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Original Research Article

Chemical Composition and Antifungal Activity of the Essential Oil from *Deverra Tortuosa* against Phytopathogenic Fungi

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The hydrodistilled essential oil of *Deverra tortuosa*was DC. Analyzed by GC–MS. Twenty five compounds were identified, of which terpinene-4-ol (24.21 %), bisabolene (12.61 %), apiol (11.56 %), 2-allyl-p-cresol (6.44 %), bornyl acetate (4.77 %), σ-Elemene (4.04 %),myristicin (3.80 %),iso thymol methyl ether (3.61 %) and methyl eugenol (3.05 %) were the major compounds. Essential oil of *Deverra tortuosa* was tested for anti-fungal activity, which was determined by disc diffusion and minimum inhibitory concentration (MIC) determination methods. The oil displayed great potential of anti-fungal activity as a mycelial growth inhibitor against the tested phytopathogenic fungi such as *Fusarium oxysporum*, *Fusarium moniliforme*, *Fusarium solani*, and *Botrytis fabaea*. It showed a complete inhibition in radial growth at 4µl/ml PDA.

Keywords Antifungal activity, *Deverra tortuosa* DC, essential oil, GC–MS.

INTRODUCTION

The increasing economic implications caused by phytopathogenic fungi increased the need to produce safer food crops and to develop new anti-fungal agents. In recent years, interest has been generated in the development of safer antifungal agents such as plant-based essential oils and extracts to control phytopathogens in agriculture (Costa *et al*., 2000).

In general, plant-derived extracts and essential oils are considered as non-phytotoxic compounds and potentially effective against plant pathogenic fungi (Pandey *et al*., 1982). The botanicals are non pollutive, cost effective, non-hazardous and do not disturb the ecological balance (Joseph *et al*., 2008 *Deverra tortuosa*) is a perennial bushy plant from family Apiaceae**.** It grows naturally in sandy and stony plains (Boulos, 2000). Several phytochemical studies have demonstrated the presence of important bioactive compounds in different parts of the plant. flavonoidal glycosides, essential oil, coumarins and unsaturated sterols (Ahmed *et al*, 1969).

The plant is used traditionally as carminative, diuretic, anti-asthmatic analgesic; it is also used to relieve stomach pain, and against intestinal parasites (Boukef, 1982; Mahran *et al*, 1989). Therefore, the aim of the present study is (a) To examine the chemical composition of the essential oil isolated from the aerial parts of *D. tortuosa* by GC–MS; (b) To evaluate the anti-fungal activity of essential oil against certain important phytopathogens.

MATERIALS AND METHODS

Plant Material

The aerial parts of *Deverra tortuosa* were collected from the Sadat city desert, Menoufia governorate at flowering stage in April 2011. Fresh plant material was freed from foreign materials, washed, shade dried and then crushed using electrical blender and stored in airtight bottles.

Isolation Of The Essential Oil

The air-dried plant material weighing (100 g) of *D. The tortuosa* were subjected to hydrodistillation for 3 hours using a Clevenger type apparatus. The oil preserved in a sealed vial at 4 ˚C until further analysis.

Gas Chromatography–Mass Spectrometry

The analysis of the essential oil was performed using an HP 6890 Series Gas Chromatograph System with an HP 5973 Mass Selective Detector equipped with TR-FAME (Thermo 260

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M142 P) (70% cyanopropyl polysilphenylene-siloxane) capillary column (30 m× 0.25 mm i.d., 0.25 µm). For GC-MS detection, an electron ionization system with ionization energy of 70 EV was used. Helium was used as the carrier gas at a flow rate of 1.5 ml/min. The injector and MS transfer line temperatures were set at 200°C and 250°C, respectively. Essential oil solution l µl (5 µl/1 ml chloroform) was injected and analyzed with the column held initially at 80°C for 2 min and then increased to 230°C with a 3°C/min heating ramp and subsequently kept at 230 °C for 5 min. Qualitative Identification of the different constituents was performed by comparison of their relative retention times and mass spectra with those of authentic reference compounds (fatty acid methyl esters, purity 98% by GC). Also, probability merges, search software and the NIST MS spectra search program were used.

Fungal Pathogens

The plant pathogenic fungi were provided by the department of microbiology, Faculty of science, Mansoura University. The Pure cultures of the fungal species from agar slants were sub-cultured onto the PDA and incubated at 25°c for 3-7 days. The Petri dishes were flooded with 8-10 ml of sterile distilled water and the spores were scraped using a sterile spatula (Mahesh and Satish, 2008). Then, the spore suspensions were adjusted to a final concentration of 1×106 spores/ ml using a haemocytometer.

Antifungal Activity Of Essential Oil

The inhibitory effect of essential oil on the mycelial radial growth of fungi was tested by the agar dilution method as described by Zabka*et al*, (2009). Essential oil was diluted in PDA medium at concentrations (2, 3, 4 µl/ml). The prepared petri dishes (9.0cm diameter) were aseptically inoculated with assay disc (0.7 cm) cuts from the periphery of 7-day-old culture of the target fungi. The plates were incubated at 25 ºC for 3– 7 days, until the growth in the control plates reached the edge of the plates. The control sets were prepared subsequently using sterile distilled water instead of oil. The plates were prepared in triplicates for each treatment.

The relative growth inhibition of treatment compared to negative control was calculated by percentage using the formula: Inhibition (%) = [(C-T)/C] x100, Where C and T are the radial growth (mm) of the fungus in the control and treated plates, respectively. Minimum inhibition concentration of essential oil was determined by the method of graded concentrations of the oil in PDA. Essential oil incorporated into the medium to obtain the following concentrations :(2.5, 2.6, 2.7…To 4 µl/ml) in the PDA. The prepared petri dishes (9.0cm diameter) were aseptically inoculated with assay disc (0.7 cm) cuts from the periphery of 7-day-old culture of the target fungi. The control sets were kept parallel to the treatment sets without essential oil.

The inoculated petri plates were incubated at 25 ◦C for 3-7 days until the growth in the control plates reached the edge of the plates. The MIC was regarded as the lowest concentration of oil that did not permit any visible growth when compared with control sets (Zabka *et al*., 2009). The nature of toxicity (fungistatic/fungicidal) of the essential oil was determined as described by (Kumar *et al*., 2007). Inhibited fungal discs of oil treated plates were re-inoculated on fresh medium and the revival of their growth was observed.

Statistical analysis

All experiments were set up in a completely randomized design. Data were expressed as mean ± standard deviation. One way analysis of variance (ANOVA) was used to analyze the obtained results with SPSS 17.0 software package. Difference on statistical analysis of the data were considered significant at P<0.05.

RESULTS

Composition Of The Essential Oil

The chemical composition of the essential oil used as determined by GC-MS analysis is shown in Table 1. The essential oil was characterized by the presence of major compounds such as apiol bisabolene and terpinene-4-ol.

Antifungal Activity Of Essential Oil On Mycelial Growth

The effects of different concentrations of the essential oil on mycelial growth of tested fungi are shown in Table2. The essential oil inhibited the growth of fungi in a dose dependent manner. *F. oxysporum* was the most susceptible fungus to the essential oil. It showed a complete inhibition (100%) in radial growth at 3µl/ml followed by *F. oxysporum* with radial growth inhibition (88.9%) at 3µl/ml. The essential oil showed different MICs on tested fungi ranged from 3.1 µl/ml to 3.4µl/ml. The MFCs obtained are 3.1, 3.4, 3.5 and 3.6µl/ml for *F. moniliforme, F. oxysporum, F. solani* and *B. fabae* respectively. *F.o: fusarium oxysporum, F.m: fusarium moniliforme, F.s: fusarium solani, B.f: botrytis fabae*. RG: radial growth, I: inhibition percent, Means having the same letters in the same column are not significantly different at (p=0.05) level.

DISCUSSION

In this study, we found that the essential oil from *Deverra tortuosa* inhibited the mycelial growth of *F.oxysporum, F.moniliforme, F.solani* and *B.fabae*. These results confirm the literature data about the effectiveness of plant essential oil extracted by hydro-distillation on the growth of plant pathogenic fungi (Daferera *et al*., 2003). These inhibitory activities may be linked to the chemical composition of essential oil of *Deverra tortuosa*. In our study, the major constituents of plant essential oil were terpinen-4-ol; apiol and bisabolene. Previous work of (Morciaa *et al*, 2012) indicated that terpinene-4-ol has toxic effects on mycelium growth of phyto pathogenic fungi *in vitro*. Also, previous investigations demonstrated that apiol possesses antifungal activity against some agriculturally important fungi (Meepagala *et al.,* 2005; Rodrigo *et al*, 2012).

Table 1: Chemical composition of the essential oil from *Deverra tortuosa* essential oil as determined by GC-MS

PK#	COMPOUND	RT	Area	Quality	Ref
1	Linalool	6.01	0.14	95	NIST08.L
2	(E)-p-2-Menthen-1-ol	7.27	2.75	96	NIST08.L
3	Isothymol methyl ether	7.71	3.61	90	NIST08.L
4	Terpinene-4-ol	8.23	24.21	94	NIST08.L
5	(z) p- menth-2- en-1-ol	8.56	1.77	91	NIST08.L
6	Bornyl acetate	8.94	4.77	98	NIST08.L
7	Bisabolene	9.59	12.61	96	NIST08.L
8	σ-Elemene	10.27	4.04	84	NIST08.L
9	(z)p-menth-3-en-1-ol	10.92	1.52	91	NIST08.L
10	(R)-Citronellol	11.13	0.94	95	NIST08.L
11	Cis-Thujanol	12.73	0.85	72	NIST08.L
12	p-cymen-8-ol	14.15	0.85	87	NIST08.L
13	Propanal 2-methyl-3-phenyl	14.30	0.75	90	NIST08.L
14	Citronellyl ester	18.69	0.59	92	NIST08.L
15	Methyl eugenol	20.03	3.05	98	NIST08.L
16	p-cymene-7-ol	20.44	1.70	96	NIST08.L
17	p-thymol	21.78	1.42	95	NIST08.L
18	Anisol	24.26	1.53	50	NIST08.L
19	Myristicin	24.97	3.80	98	NIST08.L
20	Apiol	26.64	11.56	76	NIST08.L
21	Tropone	32.37	0.53	64	NIST08.L
22	Butylidenephthalide	34.40	2.77	97	NIST08.L
23	2-allyl-p-cresol	37.02	6.44	50	NIST08.L

Rt : Retention time

Table 2: Effect of different concentrations of *Deverra tortoise* essential oil on mycelial growth of tests fungi

Fungi	Radial growth and % inhibition of target fungi (mean±SE)							
	2 µl/ml		3 µl/ml		4 µl/ml		control	
	RG (cm)	I (%)	RG (cm)	I (%)	RG (cm)	I (%)	RG	I
F.o	4.60±0.0 [a]	48.8 ± 0.79	1.03±0.05 [b]	88.92±0.06	0.0±0.0 [c]	100.0±0.0	9±0 [d]	0
F.m	4.73±0.05 [e]	47.07±0.64	1.29±0.005 [f]	85.59±.06	0.0±0.0 [c]	100.0±0.0	9±0 [d]	0
F.s	4.23±0.05 [g]	52.22±0.63	1.45±0.05 [h]	83.88±0.55	0.0±0.0 [c]	100.0±0.0	9±0 [d]	0
B. f	4.86±0.02 [j]	45.92±0.32	2.51±0.03 [k]	82.18±0.35	0.0±0.0 [c]	100.0±0.0	9±0 [d]	0

Table 3: MIC and MFC of essential oil of *Deverra tortuosa* on tests fungi

fungus	MIC (µl/ml PDA)	MFC (µl/ml PDA)
F.oxysporum	3.2	3.4
F.moniliforme	3.1	3.1
F.solani	3.4	3.5
B.fabae	3.4	3.6

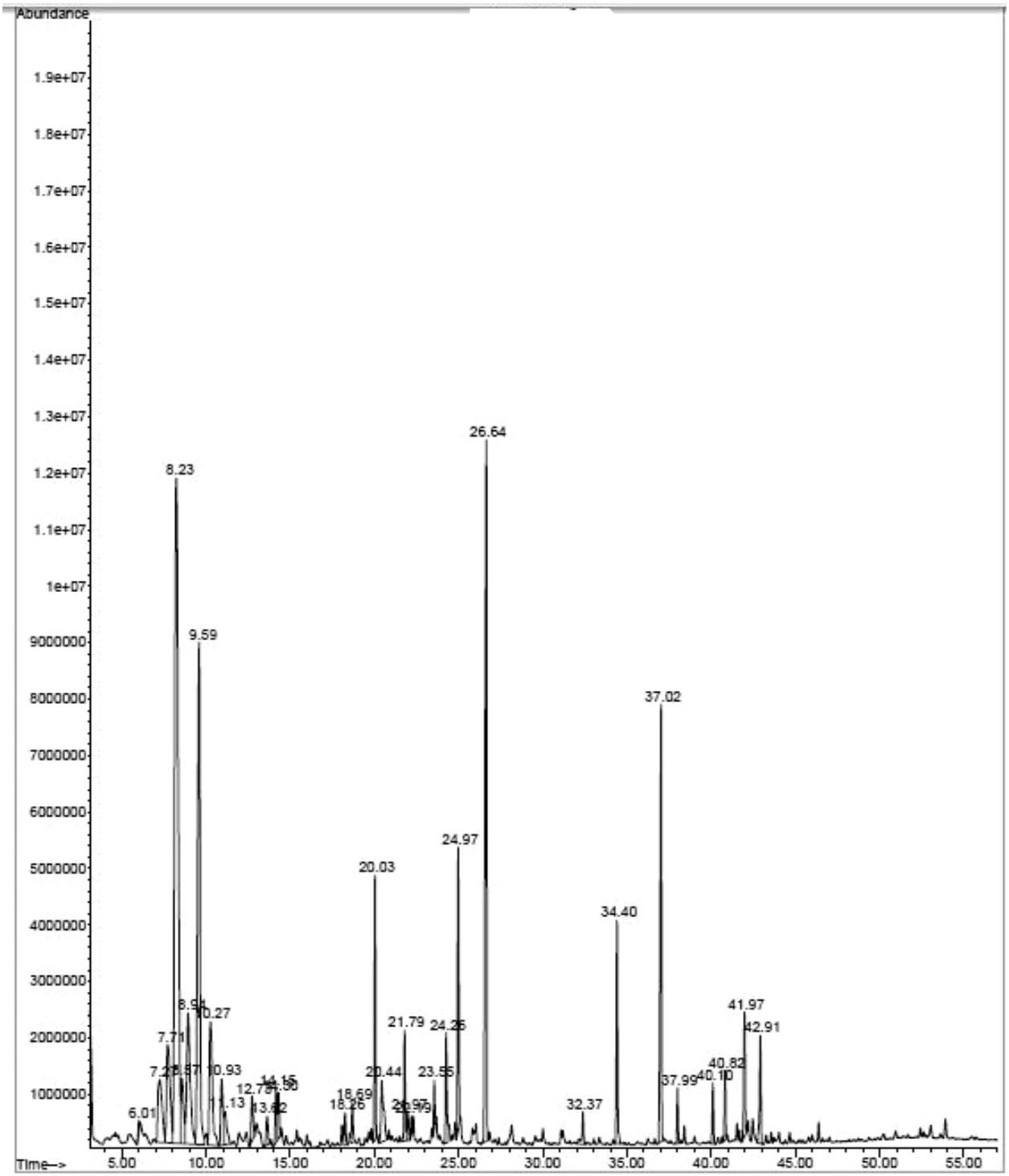

Figure 1: Total chromatogram of volatile components in *Deverra tortuosa* Essential oil.

Data for essential oil composition of *D. tortuosa* is abundant with variable results in terms of chemical composition. Abdel-Ghani and Hafez, (1995) reported that the main constituent of Dill oil is apiol (94.76 %). While, Al-Gaby and Allam, (2000) found that *D. tortuosa* aerial parts collected from Southern Sinai constitute of camphene (31.0%), pinene ,carvacrol, borneol, bornyl acetate and thymol as the major constituent. The presence of terpinen-4-ol in essential oil of *D. tortuosa* as a major compound is consistent with previous reports of (Abdelwahed *et al*., 2006). In conclusion, *Deverra tortuosa* essential oil could be applied as an alternative to synthetic fungicides for the control of tested fungi. It could also be screened to develop novel types of natural fungicides in the safe control of many agricultural plant pathogens causing crop losses.

CONCLUSION

All extracts of *Deverra tortuosa* DC that grows wild in Sadat city had antimicrobial activity against tested organisms and can

be used as a source of natural antimicrobial agents. Based on the results obtained from this study, wide availability of plant in Egypt and ease of application, It can safely be concluded that, the essential oil of the plant can be used on a wide scale in the organic farms for bean cultivation as potential alternative fungicides for seed treatment to protect them against soil borne pathogen or lessen the impact of the pathogen on faba bean crop.

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