3*

Figure 5. (Continued.)



(d) Fungal strain F4

Figure 5. (Continued.)

Table 2. Inhibition zones in mm

Strain	Sample	Solvent		
		Methanol	Ethanol	Water
F1	Barks	22.05	17.57	11.62
	Branches	26.01	21.83	13.03
	Leaves		20.52	
	Synergistic		18.6	
F2	Barks	21.63	21.23	24.61
	Branches	24.62	20.55	21.69
	Leaves		28.28	
	Synergistic		21.22	
F3	Barks	20.61	17.49	16.49
	Branches	27.0	33.47	15.29
	Leaves		21.72	
	Synergistic		23.39	
F4	Barks	17.29	15.77	12.42
	Branches	16.73	18.36	12.93
	Leaves		15.22	
	Synergistic		20.41	

demonstrated significant antifungal activity against *Gloeophyllum trabeum*, *Postia placenta*, *Trametes versicolor*, and *Irpex lacteus*, suggesting a potential role for cedrene in the antifungal properties of these extracts³⁴. Moreover, germacrene D was identified as a major constituent of the essential oil of *Bursera delpechiana*, which exhibited antifungal activity against *C. albicans* and *Cryptococcus neoformans*, suggesting a potential role for germacrene D in the antifungal properties of this medicinal plant³⁵. a-Murolene was also isolated from the soft coral *Heteroxenia* sp., and it exhibited antifungal activity against *Cladosporium cucumerinum*, highlighting its potential contribution to the antifungal properties³⁶. Finally, δ -cadinene (1-Isopropyl-4,7-dimethyl-1,2,4a,5,8,8a-hexahydronaphthalene³⁸) was identified as a major

constituent of the essential oils of *Artemisia terrae-albae* and *Juniperus sabina*. These oils exhibited significant antifungal activity against *F. avenaceum*, *F. oxysporum*, *A. niger*, and *A. fumigatus*, suggesting a potential role for δ -cadinene in the antifungal properties of these essential oils³⁷.

Other identified compounds may also be associated with fungal growth inhibition, directly or as major components of essential oils that inhibit fungal growth. Additional compounds were associated with other biological properties, including uvaol's antileishmanial properties³⁹, suggesting their possible contribution to the inhibition observed in this study.

The GC-MS results showed that the most prevalent compound was thunbergol, followed by ursolic aldehyde,

Inhibition Zones of Fungal Growth

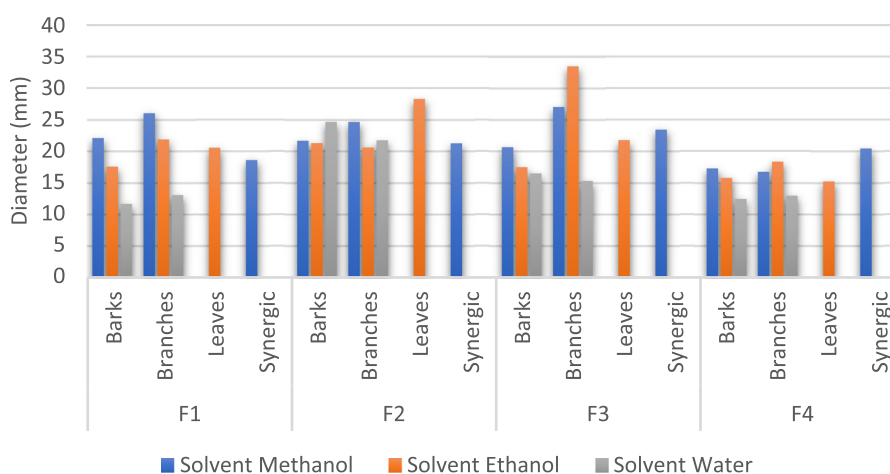


Figure 6. Inhibition zones of plant parts and solvents per fungal strain

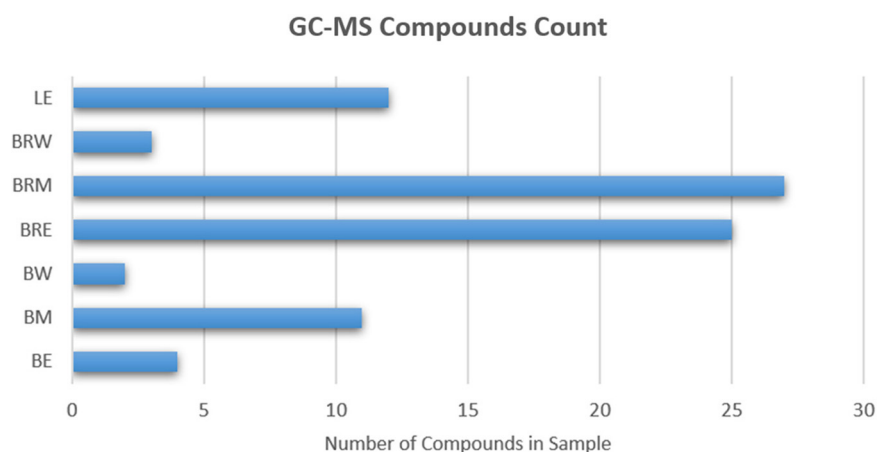


Figure 7. Qualitative summary of the phytochemical composition of *C. gileadensis* extracts via GC-MS

Table 3. GC-MS analysis of *C. gileadensis* extracts.*

Peak Name	Retention Time (min)	Area (counts*min)	Relative Area (%)
BE			
Ethane, hexachloride-	12.1	135391	0.21
S-Methyl methanethiosulphonate	13.07	54279	0.08
geranyl-a-terpinene	35.84	36694	0.06
Thunbergol	39.82	42967	0.07
BM			
Ethane, hexachloro-	12.13	184659	0.32
S-Methyl methanethiosulphonate	13	80246	0.14
1-Isopropyl-4,7-dimethyl-1,2,3,5,6,8a-hexahydronaphthalene	24.56	65418	0.12
trans-Calamenene	24.86	16892	0.03
(-)-Spathulenol	26.39	30478	0.05
1,2,2-Trimethyl-3-phenyl-cyclopent-3-enol	27.38	17063	0.03
1,3a-Ethano(1H)inden-4-ol, octahydro-2,2,4,7a-tetramethyl-	28.21	25292	0.04
geranyl-a-terpinene	35.83	52572	0.09
Thunbergol	39.82	168057	0.3
Ursolic aldehyde	59.27	90039	0.16
Uvaol	60.4	53311	0.09
BW			
Ethane, hexachloro-	12.11	184598	0.32
S-Methyl methanethiosulphonate	13.15	62816	0.11
BRE			
Cyclopropane, trimethyl(2-methyl-1-propenylidene)-	10.35	31277	0.02
Cyclohexene, 4-methylene-1-(1-methylethyl)-	11.67	42629	0.03
Cyclohexanol, 1-methyl-4-(1-methylethenyl)-, cis-	15.61	17823	0.01
Cedrene	20.82	10740	0.01
Germacrene D	23.76	16519	0.01

*B, BR, and L refer to bark, branches, and leaves, while M, E, and W refer to methanol, ethanol, and water, respectively

Table 3. (Continued.)

Peak Name	Retention Time (min)	Area (counts*min)	Relative Area (%)
a-Muurolene	24.18	13821	0.01
1-Isopropyl-4,7-dimethyl-1,2,3,5,6,8a-hexahydronaphthalene	24.57	25717	0.02
trans-Calamenene	24.87	20308	0.01
a-Calacorene	25.42	13706	0.01
(-)-Spathulenol	26.39	71434	0.05
(-)-Globulol	26.82	26360	0.02
7-epi-cis-sesquisabinene hydrate	27.57	35472	0.03
.tau.-Cadinol	27.91	16737	0.01
Benzoic acid, 4-hydroxy-3,5-dimethoxy-	31.79	100218	0.07
1-Heptatriacotanol	33.6	23641	0.02
Hexadecanoic acid, ethyl ester	34.67	13358	0.01
Butyl 6,9,12,15-octadecatetraenoate	35.67	13021	0.01
geranyl-a-terpinene	35.82	21245	0.02
Butyl 4,7,10,13,16,19-docosahexaenoate	37.09	54383	0.04
i-Propyl 5,8,11,14,17-eicosapentaenoate	39.02	13005	0.01
Thunbergol	39.83	342328	0.24
1-Heptatriacotanol	39.94	16595	0.01
Methyl 2-hydroxy-octadeca-9,12,15-trienoate	45.49	17053	0.01
Ursolic aldehyde	59.27	128749	0.09
Uvaol	60.38	103514	0.07
BRM			
Cyclohexene, 4-methylene-1-(1-methylethyl)-	11.73	25985	0.06
S-Methyl methanethiosulphonate	13.01	52310	0.11
Aspidofractinine-3-carboxylic acid, methyl ester, (2a,3 β ,5a)-	13.32	32497	0.07
Phenol, 2-methoxy-5-(1-propenyl)-, (E)-	20.72	38230	0.08
Cedrene	20.83	9737	0.02
1-Isopropyl-4,7-dimethyl-1,2,3,5,6,8a-hexahydronaphthalene	24.57	96788	0.21
trans-Calamenene	24.86	42201	0.09
a-Calacorene	25.43	34783	0.08
(-)-Spathulenol	26.4	84495	0.19
(-)-Globulol	26.84	33421	0.07
1,2,2-Trimethyl-3-phenyl-cyclopent-3-enol	27.4	50314	0.11
.tau.-Cadinol	27.92	26408	0.06
1,3a-Ethano(1H)inden-4-ol, octahydro-2,2,4,7a-tetramethyl-	28.23	65654	0.14
Benzoic acid, 4-hydroxy-3,5-dimethoxy-	31.82	175469	0.38
1-Heptatriacotanol	33.61	54308	0.12
Butyl 6,9,12,15-octadecatetraenoate	35.67	23579	0.05
geranyl-a-terpinene	35.83	60453	0.13
Butyl 4,7,10,13,16,19-docosahexaenoate	37.1	87757	0.19
i-Propyl 5,8,11,14,17-eicosapentaenoate	39.04	25993	0.06
2H-Pyran, 2-(7-heptadecynyloxy)tetrahydro-	39.17	13988	0.03

Table 3. (Continued.)

Peak Name	Retention Time (min)	Area (counts*min)	Relative Area (%)
Thunbergol	39.83	453957	1
1-Heptatriacotanol	39.94	29285	0.06
Methyl 2-hydroxy-octadeca-9,12,15-trienoate	45.5	37159	0.08
4-Methoxy-6-methyl-5-nitroisobenzofuran-1,3-dione	47.83	19886	0.04
1-Heptatriacotanol	48.46	11063	0.02
Ursolic aldehyde	59.29	173258	0.38
Uvaol	60.38	119214	0.26
BRW			
Cyclopropane, trimethyl(2-methyl-1-propenylidene)-	10.38	871870	1.46
S-Methyl methanethiosulphonate	13.04	55293	0.09
1,3a-Ethano(1H)inden-4-ol, octahydro-2,2,4,7a-tetramethyl-	28.16	21868	0.04
LE			
Cyclopropane, trimethyl(2-methyl-1-propenylidene)-	10.34	14083	0.01
Cyclohexene, 4-methylene-1-(1-methylethyl)-	11.65	21700	0.01
(-)-Spathulenol	26.39	39632	0.02
(-)-Globulol	26.83	19620	0.01
l-(+)-Ascorbic acid 2,6-dihexadecanoate	34.1	32937	0.02
Hexadecanoic acid, ethyl ester	34.68	77422	0.04
geranyl-a-terpinene	35.88	32409	0.02
Butyl 4,7,10,13,16,19-docosaheptaenoate	37.11	25141	0.01
Thunbergol	39.83	160317	0.09
Methyl 2-hydroxy-octadeca-9,12,15-trienoate	45.49	9343	0.01
Ursolic aldehyde	59.28	56827	0.03
Uvaol	60.39	62278	0.04

spathulenol, hexadecanoic acid ethyl ester, and geranyl-a-terpinene.

The present study has several limitations that should be considered when interpreting these findings. The antifungal experiments were performed without experimental replicates, and due to the limited amount of leaf material, only the ethanolic leaf extract was evaluated, preventing a complete solvent-based statistically supported comparison among all plant parts. In addition, the isolated fungal strains were identified using only morphological, biochemical, and physiological characteristics without molecular confirmation, limiting species-level identification. Furthermore, minimum inhibitory and fungicidal concentrations were not determined, and a positive antifungal control was not present, limiting direct comparison with established antifungal agents. Finally, although GC-MS analysis identified several compounds previously associated with antifungal activity, the individual compounds were not isolated and tested; therefore, their direct contribution to the observed inhibition cannot be confirmed.

4. CONCLUSIONS

This study explored the antifungal activity of *C. gileadensis* extracts and assessed the effects of different parts and/or solvents

on yield and inhibition. In addition, the phytochemical composition was investigated using GC-MS. The following key findings were observed: the branches showed the highest bioactive content and antifungal properties among the tested parts of *C. gileadensis*, whereas the methanolic extracts showed the highest inhibitory activity. The most prominent compounds were thunbergol, ursolic aldehydes, and spathulenol. Finally, the synergistic effect between the methanolic extract of the branches and the ethanolic extract of the leaves was not significant. In brief, all tested samples were effective and showed notable inhibition compared to the negative control.

The outcome of this study may contribute to the discovery of natural antifungal agents to overcome fungal resistance. Future work suggests using molecular identification tools of the isolated fungal strains to ensure accurate confirmation and molecular identification of the amplification and sequencing of the internal transcribed spacer (ITS) region of rDNA using universal primers (ITS1 and ITS4). The obtained sequences should be compared to reference sequences from the NCBI GenBank database using BLAST analysis. Future research should also examine the minimum inhibitory, minimum fungicidal, and minimum biofilm inhibitory concentrations of the extracts and incorporate Fourier transform infrared spectroscopic characterization and

scanning electron microscopy imaging to further elucidate the mechanisms underlying the antifungal activity of each suspected fungicidal agent. An integrated approach combining biological activity assays with phytochemical characterization is strongly recommended, adding depth beyond previously published studies that have focused on either aspect alone.

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