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RESEARCH ARTICLE

Essential Oils From Lamiaceae and Myrtaceae Families: Chemical, Antifungal, Antioxidant, and Multivariate Analysis for Multifunctional Purposes

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ABSTRACT

Essential oils (EOs) from three Lamiaceae species (*Lavandula dentata*, *Salvia rosmarinus*, and *Thymus vulgaris*) and seven *Eucalyptus* species (Myrtaceae) were evaluated for yield, chemical composition, antioxidant capacity, and antifungal activity. Oil yields ranged from 0.18% to 3.6%, with *L. dentata* producing the highest. Chemical composition was determined using gas chromatography, revealing high levels of oxygenated monoterpenes (39.95%–88.31%). The main constituents were 1,8-cineole, α-pinene, β-pinene, *p*-cymene, and camphor, except for the EO of *T. vulgaris*, which was dominated by thymol (72.37%). Antifungal assays showed strong activity for *T. vulgaris*, moderate activity for others, and the lowest for *E. lehmannii*. Antioxidant activity was also highest in *T. vulgaris*, surpassing the Trolox standard. Multivariate statistical analyses, including principal component analysis, hierarchical clustering, Pearson correlation, and partial least squares regression, revealed strong negative correlations ($r \leq -0.87$, $p < 0.05$) and high variable importance (VIP ≥ 2) for thymol, *cis*-linalool oxide, γ-terpinene, α-terpinene, and 3-carene. These findings support the multifunctional potential of bioactive EOs from Lamiaceae and Myrtaceae as eco-friendly agents for integrated crop protection and antioxidant applications.

1 | Introduction

Phytopathogenic fungi represents a major global agricultural threat, causing substantial yield losses and economic hardships for farmers worldwide [1]. These pathogens affect a wide variety of crops, causing diseases that manifest as wilting, rotting, leaf spots, and other symptoms [2, 3]. As these infections spread, they reduce crop quality and quantity, compromise nutritional value and directly impact farm income and food security [4, 5]. Farmers often rely on chemical fungicides to manage these diseases, further increasing production costs and environmental risks [6]. Taken together, these impacts emphasize the critical need for sustainable and eco-friendly strategies to manage fungal pathogens [7].

Essential oils (EOs) are rich in secondary metabolites, compounds produced by plants with diverse biological functions, including defense against pests and pathogens [8]. EOs have

gained attention as natural alternatives to synthetic fungicides, offering a sustainable solution to combat the growing problem of resistance in agriculture [9, 10]. Their ability to target a broad spectrum of pathogens, while being less prone to resistance development and minimizing environmental impact, makes them promising agents for sustainable agriculture [11, 12].

EOs extracted from plants of the Lamiaceae family (*Lavandula dentata*, *Salvia rosmarinus*, and *Thymus vulgaris*) are recognized for their diverse biological activities [13]. They exhibit potent antioxidant properties and strong antimicrobial effects, making them valuable in the fight against various microbial infections [14, 15]. Due to these multifunctional properties, EOs from Lamiaceae species continue to draw scientific interest for their potential applications in both human health and sustainable agriculture [16].

The *Eucalyptus* genus, a diverse and globally recognized group of flowering trees, is primarily native to Australia, where it thrives in a wide range of habitats [17]. However, these evergreen species have also spread across many regions, including South America, Africa, and Southeast Asia [18]. *Eucalyptus* leaves are rich in EOs known for their distinctive aroma, their antioxidant and biological properties, making them valuable in the fields of natural medicine and industrial applications [19–21].

The present study aimed to characterize the chemical composition of EOs extracted from three Lamiaceae species (*L. dentata*, *S. rosmarinus*, and *T. vulgaris*) and seven Myrtaceae species of the genus *Eucalyptus* (*E. camaldulensis*, *E. cinerea*, *E. grandis*, *E. lehmannii*, *E. leucoxylon*, *E. saligna*, and *E. sideroxylon*), and to evaluate their antifungal activity against six phytopathogenic fungal strains: *Fusarium culmorum*, *Fusarium oxysporum*, *Fusarium proliferatum*, *Phoma* sp., *Rhizoctonia solani*, and *Sclerotinia sclerotiorum*. The antioxidant potential of the EOs was assessed using 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2′-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid (ABTS) assays.

To examine the relationships between chemical composition and antifungal efficacy, multivariate statistical techniques, including principal component analysis (PCA), hierarchical cluster analysis (HCA), Pearson correlation, and partial least squares (PLS) regression, were applied. These analyses allowed the identification of specific volatile compounds that are strongly correlated with antifungal activity, providing a direct link between chemical profiles and observed bioactivity. In addition, the clustering of EOs based on both their chemical composition and biological effects highlighted patterns of activity that can be attributed to a particular group of compounds. This integrative analysis provides deeper insights into the key bioactive constituents responsible for the observed antifungal activity, supporting the potential use of these EOs in sustainable crop protection and plant health management.

2 | Results and Discussion

2.1 | EO Yields and Chemical Composition

EO yields are reported as mean ± standard deviation ($n = 3$), and differences among species were assessed using one-way ANOVA followed by Fisher's LSD test at $p < 0.05$. The EO yields of the selected species from the Lamiaceae and Myrtaceae families exhibited significant variation highlighting the variation in oil productivity (Table 1). For the Lamiaceae family, *L. dentata* yielded the highest EO content (3.6 ± 0.3%) which aligns with the findings of El Abdali et al. [22], who reported a content of 3.46%. This yield is comparatively higher than those reported in Tunisia (1.76%), Algeria (1.18%), Morocco (0.79%) and Argentina (0.8%) [23–26]. *T. vulgaris* EO showed a yield of 2.7 ± 0.3%, which falls within the reported range of 0.3%–4% by Pavela et al. [27] and is consistent with the yield (2.5%) recorded by Dinu et al. [28]. *S. rosmarinus* EO yield was 0.66 ± 0.05%, close to reported values ranging from 0.48% to 0.87% depending on harvest season and location [29]. In the Myrtaceae family, the EO yields ranged from 0.18 ± 0.06% (*E. grandis*) to 1.94 ± 0.1% (*E. camaldulensis*). The yields of EOs from various *Eucalyptus* species can vary significantly, with reported percentages ranging from 0.1% to 7.3%, highlighting the wide diversity in oil content among different plants [30, 31]. The EO yield of *E. camaldulensis* in the present study is consistent with previous report from Thailand with yields between 1.07% and 2.23%, depending on the specific clones tested [32]. *E. cinerea* showed a yield of 1.45 ± 0.25%, which is lower than obtained in Italy (2.56%) [33]. Tum et al. [34] studied the impact of varying extraction times, finding that the yield of *E. grandis* ranged from 0.5% to 0.7% which differs from our findings (0.18 ± 0.06%). Khedhri et al. [35] found that *E. lehmannii* from a Tunisian arboretum yielded 1.91%, which is higher than the yield observed in our study (1.45 ± 0.04%). A similar observation was made for *E. leucoxylon*, as Sebei et al. [36] reported a yield of 1.61%, which exceeds the yield found in our study (1.17 ± 0.3%). Ayed et al. [37] reported that the EOs extracted from eight *Eucalyptus* species growing in Tunisia had yields ranging from 0.12% to 1.32%, with *E. saligna* yielding 0.64%. Similarly, our study recorded a yield of 0.53 ± 0.08%. In addition, Amri et al. [38] reported a yield of 1.3% for *E. sideroxylon* growing in Tunisia, which is consistent with our findings, as we also observed a yield of 1.33 ± 0.1%.

The chemical analysis of the 10 EOs allowed the identification of more than 100 compounds, accounting for from 97.23% to 99.92% of the total EOs and distributed acroos five classes of terpene and non-terpene derivatives, as determined by gas chromatography–mass spectrometry (GC–MS) identification and gas chromatography coupled with flame ionization detection (GC–FID) quantification. The high percentages of total identified compounds indicate a comprehensive characterization of the EO chemical profiles. All EOs showed a specific richness in oxygenated monoterpenes (39.95%–88.31%) and monoterpenes hydrocarbons (7.02%–53.65%), which constituted the dominant chemical classes across the analyzed oils (Table 1).

Among the Lamiaceae family, *L. dentata* EO was characterized by 85 compounds, accounting for 99.18% of the total EO composition. Oxygenated monoterpenes predominated (72.67%), with 1,8-cineole (61.8%), β-pinene (12.52%), and α-pinene (4.35%) identified as dominant compounds. The predominance of 1,8-cineole observed here is consistent with reports on *L. dentata* EO from Brazil (63%) [39], and Tunisia (35% and 33.54%, respectively) [24, 40]. In contrast, other studies reported different dominant constituents including β-eudesmol (21.17%) and linalool (47.3%) [41, 42]. In *S. rosmarinus* EO, 56 chemical components were identified, representing 99.79% of the total oil, with oxygenated

TABLE 1 | Chemical composition of EOs of *Lavandula dentata* (A), *Salvia rosmarinus* (B), *Thymus vulgaris* (C) and *Eucalyptus* species (*E. camaldulensis*, D; *E. cinerea*, E; *E. grandis*, F; *E. lehmannii*, G; *E. leucoxylon*, H; *E. saligna*, I; and *E. sideroxylon*, J).

				Yield percentage (%)									
				Lamiaceae			Myrtaceae						
No.	Compounds	Formula	RI	A	B	C	D	E	F	G	H	I	J
1	Tricyclene	$C_{10}H_{16}$	923	0.1	0.29	0.016	—	—	—	0.012	—	0.575	0.08
2	α-Thujene	$C_{10}H_{16}$	927	0.02	0.11	1.005	0.052	0.005	—	0.018	0.383	—	—
3	α-Pinene	$C_{10}H_{16}$	936	4.35	14.42	0.951	19.199	8.16	31.96	12.69	8.054	18.2	4.381
4	Camphene	$C_{10}H_{16}$	950	0.49	7.86	0.358	0.242	0.23	0.546	0.23	0.211	3.9	0.136
5	Thuja-2,4(10)-diene	$C_{10}H_{14}$	955	0.3	—	—	0.112	0.06	0.435	0.15	0.056	0.019	0.062
6	Sabinene	$C_{10}H_{16}$	973	—	0.06	0.007	—	—	1.04	—	—	0.01	—
7	β-Pinene	$C_{10}H_{16}$	977	12.52	5.8	0.154	0.469	0.067	0.627	0.18	0.361	0.1	0.238
8	1-Octen-3-ol	$C_8H_{16}O$	980	0.02	—	0.02	—	—	0.036	0.009	—	0.073	0.027
9	Myrcene	$C_{10}H_{16}$	989	0.48	1.17	1.6	0.229	0.027	0.062	0.024	0.393	0.032	0.029
10	α-Phellandrene	$C_{10}H_{16}$	1004	0.18	0.25	0.345	1.168	0.132	0.107	0.09	3.185	0.096	0.089
11	3-Carene	$C_{10}H_{16}$	1011	—	0.15	0.227	—	—	0.014	—	—	0.052	—
12	α-Terpinene	$C_{10}H_{16}$	1017	0.14	0.42	1.638	0.128	—	0.03	—	0.111	0.032	—
13	*p*-Cymene	$C_{10}H_{14}$	1024	1.63	4.86	7.112	7.190	2.528	12.82	2.15	10.844	29.37	1.863
14	1,8-Cineole	$C_{10}H_{18}O$	1031	61.8	40.75	0.136	66.473	82.753	39.627	60.74	62.95	24.36	86.261
15	γ-Terpinene	$C_{10}H_{16}$	1059	0.17	0.69	7.879	0.382	0.11	0.116	0.093	0.218	0.9	0.138
16	*cis*-Linalool oxide	$C_{10}H_{18}O_2$	1075	0.02	0.05	0.185	—	—	0.012	—	—	0.15	—
17	α-Terpinolene	$C_{10}H_{16}$	1086	0.07	0.23	0.138	0.179	0.048	0.045	—	0.094	0.17	0.022
18	*p*-Cymenene	$C_{10}H_{12}$	1087	0.12	—	—	—	—	0.053	0.035	—	0.2	—
19	o-Guaiacol	$C_7H_8O_2$	1092	0.08	—	—	—	—	—	0.11	—	0.15	—
20	α-Pinene oxide	$C_{10}H_{16}O$	1097	0.1	—	—	—	0.015	—	—	—	—	—
21	*trans*-Sabinene hydrate	$C_{10}H_{18}O$	1098	0.05	—	—	—	—	—	—	—	0.019	—
22	Linalool	$C_{10}H_{18}O$	1099	1.2	0.15	0.719	—	—	—	—	—	0.16	—
23	*endo*-Fenchol	$C_{10}H_{18}O$	1115	0.2	0.08	—	0.032	0.169	0.108	0.175	0.085	2.32	0.054
24	α-Campholenal	$C_{10}H_{16}O$	1124	0.02	0.025	—	0.021	0.024	0.282	—	0.016	0.017	0.017
25	*trans*-Rose oxide	$C_{10}H_{18}O$	1128	0.35	0.035	—	—	—	—	0.097	—	—	—

(Continues)

TABLE 1 | (Continued)

				Yield percentage (%)									
				Lamiaceae			Myrtaceae						
No.	Compounds	Formula	RI	A	B	C	D	E	F	G	H	I	J
26	*trans*-Sabinol	$C_{10}H_{16}O$	1139	0.03	—	—	—	0.971	—	—	0.713	3.64	—
27	*trans*-Pinocarveol	$C_{10}H_{16}O$	1140	2.1	—	0.007	0.525	—	4.149	—	—	—	0.532
28	Camphor	$C_{10}H_{16}O$	1143	0.6	7.87	0.006	—	—	0.286	15.2	—	0.34	—
29	Pinocarvone	$C_{10}H^{14}O$	1160	0.6	0.07	—	0.097	0.241	2.059	—	0.142	1.21	0.116
30	Borneol	$C_{10}H_{18}O$	1166	0.7	0.01	0.773	0.072	0.354	0.597	3.14	0.084	4.23	0.057
31	Lavandulol	$C_{10}H_{18}O$	1168	0.2	—	—	—	—	—	0.56	0.058	—	0.06
32	Terpinen-4-ol	$C_{10}H_{18}O$	1177	0.9	4.21	0.583	0.052	0.25	0.179	0.084	0.32	—	—
33	Cryptone	$C_9H_{14}O$	1183	0.5	0.52	—	0.464	—	—	0.123	—	—	—
34	*p*-Cymen-8-ol	$C_{10}H_{14}O$	1184	0.04	—	—	—	—	0.296	—	—	—	—
35	*α*-Terpineol	$C_{10}H_{18}O$	1189	0.16	0.06	—	0.48	0.743	0.159	0.244	0.54	1.96	0.133
36	Myrtenol	$C_{10}H_{16}O$	1194	1.76	1.18	—	—	—	0.477	0.435	—	0.34	0.851
37	Verbenone	$C_{10}H_{14}O$	1206	1.17	—	—	0.038	—	0.233	0.32	0.036	—	—
38	*trans*-Carveol	$C_{10}H_{16}O$	1217	0.17	—	—	—	—	0.11	—	—	0.023	—
39	*cis*-Carveol	$C_{10}H_{16}O$	1226	0.07	0.004	—	0.011	0.018	0.089	0.079	0.009	0.13	0.022
40	Citronellol	$C_{10}H_{20}O$	1228	0.13	—	—	—	—	0.061	—	—	0.14	0.1
41	Pulegone	$C_{10}H_{16}O$	1234	—	—	—	0.035	0.068	0.038	0.192	0.049	0.1	0.092
42	Cumin aldehyde	$C_{10}H_{12}O$	1237	0.11	—	—	0.018	0.073	0.339	0.09	0.027	0.096	0.016
43	Piperitone	$C_{10}H_{16}O$	1253	0.15	—	—	—	—	0.1	0.033	0.006	—	—
44	Geranial	$C_{10}H_{16}O$	1270	0.015	—	0.056	—	—	—	—	—	—	—
45	Phellandral	$C_{10}H_{16}O$	1274	—	—	—	—	—	0.02	—	—	0.009	—
46	Citronellyl formate	$C_{11}H_{20}O_2$	1276	0.022	—	—	—	—	—	—	—	—	—
47	Bornyl acetate	$C_{12}H_{20}O_2$	1283	—	0.76	—	—	—	—	—	—	—	—
48	*p*-Cymen-7-ol	$C_{10}H_{14}O$	1287	0.01	—	—	—	—	0.018	—	—	—	—
49	Thymol	$C_{10}H_{14}O$	1290	—	—	72.376	—	—	—	—	0.015	—	—
50	Carvacrol	$C_{10}H_{14}O$	1300	0.02	0.03	—	—	—	0.018	0.014	—	0.035	0.003

(Continues)

TABLE 1 | (Continued)

				Yield percentage (%)									
				Lamiaceae			Myrtaceae						
No.	**Compounds**	**Formula**	**RI**	**A**	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**I**	**J**
51	*p*-Vinylguaiacol	$C_9H_{10}O_2$	1317	0.07	0.006	—	—	—	0.044	0.022	—	0.056	—
52	Myrtenyl acetate	$C_{12}H_{18}O_2$	1328	0.005	—	—	—	—	—	—	—	0.006	—
53	Linalool propanoate	$C_{13}H_{22}O_2$	1336	0.01	—	—	—	0.01	—	—	0.025	0.015	0.015
54	Piperitenone	$C_{10}H_{14}O$	1340	0.1	—	—	—	—	—	—	—	0.014	—
55	α-Terpinyl acetate	$C_{12}H_{20}O_2$	1347	0.01	0.007	—	1.404	2.718	0.022	0.008	0.028	0.02	—
56	α-Cubebene	$C_{15}H_{24}$	1351	0.03	0.009	—	—	—	—	—	1.153	0.009	0.01
57	*cis*-Carvyl acetate	$C_{12}H_{18}O_2$	1362	0.01	—	—	—	—	—	—	—	0.09	—
58	α-Ylangene	$C_{15}H_{24}$	1369	0.01	—	—	—	—	—	—	0.093	0.43	0.018
59	Carvacrol acetate	$C_{12}H_{16}O_2$	1373	—	0.012	—	—	—	—	—	—	—	—
60	α-Copaene	$C_{15}H_{24}$	1376	0.03	0.095	—	—	—	—	—	—	0.01	—
61	β-Cubebene	$C_{15}H_{24}$	1386	0.02	—	—	—	—	—	—	—	0.04	—
62	β-Elemene	$C_{15}H_{24}$	1390	—	—	—	—	—	—	—	0.068	—	0.019
63	Methyl eugenol	$C_{11}H_{14}O_2$	1401	—	0.011	—	—	—	—	—	—	—	0.018
64	α-Gurjunene	$C_{15}H_{24}$	1408	0.01	0.02	—	—	—	—	0.009	0.05	—	0.314
65	E-Caryophyllene	$C_{15}H_{24}$	1420	0.28	6.047	2.31	—	0.026	0.012	0.029	1.243	0.39	0.033
66	β-Cedrene	$C_{15}H_{24}$	1422	0.04	—	—	—	—	—	0.006	0.093	—	0.044
67	β-Gurjunene	$C_{15}H_{24}$	1431	0.2	0.06	—	—	0.02	0.048	0.397	0.372	—	0.791
68	Aromadendrene	$C_{15}H_{24}$	1440	—	—	—	0.262	—	0.03	—	—	0.17	—
69	α-Himachalene	$C_{15}H_{24}$	1445	0.19	0.66	—	—	0.007	0.018	—	0.469	—	0.053
70	α-Humulene	$C_{15}H_{24}$	1453	0.19	0.02	0.103	—	—	0.028	—	—	—	0.288
71	α-Patchoulene	$C_{15}H_{24}$	1457	—	—	—	0.084	—	—	0.084	—	0.07	—
72	γ-Gurjunene	$C_{15}H_{24}$	1472	—	—	—	—	—	—	0.009	0.056		0.024
73	γ-Muurolene	$C_{15}H_{24}$	1476	—	0.08	—	—	—	—	0.004	0.041	0.028	0.027
74	Ar-Curcumene	$C_{15}H_{22}$	1482,2	0.96	0.01	—	0.009	—	—	0.017	0.053	0.1	0.044
75	α-Amorphene	$C_{15}H_{24}$	1482,4	—	0.02	—	—	—	—	—	—	—	—

(Continues)

TABLE 1 | (Continued)

No.	Compounds	Formula	RI	Yield percentage (%)									
				Lamiaceae			Myrtaceae						
				A	B	C	D	E	F	G	H	I	J
76	Valencene	$C_{15}H_{24}$	1491	0.09	0.03	—	0.011	0.018	0.033	0.079	2.612	—	1.119
77	β-Bisabolene	$C_{15}H_{24}$	1508	0.01	0.05	—	—	—	—	—	0.074	0.02	—
78	β-Curcumene	$C_{15}H_{24}$	1512	0.01	—	—	—	—	—	—	0.02	—	0.013
79	γ-Cadinene	$C_{15}H_{24}$	1513	0.35	0.11	—	—	—	—	—	—	0.05	0.015
80	δ-Cadinene	$C_{15}H_{24}$	1523	0.26	0.15	—	—	—	—	—	0.019	0.17	0.011
81	(*E*)-γ-Bisabolene	$C_{15}H_{24}$	1532	0.01	—	—	—	—	—	—	—	—	—
82	α-Calacorene	$C_{15}H_{20}$	1540	0.74	0.003	—	—	0.015	0.076	0.007	0.025	0.48	—
83	Germacrene B	$C_{15}H_{24}$	1550	—	—	—	—	—	—	—	—	—	0.078
84	β-Calacorene	$C_{15}H_{20}$	1559	0.11	0.004	—	0.031	—	0.028	0.087	0.056	0.08	—
85	Ledol	$C_{15}H_{26}O$	1566	—	—	—	0.027	—	—	0.1	0.171	0.051	0.1
86	Spathulenol	$C_{15}H_{24}O$	1576	—	—	—	0.039	0.01	0.619	0.27	1.113	0.23	0.19
87	Caryophyllene oxide	$C_{15}H_{24}O$	1580	0.28	0.194	0.456	—	0.026	0.1	0.63	1.403	0.11	—
88	Globulol	$C_{15}H_{26}O$	1581	—	—	—	0.215	—		—	—	—	0.723
89	*epi*-Globulol	$C_{15}H_{26}O$	1584	0.06	—	—	0.1	0.012	0.064	0.33	0.805	0.02	0.39
90	Humulene epoxide II	$C_{15}H_{24}O$	1604	0.05	0.028	0.007	—	—	0.076	0.09	0.073	0.07	0.125
91	10-*epi*-γ-Eudesmol	$C_{15}H_{26}O$	1618	0.03	—	—	—	—	0.1	0.016	—	0.44	0.01
92	*epi*-1-Cubenol	$C_{15}H_{26}O$	1625	0.02	—	—	—	0.016	0.058	0.019	—	0.29	—
93	γ-Eudesmol	$C_{15}H_{26}O$	1630	—	0.012	0.012	—	—	0.034	—	0.073		0.02
94	α-Muurolol	$C_{15}H_{26}O$	1642	0.02	—	—	—	—	—	0.008	—	0.017	—
95	β-Eudesmol	$C_{15}H_{26}O$	1650	0.27	—	—	0.012	—	—	0.172	0.079	0.011	0.047
96	α-Cadinol	$C_{15}H_{26}O$	1651	0.08	—	0.007	—	—	0.026	—	—	—	—
97	Caryophyllenol II	$C_{15}H_{24}O$	1659	0.037	0.022	—	—	—	—	—	—	—	—
98	β-Bisabolol	$C_{15}H_{26}O$	1672	0.05	0.008	0.023	—	—	—	—	0.022	—	—
99	α-Bisabolol	$C_{15}H_{26}O$	1682	0.017	—	—	—	—	0.009	—	—	—	—
100	Eudesma-4(15),7-dien-1b-ol	$C_{15}H_{24}O$	1688	0.16	—	—	—	—		—	—	—	—

(Continues)

TABLE 1 | (Continued)

				Yield percentage (%)									
				Lamiaceae			Myrtaceae						
No.	**Compounds**	**Formula**	**RI**	**A**	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**I**	**J**
101	(2*Z*,6*E*)-Farnesol	$C_{15}H_{26}O$	1722	0.008	—	—	—	—		—	—	—	—
102	Chamazulene	$C_{14}H_{16}$	1726	0.006	—	—	—	—		—	0.029	—	0.007
103	α-Sinensal	$C_{15}H_{22}O$	1753	0.46	—	—	—	—		—	0.015	—	0.004
104	2-Heptadecanone	$C_{17}H_{34}O$	1903	0.029	0.005	—	—	—		—	—	—	—
105	Methyl hexadecanoate	$C_{17}H_{34}O_2$	1924	—	0.007	—		—		—	—	—	
Yield (w/w %)*				3.6 ± 0.3^{g}	0.66 ± 0.05^{b}	2.7 ± 0.3^{f}	1.94 ± 0.1^{e}	1.45 ± 0.25^{d}	0.18 ± 0.06^{a}	1.45 ± 0.04^{d}	1.17 ± 0.3^{c}	0.53 ± 0.08^{b}	1.33 ± 0.1^{cd}
Monoterpene hydrocarbons (%)				20.57	36.31	21.44	29.34	11.36	47.85	15.63	23.91	53.65	7.02
Oxygenated monoterpenes (%)				72.67	54.52	74.84	67.85	85.67	49.25	81.43	65.05	39.95	88.31
Sesquiterpene hydrocarbons (%)				2.69	7.35	2.4	0.39	0.07	0.17	0.82	6.41	1.48	2.81
Oxygenated sesquiterpenes (%)				2.28	0.27	0.5	0.42	0.07	1.19	1.72	3.82	1.96	1.6
Non-terpene derivatives (%)				0.97	1.34	0.02	1.86	2.75	0.11	0.11	0.1	0.19	0.12
Total identified (%)				99.18	99.79	99.2	99.86	99.92	98.57	99.71	99.29	97.23	99.86

Note: Components are listed in their order of elution from an HP-5 capillary column, and their percentages were calculated from a flame ionization detector (FID)

Abbreviations: RI, retention indices; —, not detected.

*Different letters indicate significant differences (Fisher's test at $p \leq 0.05$).

monoterpenes (54.52%) and monoterpenes hydrocarbons (36.31%) as the dominant classes. The prevalent constituents were 1,8-cineole (40.75%) followed by α-pinene (14.42%) and camphor (7.87%). These findings are in line with Rathore et al. [29] where *S. rosmarinus* EO contained 1,8-cineole (45.4%–48.1%), α-pinene (10.9%–14%), and camphor (5.4%–15.8%). Similar patterns were observed in *S. rosmarinus* EO originating from Serbia and Russia with varying relative abundances of α-pinene (23% and 17.76%), 1,8-cineole (17.79% and 23.4%), and camphor (14.39% and 17.17%), respectively [43]. The chemical analysis of the *T. vulgaris* EO revealed 30 compounds representing 99.2% of the total oil, with oxygenated monoterpenes (74.84%) as the primary constituents. The major components were thymol (72.37%), γ-terpinene (7.87%), and *p*-cymene (7.11%). Comparable chemical profiles were reported for *T. vulgaris* EO from different origins supporting the reproducibility of the observed chemotype while acknowledging quantitative variation among samples [44, 45].

The chemical analysis of the EOs extracted from the Myrtaceae family allowed the identification of 35 compounds from *E. camaldulensis*, 33 from *E. cinerea*, 51 from both *E. grandis* and *E. lehmannii*, 55 from *E. leucoxylon*, 64 from *E. saligna*, and 53 from *E. sideroxylon*, representing 99.86%, 99.92%, 98.57%, 99.71%, 99.29%, 97.23%, and 99.86% of the entire EO constituents, respectively. The predominant EO's constituents for *Eucalyptus* species (*E. camaldulensis*, *E. cinerea*, *E. grandis*, and *E. sideroxylon*) were 1,8-cineole (39.62%–86.26%) as oxygenated monoterpenes, followed by α-pinene (4.38%–31.96%) and *p*-cymene (1.86%–12.82%) as monoterpenes hydrocarbons. Camphor (15.2%) was also identified in *E. lehmannii* in addition to 1,8-cineole (60.74%) and α-pinene (12.69%). The key constituents of *E. leucoxylon* were 1,8-cineole (62.95%) followed by *p*-cymene (10.84%) and α-pinene (8.05%). The the main compound in *E. saligna* EO (29.37%) was *p*-cymene followed by 1,8-cineole (24.36%) and α-pinene (18.2%). *Eucalyptus* EOs exhibited significant chemical variability across different geographical origins as evidenced by various studies with 1,8-cineole being the major constituent which is in line with our findings [46, 47]. In Brazil, *E. camaldulensis* EO contained primarily 1,8-cineole (76.93%), β-pinene (11.49%), and α-pinene (7.15%) [48]. In another Brazilian study, the same plant species yielded different chemical profiles, with 1,8-cineole (41.61%), α-terpineol (19.87%), and α-pinene (15.81%) as the predominant components [49]. However, when using the aerial parts of *E. camaldulensis* in Egypt, the main constituents shifted to spathulenol (20.84%), *p*-cymene (15.16%), and 1,8-cineole (12.01%) [50]. In Pakistan, the leaves of *E. camaldulensis* had a distinctive profile, with linalool (17%), 1,8-cineole (16%), and *p*-cymene (12.2%) being the major constituents [51]. These variations extend to other regions like Morocco, Syria, Turkey, and Thailand showing *p*-cymene and γ-terpinene as the major components [32, 52–58]. EO extracted from the leaves and stems of *E. cinerea* from Brazil, contained mainly 1,8-cineole (55.24%), α-terpinyl acetate (21.64%), and α-pinene (7.94%) [59], while in Italy, the same species yielded 1,8-cineole (67.7%), α-pinene (7.3%), and α-terpinyl acetate (5.2%) when only the leaves were used [33]. Similarly, Sebei et al. [36] reported that the major constituents of *E. lehmannii* growing in Tunisia were 1,8-cineole (49.07%), α-pinene (26.35%), and α-terpinyl acetate (5.64%) while *E. leucoxylon* was characterized by the dominance of 1,8-cineole (77.76%), α-pinene (5.85%), and *trans*-pinocarveol (3.23%). Caetano et al. [49] reported that *E. grandis* EO main components were 1,8-cineole (37.43%), α-pinene (36.35%), and α-terpineol (8.71%) as the predominant components. Ayed et al. [37] characterized *E. saligna* EO extracted from Tunisia identifying 1,8-cineole (20.36%), *p*-cymene (15.27%), and isoborneol (10.54%) as the primary compounds. In contrast, the present study found that *p*-cymene was the principal component in *E. saligna* EO (29.37%), followed by 1.8-cineole (24.36%) and α-pinene (18.2%). Similarly, Amri et al. [38] analyzed the *E. sideroxylon* EO growing in Tunisia, with the main compounds were 1,8-cineole (65.4%), globulol (7.4%), and aromadendrene (2.1%). These findings differ from those observed in the current study, where the dominant compound was 1,8-cineole (86.26%), followed by α-pinene (4.38%) and *p*-cymene (1.86%), highlighting a distinct chemical composition between the two studies.

The detailed qualitative and quantitative characterization of the EOs provides a robust chemical dataset that forms the basis for subsequent multivariate analyses investigating relationships between EO composition and biological activities.

2.2 | Antioxidant Activity

The antioxidant potential of the 10 EOs were evaluated by DPPH and ABTS assays, and the results are presented graphically in Figure 1, while exact IC_{50} values are provided in Table S1.

Among the tested species, *T. vulgaris* EO revealed the strongest antioxidant activity in both DPPH and ABTS assays, with an IC_{50} of 3.06 ± 0.04 and 1.5 ± 0.08 μg mL^{-1}, respectively, exceeding the activity of the standard Trolox. This high activity is consistent with its rich thymol content, a phenolic compound well-known for its strong antioxidant and antimicrobial properties [60, 61]. Findings of the present study showed a higher antiradical capacity than reported by Chahboun et al. [62] reporting IC_{50} values of 5.94 ± 0.22 and 3.03 ± 0.17 μg mL^{-1} in DPPH and ABTS tests, respectively. Pilozo et al. [63] demonstrated a significant antioxidant profile for *T. vulgaris* with a radical neutralizing potential (DPPH) of IC_{50} = 1.11 ± 0.019 mg mL^{-1} and ferric ion reducing power of 93.05 ± 0.52 mg equivalent Trolox g^{-1}. *p*-Cymene, an intermediate in thymol biosynthesis [64], represented 29.37% of *E. saligna* EO in this study and likely contributed to its significant antioxidant activity (IC_{50} of 18.5 ± 1 and 5 ± 0.9 μg mL^{-1} in DPPH and ABTS, respectively). However, compared to thymol and *p*-cymene, 1,8-cineole exhibited lesser antioxidant potential. Literature reports suggest that 1,8-cineole, the primary constituent found in the EOs of *L. dentata*, *S. rosmarinus*, and some *Eucalyptus* species, generally demonstrates moderate antioxidant potential [22, 65]. *L. dentata* EO was the least active, showing IC_{50} values of 765.26 ± 141.05 and 102.5 ± 1.7 μg mL^{-1} in DPPH and ABTS assays, respectively. Lower IC_{50} values for this EO were previously reported by Dridi et al. [41] in DPPH, ABTS and reducing power test. In the present study, the antioxidant activity of *S. rosmarinus* EO (42.32 ± 3.06 μg mL^{-1} DPPH assay) falls within the range reported for EOs from five different sites in Palestine (IC_{50} from 10.23 ± 0.11 to 158.48 ± 0.87 μg mL^{-1}) [66]. The results obtained by Dammak et al. [24] showed that *S. rosmarinus* EO exhibited better antioxidant activity (IC_{50} = 11.12 μg mL^{-1}) than *L. dentata* (IC_{50} = 14.03 μg mL^{-1}) using DPPH test which are also consistent with our findings.

The EOs from *E. camaldulensis*, *E. grandis*, and *E. leucoxylon* were also active with IC_{50} values ranging from 100.1 ± 6.2

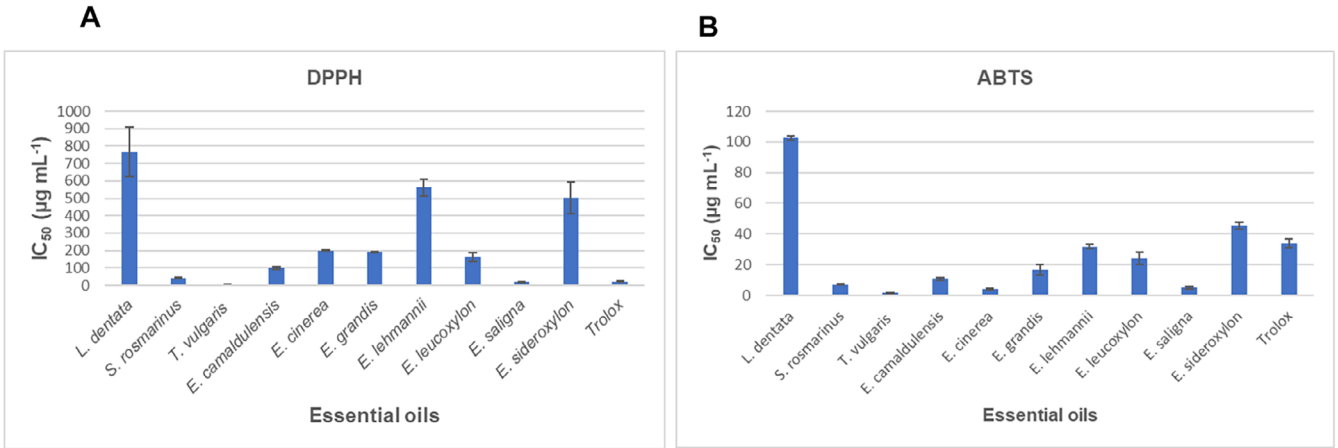

FIGURE 1 | Antioxidant activity of EOs of *Lavandula dentata*, *Salvia rosmarinus*, *Thymus vulgaris*, and *Eucalyptus* species (*E. camaldulensis*, *E. cinerea*, *E. grandis*, *E. lehmannii*, *E. leucoxylon*, *E. saligna*, and *E. sideroxylon*) evaluated by (A) DPPH and (B) ABTS assays.

to 190.7 ± 2.6 µg mL^{-1} in DPPH and from 10.8 ± 0.8 to 24.3 ± 4.1 µg mL^{-1} in ABTS assay, respectively. Moreover, *E. lehmannii* and *E. sideroxylon* revealed moderate antioxidant activities. No significant differences between these EOs were observed in the DPPH assay. Conversely, these differences were statistically significant in the ABTS assay. Several studies reported moderate antioxidant activities for *Eucalyptus* sp. EOs, with some differences depending on the species [67–69]. Limam et al. [70] found that EOs extracted from 13 Tunisian species of the *Eucalyptus* genus exhibited a moderate antioxidant activity at concentration of 10 µg mL^{-1}, with inhibition percentages of free radical DPPH ranging from 10.75 ± 1.05% to 52.69 ± 4.59%. Sadraoui Ajmi et al. [71] revealed that *E. cinerea* EO collected from Tunisia exhibited a similar DPPH radical scavenging activity (RSA) with an IC_{50} value of 161.59 µg mL^{-1} compared to our results (IC_{50} = 200.5 ± 4.1 µg mL^{-1}). Kouki et al. [68] concluded that EOs from three Tunisian *Eucalyptus* species (*E. oleosa*, *E. pimpiniana*, and *E. polyanthemos*) exhibited moderate antioxidant activities. Significant differences between the EOs were observed in the DPPH assay, with *E. oleosa* being the most active (IC_{50} = 92.179 µg mL^{-1}).

2.3 | Antifungal Activity

The effects of increasing concentrations of the 10 tested EOs on mycelium growth of the different fungal strains are summarized in Table 2. Statistically significant differences were observed among doses and fungal strains, as indicated by Fisher's LSD test ($p \leq 0.05$). Most of the EOs inhibited the growth of the tested fungal strains in a dose-dependent manner, confirming a clear concentration–response relationship. Significant differences in fungal sensitivity were observed, with certain strains, such as *R. solani* and *S. sclerotiorum*, showing higher susceptibility to the EOs compared to *Fusarium* species. These differences in sensitivity were statistically supported, highlighting the species-specific response of fungal strains to the EOs tested.

The highest inhibitory activity was exhibited by the *T. vulgaris* EO, with total inhibition of mycelium growth at 2 µL mL^{-1} for all fungal strains. *T. vulgaris* EO has shown a fungicidal effect for all the tested strains with minimum fungicidal concentration (MFC) values ranging between 2 and 12 µL mL^{-1} while it was fungistatic against *Phoma* sp. confirming species-specific sensitivity among fungal strains. Several reports have documented the antifungal effect of different species of *Thymus* against various microorganisms with thymol reported as a major contributor to its antifungal activity, which is consistent with the strong inhibition observed in the present study [72–74]. In conformity with our findings, the antifungal potential of different thyme species (*Thymus convolutes*, *T. pectinatus*, and *T. vulgaris*) has been demonstrated against plant pathogens including *F. oxysporum* f. sp. *radicis-lycopersici*, *Phytophthora infestans*, and *R. solani* [75]. The EOs of *T. vulgaris* and *T. pectinatus* completely inhibited the growth of all the fungal strains at a dose of 4 µL per Petri dish, confirming their pronounced antifungal effectiveness. Conversely, other *Thymus* species, such as *T. convolutes* EO showed lower antifungal efficacy, with only partial inhibition of *F. oxysporum* f. sp. *radicis-lycopersici* and no effect on *R. solani* and *P. infestans* suggesting that antifungal efficacy is strongly influenced by the chemical profile of each *Thymus* species. Fonseca-Guerra et al. [76] demonstrated that *T. vulgaris* EO completely inhibited the *in vitro* growth of *Fusarium* isolates originating from *Chenopodium quinoa* crops at higher concentrations (MIC value of 10 µL mL^{-1}) than those required in our study, highlighting variability in fungal sensitivity depending on isolate origin and experimental conditions. While Bounar et al. [77] demonstrated strong inhibition of four *Fusarium* species causing rot in potato tubers at very low concentrations (0.156–0.313 µL mL^{-1}), Divband et al. [78] reported higher MIC and MFC values (up to 30 mg mL^{-1}) against 20 wild-type strains of *F. oxysporum* isolates.

S. rosmarinus totally inhibited the growth of all fungal strains at concentrations ranging from 4 to 8 µL mL^{-1}. It was fungicidal for both *R. solani* and *S. sclerotiorum* at 8 and 10 µL mL^{-1}, respectively, indicating high antifungal potency against these strains. These results are consistent with those of ElYacoubi et al. [79], who showed that *S. rosmarinus* EO achieved complete inhibition of a broad-spectrum of phytopathogenic fungi when applied at the highest tested concentration (1/100 v/v). Ben Kaab et al. [80] found that *S. rosmarinus* EO significantly inhibited the spore germination of *F. culmorum* (85.99%), *F. oxysporum* (100%), and *Penicillium italicum* (95.40%) at slightly higher concentration (6 µL mL^{-1}) than that used in our study. Similarly, Hussein et al.

TABLE 2 | Antifungal activity of *Lavandula dentata*, *Salvia rosmarinus*, *Thymus vulgaris*, and *Eucalyptus* species (*E. camaldulensis*, *E. cinerea*, *E. grandis*, *E. lehmannii*, *E. leucoxylon*, *E. saligna*, and *E. sideroxyon*) EOs against phytopathogenic fungi.

Growth inhibition percentage (*I* %)							
Essential oil	**Dose (μL mL^{-1})**	***Fusarium oxysporum***	***Fusarium proliferatum***	***Fusarium culmorum***	***Rhizoctonia solani***	***Phoma* sp.**	***Sclerotinia sclerotiorum***
L. dentata	0	0 ± 0^{a}	0 ± 0^{a}	0 ± 0^{a}	0 ± 0^{a}	0 ± 0^{a}	0 ± 0^{a}
	2	28.26 ± 3.8^{bA}	29.55 ± 3.9^{bA}	36.96 ± 6.8^{bAB}	45.10 ± 6.8^{bBC}	49.02 ± 12.2^{aC}	50.98 ± 13.6^{bC}
	4	45.65 ± 3.8^{cA}	50 ± 3.9^{cA}	45.65 ± 3.8^{bA}	74.12 ± 6.2^{cC}	64.31 ± 5.6^{bB}	80.39 ± 15.1^{cC}
	6	56.52 ± 10^{dA}	60 ± 1.6^{dA}	77.83 ± 1.3^{cB}	81.96 ± 5.3^{dB}	74.90 ± 1.4^{cB}	96.08 ± 6.8^{dC}
	8	80 ± 2^{eA}	69.55 ± 3.4^{eA}	92.61 ± 12.8^{dB}	100 ± 0^{eB}	94.12 ± 10.2^{dB}	100 ± 0^{dB}
	10	100 ± 0^{fB}	73.64 ± 1.6^{eA}	100 ± 0^{dB}	100 ± 0^{eB}	100 ± 0^{eB}	100 ± 0^{dB}
	12	100 ± 0^{f}	100 ± 0^{f}	100 ± 0^{d}	100 ± 0^{e}	100 ± 0^{e}	100 ± 0^{d}
	Fongicide	100 ± 0^{f}	100 ± 0^{f}	100 ± 0^{d}	100 ± 0^{e}	100 ± 0^{e}	100 ± 0^{d}
MIC	(μL mL^{-1})	10	12	10	10	8	8
MFC	(μL mL^{-1})	> 12	> 12	> 12	> 12	> 12	> 12
S. rosmarinus	0	0 ± 0^{a}	0 ± 0^{a}	0 ± 0^{a}	0 ± 0^{a}	0 ± 0^{a}	0 ± 0^{a}
	2	57.61 ± 4.6^{bAB}	63.86 ± 2.9^{bBC}	54.35 ± 9.2^{bA}	79.41 ± 4.2^{bD}	67.65 ± 4.2^{bC}	94.12 ± 8.3^{bE}
	4	73.91 ± 9.2^{cA}	79.55 ± 9.6^{cAB}	81.74 ± 1.8^{cAB}	94.12 ± 8.3^{cC}	84.71 ± 1.7^{cB}	100 ± 0^{cC}
	6	77.83 ± 1.8^{cA}	80.91 ± 1.9^{cA}	93.48 ± 9.2^{dB}	100 ± 0^{dB}	94.12 ± 8.3^{dB}	100 ± 0^{cB}
	8	100 ± 0^{d}	100 ± 0^{d}	100 ± 0^{e}	100 ± 0^{d}	100 ± 0^{e}	100 ± 0^{c}
	10	100 ± 0^{d}	100 ± 0^{d}	100 ± 0^{e}	100 ± 0^{d}	100 ± 0^{e}	100 ± 0^{c}
	12	100 ± 0^{d}	100 ± 0^{d}	100 ± 0^{e}	100 ± 0^{d}	100 ± 0^{e}	100 ± 0^{c}
	Fongicide	100 ± 0^{d}	100 ± 0^{d}	100 ± 0^{e}	100 ± 0^{d}	100 ± 0^{e}	100 ± 0^{c}
MIC	(μL mL^{-1})	8	8	8	6	8	4
MFC	(μL mL^{-1})	> 12	> 12	> 12	8	> 12	10
T. vulgaris	0	0 ± 0^{a}	0 ± 0^{a}	0 ± 0^{a}	0 ± 0^{a}	0 ± 0^{a}	0 ± 0^{a}
	2	100 ± 0^{b}	100 ± 0^{b}	100 ± 0^{b}	100 ± 0^{b}	100 ± 0^{b}	100 ± 0^{b}
	4	100 ± 0^{b}	100 ± 0^{b}	100 ± 0^{b}	100 ± 0^{b}	100 ± 0^{b}	100 ± 0^{b}
	6	100 ± 0^{b}	100 ± 0^{b}	100 ± 0^{b}	100 ± 0^{b}	100 ± 0^{b}	100 ± 0^{b}
	8	100 ± 0^{b}	100 ± 0^{b}	100 ± 0^{b}	100 ± 0^{b}	100 ± 0^{b}	100 ± 0^{b}
	10	100 ± 0^{b}	100 ± 0^{b}	100 ± 0^{b}	100 ± 0^{b}	100 ± 0^{b}	100 ± 0^{b}

(Continues)

TABLE 2 | (Continued)

Growth inhibition percentage (*I* %)							
Essential oil	**Dose (μL mL^{-1})**	***Fusarium oxysporum***	***Fusarium proliferatum***	***Fusarium culmorum***	***Rhizoctonia solani***	***Phoma* sp.**	***Sclerotinia sclerotiorum***
	12	100 ± 0^{b}	100 ± 0^{b}	100 ± 0^{b}	100 ± 0^{b}	100 ± 0^{b}	100 ± 0^{b}
	Fongicide	100 ± 0^{b}	100 ± 0^{b}	100 ± 0^{b}	100 ± 0^{b}	100 ± 0^{b}	100 ± 0^{b}
MIC	(μL mL^{-1})	2	2	2	2	2	2
MFC	(μL mL^{-1})	6	2	2	2	> 12	12
E. camaldulensis	0	0 ± 0^{a}	0 ± 0^{a}	0 ± 0^{a}	0 ± 0^{a}	0 ± 0^{a}	0 ± 0^{a}
	2	32.61 ± 3.7^{bB}	36.36 ± 3.9^{bB}	10.87 ± 7.5^{bA}	17.65 ± 5.8^{bA}	85.49 ± 2.9^{bC}	11.76 ± 5.8^{bA}
	4	58.7 ± 13.5^{cBC}	65.91 ± 6.8^{cC}	36.96 ± 13.5^{cA}	47.45 ± 5.9^{cAB}	96.08 ± 6.7^{cD}	100 ± 0^{cD}
	6	75.22 ± 4.7^{dA}	95.45 ± 7.8^{dB}	75.22 ± 2.2^{dA}	79.61 ± 17.7^{dA}	100 ± 0^{cB}	100 ± 0^{cB}
	8	80.43 ± 7^{deA}	100 ± 0^{dB}	80.87 ± 4.5^{dA}	100 ± 0^{eB}	100 ± 0^{cB}	100 ± 0^{cB}
	10	86.96 ± 3^{eA}	100 ± 0^{dB}	100 ± 0^{eB}	100 ± 0^{eB}	100 ± 0^{cB}	100 ± 0^{cB}
	12	100 ± 0^{f}	100 ± 0^{d}	100 ± 0^{e}	100 ± 0^{e}	100 ± 0^{c}	100 ± 0^{c}
	Fongicide	100 ± 0^{f}	100 ± 0^{d}	100 ± 0^{e}	100 ± 0^{e}	100 ± 0^{c}	100 ± 0^{c}
MIC	(μL mL^{-1})	12	8	10	8	6	4
MFC	(μL mL^{-1})	> 12	> 12	12	10	> 12	4
E. cinerea	0	0 ± 0^{a}	0 ± 0^{a}	0 ± 0^{a}	0 ± 0^{a}	0 ± 0^{a}	0 ± 0^{a}
	2	47.83 ± 2.61^{bA}	55.91 ± 2.8^{bB}	45.65 ± 1.9^{bA}	62.35 ± 2.3^{bC}	66.27 ± 1.8^{bC}	82.35 ± 2.35^{bD}
	4	53.04 ± 3.45^{cA}	62.27 ± 3.4^{cB}	59.13 ± 4.1^{cB}	73.33 ± 1.8^{cC}	69.02 ± 1.8^{cC}	100 ± 0^{cD}
	6	59.57 ± 4.7^{dA}	75.45 ± 1.3^{dC}	70.43 ± 3.2^{dB}	91.37 ± 2.9^{dD}	70.59 ± 1.1^{cBC}	100 ± 0^{cE}
	8	63.91 ± 4.1^{dA}	82.27 ± 2.7^{eC}	73.91 ± 1.3^{dB}	100 ± 0^{eD}	74.90 ± 1.8^{dB}	100 ± 0^{cD}
	10	72.61 ± 1.3^{eA}	100 ± 0^{fC}	100 ± 0^{eC}	100 ± 0^{eC}	78.43 ± 1.8^{eB}	100 ± 0^{cC}
	12	76.09 ± 2.72^{eA}	100 ± 0^{fB}	100 ± 0^{eB}	100 ± 0^{eB}	100 ± 0^{fB}	100 ± 0^{cB}
	Fongicide	100 ± 0^{f}	100 ± 0^{f}	100 ± 0^{e}	100 ± 0^{e}	100 ± 0^{f}	100 ± 0^{c}
MIC	(μL mL^{-1})	> 12	10	10	8	12	4
MFC	(μL mL^{-1})	> 12	> 12	12	> 12	> 12	6
E. grandis	0	0 ± 0^{a}	0 ± 0^{a}	0 ± 0^{a}	0 ± 0^{a}	0 ± 0^{a}	0 ± 0^{a}
	2	26.09 ± 3.77^{bC}	38.64 ± 6.82^{bD}	8.7 ± 3^{bB}	0 ± 0^{aA}	75.29 ± 1.18^{bE}	0 ± 0^{aA}
	4	52.17 ± 7.53^{cB}	61.36 ± 3.94^{cC}	23.04 ± 2.26^{cA}	29.41 ± 5.88^{bA}	81.18 ± 2.35^{cD}	100 ± 0^{bE}

(Continues)

TABLE 2 | (Continued)

Growth inhibition percentage (*I* %)							
Essential oil	**Dose (μL mL^{-1})**	***Fusarium oxysporum***	***Fusarium proliferatum***	***Fusarium culmorum***	***Rhizoctonia solani***	***Phoma* sp.**	***Sclerotinia sclerotiorum***
	6	54.35 ± 11.3^{cA}	68.64 ± 2.36^{dB}	56.52 ± 4.58^{dA}	68.63 ± 8.99^{cB}	85.49 ± 2.96^{dC}	100 ± 0^{bD}
	8	58.7 ± 4.98^{cA}	80.91 ± 6.25^{eB}	63.04 ± 3.77^{eA}	87.45 ± 4.75^{dB}	100 ± 0^{eC}	100 ± 0^{bC}
	10	78.26 ± 3.77^{dB}	100 ± 0^{fC}	73.91 ± 1.3^{fA}	100 ± 0^{eC}	100 ± 0^{eC}	100 ± 0^{bC}
	12	80.43 ± 6.5^{dA}	100 ± 0^{fB}	100 ± 0^{gB}	100 ± 0^{eB}	100 ± 0^{eB}	100 ± 0^{bB}
	Fongicide	100 ± 0^{e}	100 ± 0^{f}	100 ± 0^{g}	100 ± 0^{e}	100 ± 0^{e}	100 ± 0^{b}
MIC	(μL mL^{-1})	> 12	10	12	10	8	4
MFC	(μL mL^{-1})	> 12	> 12	> 12	10	> 12	6
E. lehmannii	0	0 ± 0^{a}	0 ± 0^{a}	0 ± 0^{a}	0 ± 0^{a}	0 ± 0^{a}	0 ± 0^{a}
	2	23.91 ± 3.8^{bB}	21.82 ± 3.4^{bB}	19.57 ± 10^{bB}	0 ± 0^{aA}	0 ± 0^{aA}	62.75 ± 3.4^{bC}
	4	32.61 ± 3.8^{bD}	36.36 ± 3.9^{cD}	26.09 ± 3.8^{bcC}	9.80 ± 3.4^{bB}	0 ± 0^{aA}	75.69 ± 1.4^{cE}
	6	60.87 ± 13^{cC}	53.64 ± 4.9^{dC}	34.78 ± 6.5^{cB}	91.37 ± 7.6^{cD}	0 ± 0^{aA}	100 ± 0^{dD}
	8	65.22 ± 3.8^{cdC}	58.18 ± 1.6^{dB}	67.39 ± 6.5^{dC}	100 ± 0^{dD}	17.65 ± 5.9^{bA}	100 ± 0^{dD}
	10	71.74 ± 7.5^{dB}	65.91 ± 6.8^{eB}	$73.04 \pm 1.5\mathrm{d}^{eB}$	100 ± 0^{dC}	37.25 ± 3.4^{cA}	100 ± 0^{dC}
	12	71.74 ± 3.8^{dB}	76.36 ± 3.4^{fB}	80.43 ± 2.6^{eB}	100 ± 0^{dC}	41.18 ± 11.8^{cA}	100 ± 0^{dC}
	Fongicide	100 ± 0 e	100 ± 0 g	100 ± 0 f	100 ± 0 d	100 ± 0 d	100 ± 0 d
MIC	(μL mL^{-1})	> 12	> 12	> 12	8	> 12	6
MFC	(μL mL^{-1})	> 12	> 12	> 12	10	> 12	6
E. leucoxylon	0	0 ± 0^{a}	0 ± 0^{a}	0 ± 0^{a}	0 ± 0^{a}	0 ± 0^{a}	0 ± 0^{a}
	2	49.13 ± 1.3^{bA}	58.64 ± 2^{bB}	49.57 ± 1.9^{bA}	59.22 ± 1.8^{bB}	73.73 ± 2.96^{bC}	82.35 ± 2.3^{bD}
	4	59.13 ± 4.19^{cB}	64.55 ± 3.6^{cB}	55.22 ± 2.7^{cA}	75.69 ± 1.8^{cC}	78.43 ± 0.68^{cC}	100 ± 0^{cD}
	6	64.35 ± 1.9^{dB}	65.91 ± 2.7^{cB}	60.43 ± 1.9^{dA}	76.47 ± 3.1^{cC}	85.1 ± 1.36^{dD}	100 ± 0^{cE}
	8	68.26 ± 2.7^{deA}	67.27 ± 1.3^{cA}	64.78 ± 2.6^{dA}	85.1 ± 2.9^{dB}	86.27 ± 2.45^{dB}	100 ± 0^{cC}
	10	70 ± 2.6^{eA}	100 ± 0^{dB}	73.48 ± 5.4^{eA}	100 ± 0^{eB}	100 ± 0^{eB}	100 ± 0^{cB}
	12	76.52 ± 3.4^{fB}	100 ± 0^{dC}	73.48 ± 1.9^{eA}	100 ± 0^{eC}	100 ± 0^{eC}	100 ± 0^{cC}
	Fongicide	100 ± 0^{g}	100 ± 0^{d}	100 ± 0^{f}	100 ± 0^{e}	100 ± 0^{e}	100 ± 0^{c}
MIC	(μL mL^{-1})	> 12	10	>12	10	10	4
MFC	(μL mL^{-1})	> 12	> 12	>12	> 12	> 12	4

(Continues)

TABLE 2 | (Continued)

Growth inhibition percentage (*I* %)							
Essential oil	**Dose (μL mL^{-1})**	***Fusarium oxysporum***	***Fusarium proliferatum***	***Fusarium culmorum***	***Rhizoctonia solani***	***Phoma* sp.**	***Sclerotinia sclerotiorum***
E. saligna	0	0 ± 0^{a}	0 ± 0^{a}	0 ± 0^{a}	0 ± 0^{a}	0 ± 0^{a}	0 ± 0^{a}
	2	73.04 ± 1.5^{bAB}	69.55 ± 3.4^{bA}	73.04 ± 1.5^{aAB}	95.69 ± 7.5^{bC}	78.43 ± 1.8^{bB}	100 ± 0^{bC}
	4	78.26 ± 2^{cAB}	75.91 ± 6.3^{bcA}	85.22 ± 1.5^{bC}	100 ± 0^{bD}	81.18 ± 1.2^{bBC}	100 ± 0^{bD}
	6	95.65 ± 7.5^{dB}	82.73 ± 5.2^{cA}	92.17 ± 6.8^{cB}	100 ± 0^{bB}	96.47 ± 6.1^{cB}	100 ± 0^{bB}
	8	100 ± 0^{d}	95 ± 8.7^{d}	100 ± 0^{d}	100 ± 0^{b}	100 ± 0^{c}	100 ± 0^{b}
	10	100 ± 0^{d}	100 ± 0^{d}	100 ± 0^{d}	100 ± 0^{b}	100 ± 0^{c}	100 ± 0^{b}
	12	100 ± 0^{d}	100 ± 0^{d}	100 ± 0^{d}	100 ± 0^{b}	100 ± 0^{c}	100 ± 0^{b}
	Fongicide	100 ± 0^{d}	100 ± 0^{d}	100 ± 0^{d}	100 ± 0^{b}	100 ± 0^{c}	100 ± 0^{b}
MIC	(μL mL^{-1})	8	10	8	4	8	2
MFC	(μL mL^{-1})	> 12	> 12	> 12	> 12	> 12	2
E. sideroxylon	0	0 ± 0^{a}	0 ± 0^{a}	0 ± 0^{a}	0 ± 0^{a}	0 ± 0^{a}	0 ± 0^{a}
	2	57.83 ± 3.28^{bA}	63.18 ± 1.36^{bB}	58.26 ± 2.61^{bA}	60.78 ± 3.4^{bAB}	79.61 ± 0.68^{bC}	100 ± 0^{bD}
	4	72.61 ± 8.5^{cAB}	77.73 ± 1.57^{cB}	76.09 ± 1.99^{cB}	68.24 ± 1.18^{cA}	87.06 ± 2.04^{cC}	100 ± 0^{bD}
	6	80.43 ± 6.5^{dBC}	85.45 ± 0.79^{dC}	78.7 ± 0.75^{cdAB}	73.73 ± 2.96^{dA}	100 ± 0^{dD}	100 ± 0^{bD}
	8	81.74 ± 1.3^{dAB}	86.36 ± 2.73^{dB}	80.43 ± 1.3^{dA}	92.94 ± 6.2^{eC}	100 ± 0^{dD}	100 ± 0^{bD}
	10	83.91 ± 0.75^{dB}	90.91 ± 2.08^{eC}	80.87 ± 0.75^{dA}	100 ± 0^{fD}	100 ± 0^{dD}	100 ± 0^{bD}
	12	85.22 ± 1.5^{dA}	91.36 ± 2.84^{eB}	85.65 ± 2.26^{eA}	100 ± 0^{fC}	100 ± 0^{dC}	100 ± 0^{bC}
	Fongicide	100 ± 0^{e}	100 ± 0^{f}	100 ± 0^{f}	100 ± 0^{f}	100 ± 0^{d}	100 ± 0^{b}
MIC	(μL mL^{-1})	> 12	> 12	> 12	10	6	2
MFC	(μL mL^{-1})	> 12	> 12	> 12	> 12	> 12	4

Note: Means with different lowercase letters $^{(a\text{-}g)}$ in the same column and for the same tested oil compare the difference between doses, and means with different capital letters $^{(A\text{-}E)}$ in the same line and for the same dose compare the different sensitivities between fungi strains according to Fisher's test at $p \leq 0.05$.

[81] confirmed that *S. rosmarinus* EO was active against six major ginseng pathogens with MIC ranging from 0.1% to 0.5% (v/v), supporting the broad-spectrum antifungal potential of this oil. *L. dentata* EO showed moderate antifungal activity against the strains tested. The MIC values ranged between 8 and 12 μL mL^{-1}, however these applied doses were not lethal for all tested strains.

Among *Eucalyptus* species, *E. saligna*, even at the lowest concentration (2 μL mL^{-1}), exerted a total growth inhibition and fungicidal effect on *S. sclerotiorum* strain while it showed a strong antifungal activity with inhibition percentages ranging from 69.55 ± 3.4% to 95.69 ± 7.5% at the same concentration. Total inhibition was recorded for all strains when *E. camaldulensis* EO was applied at concentrations ≥ 4 μL mL^{-1}, with *S. sclerotiorum*, *R. solani*, and *F. culmorum* being the most sensitive strains, as indicated by their MFC ranging between 4 and 12 μL mL^{-1}. Similarly, *E. grandis* EO completely inhibited the growth of all strains at concentrations ≥ 4 μL mL^{-1}, except for *F. oxysporum*, which showed an inhibition percentage of 80.43 ± 6.5% at the highest concentration. Among the strains, *S. sclerotiorum* and *R. solani* exhibited the highest sensitivity to *E. grandis* EO, with MFC of 6 and 10 μL mL^{-1}, respectively. This indicates that both *E. camaldulensis* and *E. grandis* exert significant antifungal activity, with some variability in their effectiveness against different fungal species. Growth inhibition percentages between 49.13 ± 1.3% and 82.35 ± 2.3% were observed in all fungal strains when *E. leucoxylon* EO was used at the lowest concentration of 2 μL mL^{-1}. Complete inhibition was achieved for all strains, with MIC ranging from 4 to 12 μL mL^{-1}, except for *F. oxysporum* and *F. culmorum*, with 76.52 ± 3.4% and 73.48 ± 1.9% inhibition at the highest dose of 12 μL mL^{-1}. *E. leucoxylon* EO exhibited fungicidal effect against *S. sclerotiorum* with MFC of 4 μL mL^{-1}. Likewise, *E. cinerea* EO totally inhibited all strains growth at concentrations ranging between 4 and 12 μL mL^{-1} except for *F. oxysporum*. Both *S. sclerotiorum* and *F. culmorum* are the most sensitive strains with MFC of 6 and 12 μL mL^{-1}, respectively. *E. sideroxylon* EO at the lowest concentration (2 μL mL^{-1}) inhibited the growth of all tested strains from 57.83 ± 3.2% to 79.61 ± 0.6%, except for *S. sclerotiorum* which was totally inhibited at this concentration. Meanwhile, *Phoma* sp., and *R. solani* were fully inhibited at higher concentrations of 6 and 10 μL mL^{-1}, respectively. *E. sideroxylon* demonstrated a fungistatic effect only on *S. sclerotiorum* strain with MFC equal to 4 μL mL^{-1}. *E. lehmannii* EO exhibited effectiveness against both *S. sclorotiurom* and *R. solani* strains with MIC values of 6 and 8 μL mL^{-1} and MFC values of 6 and 10 μL mL^{-1}, respectively. A representative image illustrating the dose-dependent antifungal activity of *E. lehmannii* EO is shown in Figure S1.

The EOs of the seven studied *Eucalyptus* species resulted in antifungal activity for all six phytopathogenic fungal strains in a dose-dependent manner. The results of the present study are consistent with those of Ayed et al. [37] who evaluated eight *Eucalyptus* sp. against four *Fusarium* strains with *F. oxysporum* and *F. redolens* exhibiting the highest sensitivity to most of the EOs tested with complete mycelium growth inhibition at doses between 1 and 6 μL mL^{-1}. Amri et al. [38] obtained similar results with Tunisian *Eucalyptus* species at a dose of 4 μL mL^{-1}, particularly against *Fusarium* species, which appeared more susceptible than *Bipolaris sorokiniana*. Kouki et al. [68] revealed that *E. oleosa* EO showed the strongest inhibitory activity among

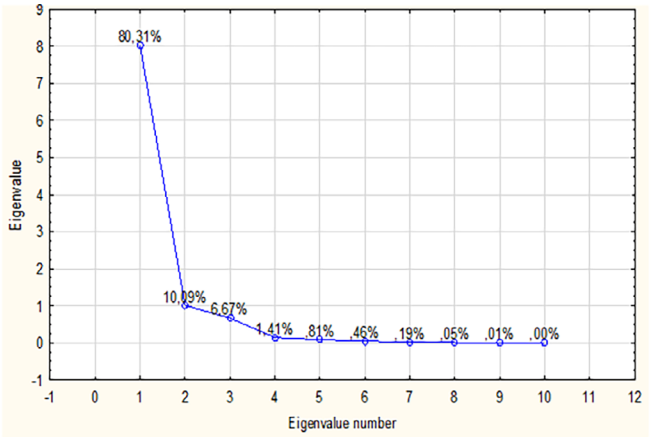

FIGURE 2 | Scree plot showing eigenvalues and variance explained by each principal component.

three Tunisian *Eucalyptus* species (*E. oleosa*, *E. pimpiniana*, and *E. polyanthemos*), fully inhibiting fungal growth at 6 μL mL^{-1}. In another study supporting these results, *E. camaldulensis* EO confirmed its fungicidal property against *Fusarium* species that affect maize with MICs and MFCs in the range of 7–10 μL mL^{-1} [56]. Caetano et al. [49] tested *Eucalyptus* EOs (*E. citriodora*, *E. camaldulensis*, *E. grandis*, and *E. microcorys*) in vivo against the agent of coffee leaf rust (*Hemileia vastatrix*), reporting good antifungal activity for most species, except *E. microcorys*. Similarly, *E. staigeriana*, *E. globulus*, and *Cinnamomum camphora* EOs were effective for both in vitro and in vivo assays against *Alternaria solani* causing early blight disease in tomato [82]. Umereweneza et al. [83] found that *E. melliodora* EO was the most effective among the tested species, inhibiting the growth of food spoilage fungi and aflatoxin-producing *Aspergillus* species with MICs varying from 3.3 to 8.1 mg mL^{-1} and complete inhibition of aflatoxins production at 6 and 7 μL mL^{-1}.

The antifungal activity observed in this study, as well as in previous reports, appears to be closely associated with the chemical composition of the EOs. Differences in bioactive compound profiles likely explain the variations in activity among EO species and fungal strains, which is reflected in the differential sensitivity observed across fungi. This relationship, along with potential interactions between constituents, is explored in detail in the following section using chemometric analyses.

2.4 | Multivariate Analysis of EO Composition

2.4.1 | PCA

PCA was applied to the relative abundance of identified EO constituents to explore compositional variability and identify potential chemotypes among the studied species. Based on the eigenvalues of the correlation matrix and considering chemical constituents as active variables, the first principal component (PCA1) accounted for 80.31% of the total variance, while the second principal component (PCA2) explained 10.09%, bringing the cumulative variance explained to 90.4% (Figure 2). The high cumulative variance explained by the first two principal components demonstrates their effectiveness in capturing the

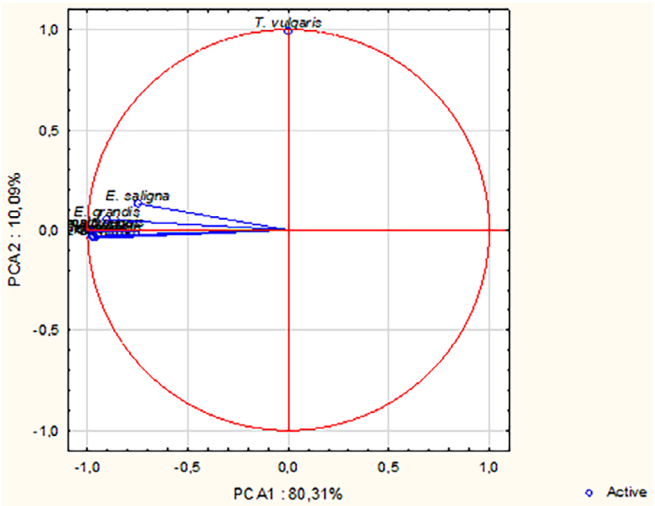

FIGURE 3 | PCA loading plot of active chemical compounds based on essential oil composition.

major sources of variation in EO composition, thereby reducing the complexity oft the dataset with minimal information loss [84]. Consistent with previous EO stuides, PCA in the present analysis transformed correlated chemical variables into orthogonal components that captured the major trends in composition, facilitating the identification of chemotypes and compositional groupings [85].

The PCA biplot (Figure 3) revealed a clear separation of *T. vulgaris*, which was strongly associated with the vertical axis (PCA2), suggesting a distinct chemical profile, consistent with literature describing it as a thymol-rich chemotype [73]. PCA1 appears to be primarily driven by the variation in 1,8-cineole content, a major component in several *Eucalyptus* species, supporting previous reports of cineole-type chemotypes within this genus [46, 47]. Meanwhile, *E. grandis* and *E. saligna* showed partial separation from the main *Eucalyptus* cluster, a pattern that may be attributed to their relatively higher concentrations of *p*-cymene, a compound less abundant in other *Eucalyptus* species. In summary, the application of PCA in this study proved to be a powerful chemometric tool for visualizing compositional variability, distinguishing species and chemotypes, and supporting chemotaxonomic classification based on EO profiles [84].

2.4.2 | HCA

The HCA based on Euclidean distances provided further insight into species relationships (Figure 4). The dendrogram revealed two major clusters: the first included all *Eucalyptus* species along with *L. dentata* and *S. rosmarinus*, forming multiple subclusters with relatively low linkage distances (< 40), indicating compositional similarity. The second cluster, clearly separated at a linkage distance > 80, comprised only *T. vulgaris*, emphasizing its chemically distinct profile. This separation in both PCA and HCA reinforces the uniqueness of *T. vulgaris*, likely due to its well-documented high content of thymol. Within the *Eucalyptus* group, *E. camaldulensis*, *E. leucoxylon*, and *E. lehmannii* formed a tight sub-cluster, supported by minimal Euclidean distances (13–19 units), suggesting a shared profile dominated by 1,8-cineole. In

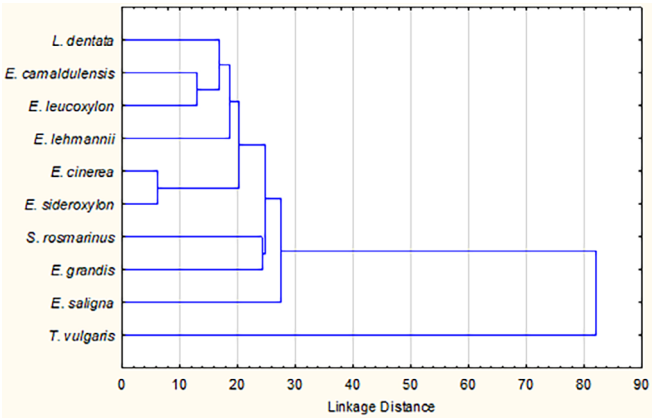

FIGURE 4 | HCA dendrogram of essential oils based on their chemical composition.

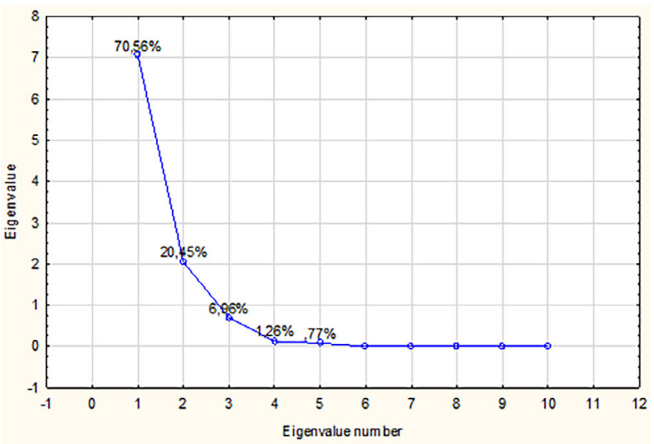

FIGURE 5 | **FIGURE 5** Scree plot showing eigenvalues and variance explained by each principal component based on antifungal activity (MIC values).

contrast, *E. saligna* and *E. grandis* diverged from this group, likely due to differing proportions of *p*-cymene.

2.5 | Multivariate Analysis of Antifungal Activity

To better understand the relationship between EO composition and antifungal efficacy, multivariate statistical methods were applied using GC–MS/FID-derived chemical composition data integrated with minimum inhibitory concentration (MIC) values. These analyses aimed to classify EOs according to their antifungal profiles, identify bioactive compounds associated with potency and evaluate the predictive potential of chemical constituents on antifungal outcomes.

2.5.1 | PCA Based on MIC Values

According to the eigenvalues of the correlation matrix, the first principal component (PCA1) accounted for 70.58% of the total variance, while the second component (PCA2) explained an additional 20.45%, resulting in a cumulative explanation of 91.01% of the overall variation in antifungal activity (Figure 5). This

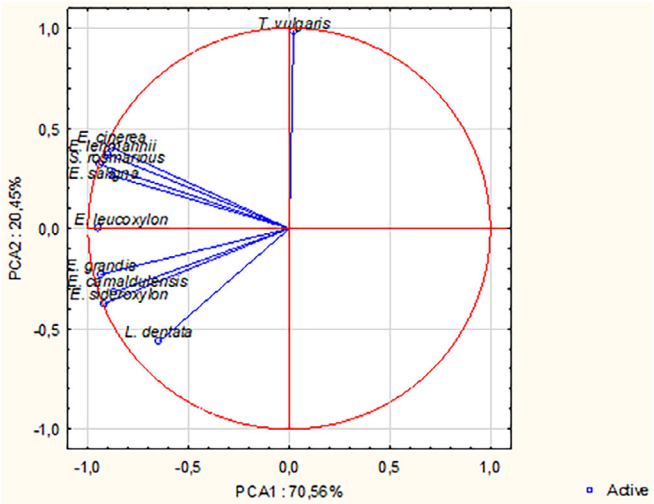

FIGURE 6 | PCA loading plot of essential oils based on antifungal activity (MIC values).

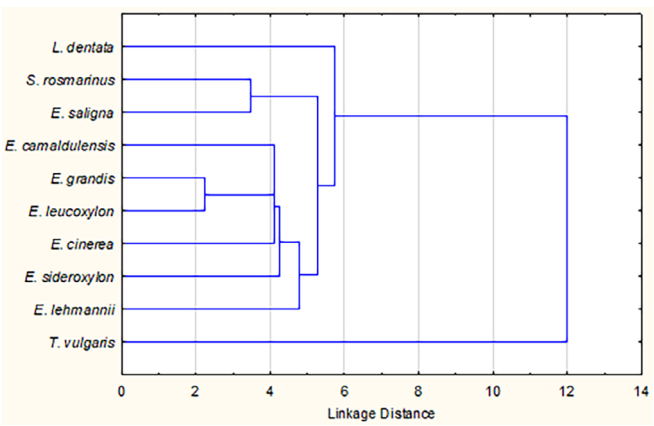

FIGURE 7 | HCA dendrogram of essential oils based on their antifungal activity (MIC values).

indicates that the variability in MIC responses across EOs is effectively captured by the first two principal components.

The PCA biplot revealed a clear separation of *T. vulgaris*, which forms a distinct group positioned strongly along PCA2, indicating its superior antifungal potency (Figure 6). This distinct positioning is likely attributable to its high levels of thymol, a compound well-documented for its broad-spectrum antifungal effects [74].

2.5.2 | HCA of Antifungal Activity

The HCA dendrogram (Figure 7), based on Euclidean distances, confirmed the PCA findings by revealing two main clusters. Cluster A included the majority of *Eucalyptus* species, *S. rosmarinus*, and *L. dentata*, forming tighter sub-groups. Within this, a close grouping of *E. camaldulensis*, *E. leucoxylon*, and *E. grandis* was observed, with minimal distances (2.2–5.8), indicating similar MIC profiles likely due to comparable levels of 1,8-cineole and other shared compounds [37]. Cluster B consisted of *T. vulgaris*, which separated at a high linkage distance (> 12), clearly reflecting its distinct and strong documented antifungal efficacy [45].

This multivariate analysis reveals that the EOs exhibit varying degrees of antifungal efficacy, with *T. vulgaris* clearly standing out for its potency. Meanwhile, moderate clustering among most *Eucalyptus* species suggests shared but limited activity, while *E. lehmannii* emerges as the least effective among the tested oils.

2.5.3 | Pearson Correlation Between MIC Values and Compound Abundance

Pearson correlation analysis was performed to assess the relationships between the chemical composition of EOs and their antifungal activity, as measured by MICs against the tested phytopathogenic fungi. Given the large number of identified compounds, only those exhibiting statistically significant ($p < 0.05$) and biologically relevant correlations were retained for interpretation. Table 3 presents the Pearson correlation coefficients (r) alongside their corresponding p values. Significant associations are highlighted to underscore the most relevant relationships between compound abundance and antifungal effectiveness.

Several compounds demonstrated strong negative correlations with MIC values, indicating a potential contribution to antifungal activity. Notably, thymol, geranial, α-terpinene, γ-terpinene, myrcene, 3-carene, and *cis*-linalool oxide exhibited highly significant inverse correlations ($r \leq -0.87$, $p < 0.05$) across multiple fungal species, suggesting their key roles in the antifungal efficacy of the EOs tested [72, 87]. Moderate negative correlations were observed for E-caryophyllene, particularly against *Fusarium* species, indicating possible species-dependent selective antifungal activity, consistent with a previous report on E-caryophyllene-rich oils [88]. Conversely, 1,8-cineole showed positive correlations with MIC values, especially against *S. sclerotiorum*, implying reduced antifungal activity or potential antagonistic effects. This finding aligns with an earlier study suggesting that 1,8-cineole may lack potent antifungal properties or interfere with the activity of other compounds [89]. Several other compounds, including *endo*-fenchol, *p*-cymenene, α-pinene oxide, verbenone, α-muurolol, and β-eudesmol, also correlated positively with MIC values, indicating that their increased presence is associated with reduced antifungal effectiveness.

2.5.4 | PLS Regression Modeling of Antifungal Activity

PLS regression analysis was performed to model the relationship between EO chemical composition and antifungal activity, with MIC values used as the dependent variable. The PLS model extracted five components, cumulatively explaining 94.5% of the variance in MIC values (R^2Y) (Figure 8). Detailed statistics for each component, including R^2X, R^2Y, eigenvalues, and number of iterations, are presented in Table S2.

Variable importance in projection (VIP) scores were used to evaluate the contribution of individual compounds to the model. A total of 27 volatile compounds had VIP scores ≥ 1, indicating significant influence on MIC variability. These compounds are listed in Table 4. The most influential volatiles included *cis*-linalool oxide (VIP = 2.117), γ-terpinene (2.115), 3-carene (2.106), α-terpinene (2.092), and thymol (2.004). The full list of all analyzed compounds and their VIP scores is available in Table S3.

TABLE 3 | Pearson correlation between chemical composition and minimum inhibitory concentrations (MIC) against various fungal species.

Compound	MIC Fo		MIC Fp		MIC Fc		MIC Rs		MIC Ps		MIC Ss	
	r	*p*	*r*	*p*	*r*	*p*	*r*	*p*	*r*	*p*	*r*	*p*
α-Thujene	**−0.874**	**0.010**	**−0.876**	**0.010**	**−0.838**	**0.019**	**−0.855**	**0.014**	−0.596	0.158	−0.480	0.275
Myrcene	**−0.974**	**0.000**	**−0.877**	**0.010**	**−0.948**	**0.001**	**−0.865**	**0.012**	−0.691	0.085	−0.293	0.524
3-Carene	**−0.956**	**0.001**	**−0.887**	**0.008**	**−0.938**	**0.002**	**−0.939**	**0.002**	−0.713	0.072	−0.479	0.277
α-Terpinene	**−0.976**	**0.000**	**−0.905**	**0.005**	**−0.961**	**0.001**	**−0.940**	**0.002**	−0.726	0.065	−0.432	0.334
1,8-Cineole	**+0.835**	**0.019**	**+0.850**	**0.015**	**+0.842**	**0.018**	**+0.817**	**0.025**	+0.503	0.250	+0.250	0.588
γ-Terpinene	**−0.964**	**0.000**	**−0.893**	**0.007**	**−0.947**	**0.001**	**−0.950**	**0.001**	−0.722	0.067	−0.475	0.281
cis-Linalool oxide	**−0.979**	**0.000**	**−0.870**	**0.011**	**−0.956**	**0.001**	**−0.923**	**0.003**	−0.717	0.070	−0.401	0.372
p-Cymenene	+0.078	0.868	+0.386	0.392	+0.114	0.808	+0.361	0.426	+0.282	0.540	**+0.846**	**0.016**
o-Guaiacol	+0.146	0.756	+0.498	0.255	+0.222	0.632	+0.128	0.785	+0.645	0.117	**+0.803**	**0.030**
α-Pinene oxide	−0.093	0.843	+0.261	0.572	−0.048	0.919	+0.258	0.576	+0.055	0.907	**+0.766**	**0.045**
trans-Sabinene hydrate	−0.093	0.843	+0.261	0.572	−0.048	0.919	+0.258	0.576	+0.055	0.907	**+0.766**	**0.045**
endo-Fenchol	+0.430	0.335	+0.711	0.073	+0.504	0.249	+0.548	0.203	**+0.757**	**0.049**	**+0.908**	**0.005**
trans-Rose oxide	−0.029	0.951	+0.368	0.417	+0.032	0.946	+0.248	0.591	+0.249	0.591	**+0.871**	**0.011**
Verbenone	+0.030	0.950	+0.383	0.397	+0.076	0.871	+0.319	0.485	+0.271	0.557	**+0.889**	**0.008**
Piperitone	+0.116	0.805	+0.346	0.447	+0.136	0.772	+0.407	0.365	+0.241	0.602	**+0.756**	**0.049**
Geranial	**−0.996**	**0.000**	**−0.818**	**0.024**	**−0.962**	**0.001**	**−0.885**	**0.008**	−0.702	0.079	−0.269	0.560
Citronellyl formate	−0.093	0.843	+0.261	0.572	−0.048	0.919	+0.258	0.576	+0.055	0.907	**+0.766**	**0.045**
Thymol	**−0.962**	**0.001**	**−0.881**	**0.009**	**−0.940**	**0.002**	**−0.947**	**0.001**	−0.711	0.074	−0.471	0.286
p-Vinylguaiacol	+0.120	0.798	+0.379	0.402	+0.145	0.757	+0.386	0.393	+0.291	0.527	**+0.796**	**0.032**
Myrtenyl acetate	−0.093	0.843	+0.261	0.572	−0.048	0.919	+0.258	0.576	+0.055	0.907	**+0.766**	**0.045**
Piperitenone	−0.093	0.843	+0.261	0.572	−0.048	0.919	+0.258	0.576	+0.055	0.907	**+0.766**	**0.045**
cis-Carvyl acetate	−0.093	0.843	+0.261	0.572	−0.048	0.919	+0.258	0.576	+0.055	0.907	**+0.766**	**0.045**
α-Copaene	−0.093	0.843	+0.261	0.572	−0.048	0.919	+0.258	0.576	+0.055	0.907	**+0.766**	**0.045**
β-Cubebene	−0.093	0.843	+0.261	0.572	−0.048	0.919	+0.258	0.576	+0.055	0.907	**+0.766**	**0.045**
E-Caryophyllene	**−0.822**	**0.023**	**−0.804**	**0.029**	**−0.766**	**0.045**	**−0.755**	**0.050**	−0.520	0.232	−0.404	0.369
Ar-Curcumene	−0.066	0.889	+0.291	0.527	−0.015	0.975	+0.289	0.530	+0.074	0.874	**+0.762**	**0.047**
(*E*)-*γ*-Bisabolene	−0.093	0.843	+0.261	0.572	−0.047	0.919	+0.258	0.576	+0.054	0.907	**+0.766**	**0.045**
α-Calacorene	−0.060	0.898	+0.274	0.551	−0.017	0.970	+0.298	0.515	+0.078	0.867	**+0.776**	**0.040**

(Continues)

TABLE 3 | (Continued)

Compound	MIC Fo		MIC Fp		MIC Fc		MIC Rs		MIC Ps		MIC Ss	
	r	*p*	*r*	*p*	*r*	*p*	*r*	*p*	*r*	*p*	*r*	*p*
β-Calacorene	+0.299	0.514	+0.520	0.231	+0.351	0.439	+0.403	0.369	+0.708	0.075	**+0.971**	**0.000**
Humulene epoxide II	+0.618	0.139	**+0.779**	**0.039**	+0.733	0.061	+0.616	0.140	+0.520	0.231	−0.007	0.987
α-Muurolol	+0.000	1.000	+0.405	0.366	+0.066	0.888	+0.237	0.608	+0.327	0.474	**+0.895**	**0.006**
β-Eudesmol	+0.168	0.718	+0.5639	0.187	+0.265	0.565	+0.347	0.444	+0.540	0.211	**+0.885**	**0.008**
Caryophyllenol II	−0.093	0.843	+0.2611	0.572	−0.047	0.919	+0.258	0.576	+0.054	0.907	**+0.766**	**0.045**
Eudesma-4(15),7-dien-1b-ol	−0.093	0.843	+0.2611	0.572	−0.047	0.919	+0.258	0.576	+0.054	0.907	**+0.766**	**0.045**
(2*Z*,6*E*)-Farnesol	−0.093	0.843	+0.2611	0.572	−0.047	0.919	+0.258	0.576	+0.054	0.907	**+0.766**	**0.045**
α-Sinensal	−0.084	0.858	+0.2671	0.563	−0.036	0.939	+0.270	0.557	+0.063	0.893	**+0.764**	**0.045**
2-Heptadecanone	−0.093	0.843	+0.2611	0.572	−0.047	0.919	+0.258	0.576	+0.054	0.907	**+0.766**	**0.045**

Note: Values represent Pearson correlation coefficients (r) and corresponding significance levels (p) between minimum inhibitory concentration (MIC) values and compound abundance. Correlations are considered significant at $p < 0.05$. Bold values indicate significant correlations ($p < 0.05$).

Abbreviations: Fc, *Fusarium culmorum*; Fo, *Fusarium oxysporum*; Fp, *Fusarium proliferatum*; Ps, *Phoma* sp.; Rs, *Rhizoctonia solani*; Ss, *Sclerotinia sclerotiorum*.

FIGURE 8 | Cumulative R^2Y and Q^2 values for the five PLS components explaining the variance in MIC values.

TABLE 4 | Key volatile compounds contributing to antifungal activity based on partial least squares (PLS) regression (VIP ≥ 1).

No.	Compound name	VIP score	Rank
16	*cis*-Linalool oxide	2.117	1
15	*γ*-Terpinene	2.115	2
11	3-Carene	2.106	3
12	*α*-Terpinene	2.092	4
48	Thymol	2.004	5
14	1,8-Cineole	1.906	6
44	Geranial	1.896	7
9	Myrcene	1.850	8
2	*α*-Thujene	1.836	9
17	*α*-Terpinolene	1.432	10
92	*β*-Eudesmol	1.293	11
87	Humulene epoxide II	1.285	12
31	Lavandulol	1.259	13
41	Pulegone	1.253	14
65	*β*-Gurjunene	1.235	15
5	Thuja-2,4(10)-diene	1.180	16
86	*epi*-Globulol	1.159	17
63	E-Caryophyllene	1.091	18
37	Verbenone	1.091	19
22	Linalool	1.087	20
81	*β*-Calacorene	1.080	21
43	Piperitone	1.061	22
75	*β*-Curcumene	1.060	23
82	Ledol	1.048	24
64	*β*-Cedrene	1.043	25
28	Camphor	1.010	26
25	*trans*-Rose oxide	1.008	27

3 | Conclusions

The EOs extracted from *L. dentata*, *S. rosmarinus*, *T. vulgaris*, and seven *Eucalyptus* species demonstrated significant potential as natural alternatives to synthetic fungicides. Chemical analyses revealed that these EOs are rich in oxygenated monoterpenes and monoterpene hydrocarbons, which underlie their antifungal and antioxidant properties. Multivariate analyses of chemical composition and antifungal activity (MIC values) distinguished two main groups. *T. vulgaris* EO, characterized by high thymol content, exhibited the strongest antifungal efficacy against all tested phytopathogenic fungi and the highest antioxidant potential, surpassing the standard antioxidant Trolox. In contrast, *L. dentata*, *S. rosmarinus*, and most *Eucalyptus* species, dominated by 1,8-cineole, clustered together and showed moderate antifungal activity, with *E. lehmannii* displaying the weakest effect. Further chemometric analyses, including PCA, HCA, Pearson correlation, and PLS regression, revealed significant inverse correlations between antifungal activity and key volatile compounds. Thymol, geranial, *cis*-linalool oxide, γ-terpinene, α-terpinene, and 3-carene were identified as the principal bioactive constituents driving antifungal effects. These findings support the use of EOs from both Lamiaceae and Myrtaceae families as eco-friendly, sustainable alternatives to synthetic fungicides. However, variations in MIC and MFC values across studies emphasize the need for standardized in vitro methods and detailed chemical characterization to optimize their application. Future research should focus on assessing field efficacy, stability, and economic feasibility, as well as exploring synergistic effects with other biocontrol agents to develop robust, environmentally friendly pest management strategies.

4 | Experimental Section

4.1 | Plant Material

Plant samples were randomly collected during the summer of 2023 from different Tunisian regions. Aerial parts of *L. dentata*, *S. rosmarinus*, and *T. vulgaris* (Lamiaceae) and leaves from seven *Eucalyptus* species (Myrtaceae): *E. camaldulensis*, *E. cinerea*, *E. grandis*, *E. lehmannii*, *E. leucoxylon*, *E.saligna*, and *E. sideroxylon* were randomly harvested from multiple plants or trees and combined to obtain a representative homogenized sample (Table 5). The plant materials were stored in a glass greenhouse for drying for 5 days. Once dried, they were kept in paper bags at room temperature until further processing. Plant species were identified by Professor Lamia Hamrouni. Voucher specimens were deposited at the herbarium division of the National Institute of Researche on Rural Engineering, Water and Forests (INRGREF).

4.2 | EO Extraction

The EOs were obtained by hydrodistillation of 100 g of dried plant material for 3 h using a Clevenger-type apparatus (SAF Wärmetechnik LabHEAT KM-ME, 1000 mL, SAF GmbH, Hamm, Germany). Distillation was performed using distilled water at a plant material-to-water ratio of 10:1 (v/w). The extracted oils were dried over anhydrous sodium sulfate and stored in amber glass vials at 4°C until use.

4.3 | GC–FID and GC–MS Analysis

Chemical composition of EOs was determined using GC–FID and GC–MS.

GC–FID analyses were assessed on an HP6890 (II) gas chromatograph (Agilent Technologies, Santa Clara, CA, USA) equipped with a FID, by using an apolar HP-5 capillary column [30 m × 0.32 mm (i.d.), 0.25-µm film thickness] (Agilent Technologies). Oil diluted in hexane was injected with a split ratio of 1:50 and a flow percentage of 1.2 mL min^{-1}. The oven temperature program was: 40°C for 1 min; 40°C–260°C at a rate of 5°C min^{-1}; 260°C isothermally for 4 min. The temperature of the injector and the detector was maintained at 250°C and 300°C, respectively.

GC–MS analyses were performed on an HP 6890 N gas chromatograph coupled to an HP 5975 mass spectrometer (Agilent Technologies). The separation of volatile compounds was assessed using an HP-5MS capillary column (60 m × 0.25 mm; 0.25 mm) (Agilent Technologies). The temperature of the oven ramped from 40°C to 280°C at a rate of 5°C min^{-1}. Helium was used as a carrier gas at a flow speed of 1.2 mL min^{-1}. Scan mass range was 50–550 m/z at a sampling speed of 1 scan s^{-1}.

A standard dilution of a C_6–C_{25} *n*-alkane series was prepared to calculate retention indices (*Ri*). The EO compounds were identified by GC–MS through comparison of their relative *Ri* and mass spectra with those from corresponding data (Wiley 275 L library) and/or reported in the literature [90]. GC–FID was used for the quantitative determination of relative abundance, with percentages of each compound calculated from the electronic integration of its relative FID peak area without including a correction factor.

4.4 | Antioxidant Activity Assays

The method of Hamdeni et al. [91] was used for the evaluation of the free RSA of EOs. Briefly, 50 µL of various dilutions of EOs were mixed with 200 µL of 0.1 mM methanolic DPPH solution (Sigma-Aldrich, St. Louis, MO, USA). The mixture was then shaken vigorously and allowed to stand at room temperature for 30 min in the dark. The decrease in absorbance at 517 nm was measured spectrophotometrically versus DPPH standard solutions. The RSA of the oil expressed as % inhibition of DPPH was calculated using the formula: % inhibition = $[(A_0 - A_s)/A_0] \times 100$, where A_0 and A_s are the absorbance values of the control and that of the sample, respectively. The concentration (µg mL^{-1}) that allowed to 50% inhibition (IC_{50}) was calculated from the graph of RSA percentage against oil concentration. All results were reported as mean ± standard deviation of three measurements.

The antioxidant activity was estimated by using the ABTS (Sigma-Aldrich) based on the reduction of $ABTS^{\bullet+}$ radicals by antioxidants present in the EOs. ABTS radical cation ($ABTS^{\bullet+}$) was produced by mixing a 7 mM ABTS solution with 2.45 mM $K_2S_2O_8$ in a ratio 1:1. The mixture was allowed to stand in the dark at room temperature for 12 h before use. For the assay, the $ABTS^{\bullet+}$ solution was diluted in methanol to an absorbance of 0.7 (±0.02) at 735 nm. Two hundred microliters of $ABTS^{\bullet+}$ solution were mixed with

TABLE 5 | Plant species, used part, period, and harvesting sites.

Species	Used part	Harvesting period	Site
Lavandula dentata	Aerial parts	April 2023	Chbedda, Ben Arous
Salvia rosmarinus			Korbous, Nabeul
Thymus vulgaris		July 2023	Krib, Siliana
Eucalyptus camaldulensis	Leaves	Mach 2023	Zarniza arboreta, Sejnane
Eucalyptus cinerea			Souinet arboreta, Ain Draham
Eucalyptus grandis			Zarniza arboreta, Sejnane
Eucalyptus lehmannii			Souinet arboreta, Ain Draham
Eucalyptus leucoxylon			Korbous arboreta, Nabeul
Eucalyptus saligna			Zarniza arboreta, Sejnane
Eucalyptus sideroxylon			Korbous arboreta, Nabeul

25 µL of the corresponding dilution of EOs. The absorbance reading was taken 30 min after initial mixing [92]. All the analyses were performed in triplicate and results were expressed as inhibition percentage of the radical cation $ABTS^+$ using the following formula: % inhibition ABTS = $[(A_0 - A_s)/A_0] \times 100$, where A_0 and A_s are the absorbance values of the control and that of the sample, respectively.

4.5 | Antifungal Activity

The antifungal effects of EOs were tested in vitro by the direct contact method on agar. The phytopathogenic fungal strains *F. culmorum*, *F. oxysporum*, *F. proliferatum*, *Phoma* sp., *R. solani*, and *S. sclerotiorum* were provided by the Plant Protection Laboratory of the National Agriculture Research Institute of Tunisia (INRAT, Tunisia).

EOs were solubilized in a Tween 20 solution (0.1%, v/v) (Sigma-Aldrich, P1379), and later they were incorporated at increasing concentrations in potato dextrose agar (PDA) medium (0, 2, 4, 6, 8, 10, and 12 µL mL^{-1}). Six-millimeter agar plugs of each fungal strain were deposited in the center of PDA plates [68]. Negative controls contained only 0.1% Tween 20 (without EOs) and positive controls consisted of PDA plates containing the synthetic fungicide mancozeb at 2 g L^{-1}. These controls were included to ensure that any observed antifungal activity was due to the EOs and not to the solubilizing agent (Tween 20) or the test conditions. All tests, including controls, were carried out in triplicate. Incubation was performed at 24°C for 5 days. The inhibition percentage of fungal growth was determined by the following formula: Inhibition of fungal growth (I %) = $[(D - D_i)/D \times 100]$ where D and D_i are the diameters of mycelial growth in control and treatment.

MIC is defined as the lowest dose at which there is complete inhibition of fungal growth. To establish the MFCs, the inhibited fungal disks were inoculated into PDA plates without EO and their growth was observed. After 3 days of incubation, MFC was obtained as the lowest MIC at which no growth observed in the plates after culturing [37].

4.6 | Statistical and Chemometric Analysis

All of the experiments were carried out in three replicates, with the results represented as mean ± standard deviation. Statistical analyses were performed with STATISTICA software 10. One-way analysis of variance (ANOVA) was conducted to evaluate the effects of treatments, followed by Fisher's least significant difference (LSD) test to compare means at a significance level of $p < 0.05$. Multivariate analyses were also performed with STATISTICA software 10.

Multivariate analyses were applied separately to chemical composition and antifungal activity data. Chemical composition data consisted of the relative abundances (%) of GC–MS/FID-identified compounds. PCA and HCA (using Euclidean distances and single linkage) explored patterns and clustering among EOs based on their volatile profiles and MIC values. Pearson correlation analysis identified significant associations between compound abundance and antifungal activity. In addition, PLS regression modeling was used to determine key bioactive compounds influencing antifungal effects, with VIP scores highlighting the most relevant constituents. These combined chemometric approaches ensured an in-depth interpretation of chemical composition data in relation to biological activity.

Author Contributions

Imtinene Hamdeni: conceptualization, writing – original draft preparation, writing – review and editing. **Ismail Amri**: conceptualization. **Mounir Louhaichi**: conceptualization, writing – review and editing. **Abdennacer Boulila**: conceptualization, writing – review and editing. **Samia Gargourie**: conceptualization. **Juan José R. Coque**: conceptualization, writing – review and editing, funding acquisition. **Lamia Hamrouni**: conceptualization, writing – review and editing, funding acquisition. All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

The authors declare no conflicts of interest

Data Availability Statement

The authors have nothing to report.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.

Supporting File 1: cbdv70972-sup-0001-SuppMat.docx.