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Volatile constituents of *Dietes bicolor* (Iridaceae) and their antimicrobial activity

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Abstract: Volatile oils from the leaves, flowers and rhizomes of *Dietes bicolor* (Steud.) Sweet ex Klatt (Iridaceae) were analyzed using GLC/FID and GLC/MS. A total of 84 compounds were identified accounting for 94.65, 95.63 and 87.09% in the hydrodistilled oils from flowers, leaves and rhizomes, respectively. Spathulenol (48.44%) represented the major component in the leaf oil, followed by dihydro-edulan I (6.25%), cubenol (6.00%) and τ-cadinol (5.90%). For the flower and rhizome oils, fatty acids, their esters, aliphatic hydrocarbons and their derivatives predominate. The antimicrobial activity of both leaf and flower oils was investigated against four bacteria in addition to four fungi using the micro-broth dilution method. The leaf oil showed a more potent antimicrobial activity as compared to the flower oil against most of the assessed bacteria and fungi, with higher activities against Gram- positive organisms showing MIC values of 115 and 460 μg/ml for *Bacillus subtilis* and *Streptococcus pneumonia*, respectively. Gram-negative bacteria were generally less susceptible (MIC > 2 mg/ml for both oils against *Escherichia coli*) and being completely ineffective against *Pseudomonas aeruginosa*. A relevant antifungal potency of the leaf oil against *Geotrichum candidum* and *Syncephalastrum racemosum* was also observed with MIC values of 115 and 920 μg/ml, respectively.

Keywords: antimicrobial activity; *Dietes bicolor*; GLC/MS; Iridaceae; volatile constituents.

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1 Introduction

Essential oils represent a fascinating mixture of plant secondary metabolites that have gained interest in both industrialized as well as developing countries owing to their remarkable therapeutic and commercial benefits. Essential oils, also known as volatile or ethereal oils, consist of a complex mixture of volatile components biosynthesized by living organisms [1]. They play a crucial role for plants such as for cooling, attraction or repellence of arthropods, in addition to protection against microbial infections [2]. Besides, they possess various biological and pharmacological properties such as antimicrobial, anti-inflammatory, antioxidant as well as cytotoxic activities [3–10], which are the main reason for being the core of their widely use in aromatherapy. Moreover, the emergence of antibiotic resistance among microorganisms constitutes a leading driving force to search for new and safe natural antimicrobials exemplified by the essential oils that can not only reduce the appearance but also the dissemination of the resistant pathogens [11].

The family Iridaceae represents one of the most important plant families for horticulture comprising about 72 genera and 2000 species [12, 13]. Members of the Iridaceae are perennial herbs or subshrubs with rhizomes, bulbs, or corms [14] that are widely grown as ornamentals owing to their beautiful, magnificent, and often fragrant flowers [15]. Traditionally, plants belonging to Iridaceae have been used for the alleviation of cold, flu, toothache and bruises, in addition to being effective in the treatment of malaria [16].

The family produces a rich diversity of secondary metabolites that includes alkaloids, isoflavonoids, flavonoids, monoterpenes, triterpenoids, naphthoquinones, anthraquinones, naphthalene derivatives, xanthones and simple phenolics [17, 18] to which many pharmacological

and biological activities embracing antibacterial, antiprotozoal, antiviral, antioxidant, antinociceptive, anti-inflammatory, cytotoxic, and immunomodulatory activities have been attributed [19, 20].

The analysis of essential oils obtained from a number of Iridaceae revealed the prevalence of oxygenated monoterpenes, aliphatic hydrocarbons and their derivatives, fatty acids and their esters, in addition to monoterpene hydrocarbons of the pinane skeleton. (+)-(*Z*)-γ-Irone and (+)-(*Z*)-α-irone were found to be predominating the essential oils of *Iris* rhizomes [15, 21, 22]. Irones, with a pleasant violet-like odor, are gradually formed in the ageing rhizomes through oxidative degradation of iridals contributing to the attractive fragrance of *Iris* oil, one of the most precious ingredients used in perfumery [23–25].

*Dietes bicolo*r (Steud.) Sweet ex Klatt is an evergreen plant with rhizomes, native to South Africa [26]. It is commonly known as Yellow Wild Iris, Butterfly Iris or Peacock Flower, characterized by having sword-like leaves and white to creamy yellow Iris-like flowers with three dark spots, each surrounded by an orange outline [27–29]. The genus name "*Dietes*" is derived from the Greek word "dis" meaning twice and the Latin one "*etum*" meaning an association for the close relationship to both *Moraea* and *Iris*. The species name "*bicolor*" means two-colored [26]. In our ongoing research regarding the phytochemistry and pharmacology of *D. bicolor*, a widely unexplored South African plant, we have recently reported antimicrobial activity of the crude alcoholic extracts of leaves, flowers and rhizomes from *D. bicolor*, which lack measurable cytotoxicity to mammalian cells [30]. Although current literature was thoroughly screened, we did not find information on the chemical composition and the biological activity of *D. bicolor* and to the best of our knowledge; no reports have addressed the antimicrobial activity and the chemical composition of the essential oils of flowers, leaves, and rhizomes from *D. bicolor*.

In this study, a comparative analysis of the volatile oil composition of different parts of *D. bicolor* was carried out and the antimicrobial activity of the leaf and flower essential oils was assessed against various Gram-positive and Gram-negative bacterial strains in addition to four fungi aiming to explore their antibacterial and antifungal activities.

2 Materials and methods

2.1 Plant material

Fresh leaves, flowers and rhizomes of *D. bicolo*r (Steud.) Sweet ex Klatt were collected in April 2011 from a private ornamental garden, Cairo, Egypt. The plant was kindly identified and authenticated by Mrs Therese Labib, Consultant of Plant Taxonomy at the Ministry of Agriculture and El-Orman Botanical Garden, Giza, Egypt and Prof. Dr. Mohamed El-Gebaly, Department of Botany, National Research Centre (NRC), Giza, Egypt. A voucher specimen was deposited in the herbarium of the Department of Pharmacognosy, Faculty of Pharmacy, Ain Shams University (voucher number: IA-10411).

2.2 Chemicals

All chemicals utilized in the chemical analysis were purchased from AppliChem (Darmstadt, Germany), Fluka (Buchs, Switzerland) and Sigma (Sigma Aldrich, Sternheim, Germany).

2.3 Essential oil isolation

The essential oils of the fresh leaves, flowers and rhizomes of *D. bicolor* were obtained individually by hydrodistillation (200 g each) for 6 h using a *Clevenger*-type apparatus. The oils were collected over *n*-hexane and then dried over anhydrous sodium sulfate and kept in separated sealed vials at −30 °C for further analyses. The yield in % (w/w) was determined in triplicate and based on the initial plant dry weight.

2.4 GLC/FID analysis

The GLC-FID analysis was performed using a Varian 3400 apparatus (Varian, Darmstadt, Germany) equipped with DB-5 fused bonded column (30 m × 0.25 mm i.d. × 0.25 µm film thickness) (Ohio Valley, OH, USA) and FID detector. Helium was used as a carrier gas at a flow rate of 2 ml/min. The initial column temperature was kept at 45 °C for 2 min (isothermal) and programmed to 300 °C at a rate of 4 °C/min, and kept constant at 300 °C for 20 min (isothermal). Detector and injector temperatures were 300 and 250 °C, respectively. The split ratio was 1:20. PeakSimple® 2000 chromatography data system (SRI Instruments, CA, USA) was used for the recording and integration of the chromatograms. Average areas under the peaks of three independent chromatographic runs were used for calculating the % composition of each component.

2.5 GLC/MS analysis

Mass spectra were recorded using a Hewlett-Packard gas chromatograph (GC 5890 II; Hewlett-Packard, Bad Homburg, Germany) equipped with a split-splitless injector and an FID detector. A DB-5 column was used and the operating conditions were similar to those applied for the GLC/FID. The capillary column was directly coupled to a quadrupole mass spectrometer (SSQ 7000; Thermo-Finnigan, Bremen, Germany). The injector and detector temperatures were maintained at 250 and 300 °C, respectively. Helium carrier gas flow rate was 2 ml/min. All the mass spectra were recorded applying the following conditions: filament emission current, 100 mA; ionization voltage, 70 eV; ion source, 175 °C. Diluted samples (0.5% v/v)

were injected with split mode (split ratio 1:15). Essential oil components were identified by comparing their retention indices and mass spectra with those listed in the commercial MS libraries: (NIST Mass Spectral Library (December 2005), Wiley Registry of Mass Spectral Data 8th edition), our own laboratory MS library in Heidelberg built from spectra corresponding to pure substances and components of known essential oils, the co-injection with an authentic sample, whenever possible and reported literature data [6, 8, 31].

2.6 Microbial strains

The antimicrobial activity of essential oils from leaves and flowers was evaluated against standard microbial species including the Gram-positive bacteria *Streptococcus pneumoniae* (RCMB 000101), *Bacillus subtilis* (RCMB 000102) and the Gram negative bacteria, *Escherichia coli* (RCMB 000107) and *Pseudomonas aeruginosa* (RCMB 000104). Moreover, the antimicrobial activity was assessed against the fungi *Aspergillus fumigatus* (RCMB 002007), *Syncephalastrum racemosum* (RCMB 005005), *Candida albicans* (RCMB 005008) and *Geotrichum candidum* (RCMB 052009). All microorganisms and culture media were supplied by the Regional Center for Mycology and Biotechnology (RCMB), Al-Azhar University, Nasr City, Cairo, Egypt.

2.7 Inocula preparation

The bacterial and fungal cultures were prepared as follows: the microbial suspension equivalent to the turbidity of 0.5 McFarland (10^8 CFU/ml) standard was taken from a fresh subculture of the tested bacteria in Mueller Hinton Broth (MHB) and the tested fungi in Sabouraud dextrose broth (SDB), then the suspensions were diluted to 10^6 CFU/ml using MHB for bacteria and SDB for fungi. The antimicrobial activity was determined using the broth microdilution assay following the methods described by the Clinical and Laboratory Standards Institute (CLSI) for bacteria [32] and yeasts [33].

2.8 Assessment of viability

The adjusted microbial inoculum (100 µl) was added to each well of a sterile 96-well flat-bottomed microtiter plate containing the serial dilutions of the tested samples (100 µl/well). Consequently, an inoculum concentration of 5×10^5 CFU/ml was obtained in each well. Three wells containing microbial suspensions with no sample using only DMSO employed for dissolving the tested samples (growth control) and two wells containing only media (background control) were included in this plate. Ampicillin, gentamicin and amphotericin B were used as positive controls for Gram-positive, Gram-negative bacteria and fungi, respectively. The tested samples as well as the used standards were used in the concentration range of 460–5000 µg/ml. Optical densities were measured at 600 nm after 24 h at 37 °C for bacteria and after 48 h at 28 °C for fungi using a multi-detection microplate reader (Sunrise-Tecan, Durham, North Carolina, USA). The percentage of growth at each sample concentration was calculated using the following equation: % growth = [(OD_{600} of wells containing the test sample/OD_{600} of the sample-free well) × 100] after subtraction of background ODs (ODs of microorganism-free wells). Data were measured in triplicates ($n = 3$) and expressed as means ± S.D.

2.9 Determination of minimum inhibitory concentration (MIC)

The MIC value was assessed applying the micro-broth dilution method according to Kaya et al. [34]. The adjusted bacterial inoculum (50 µl/well, 10^6 CFU/ml) was added to each well of a sterile U based microtiter plate containing the target concentrations of samples and standards (50 µl/well). Thus, an inoculum concentration of 5×10^5 CFU/ml was obtained in each well. Then, the plates were incubated at 37 °C for 24 h for bacteria and at 28 °C for 48 h for fungi. The lowest concentration inhibiting the visible microbial growth was determined as the MIC value of the isolate.

3 Results and discussion

GLC analyses of the volatile oils from *D. bicolor* flowers, leaves and rhizomes were carried out aiming to assess the differences in their chemical composition and their subsequent effect on the antimicrobial activity. Generally, all the oils had a yellow color displaying a characteristic odor. Nevertheless, their yields varied, to some extent, being 0.005 and 0.003% (w/w) in fresh leaves and flowers, respectively. However, only traces of volatile constituents were detected in the rhizomes. GLC/MS analysis revealed the presence of a total of 84 components, accounting for 94.65, 95.63 and 87.09% of total flower, leaf, and rhizome oils, respectively. A list of the identified components is documented in Table 1 together with their retention indices (*RIs*) on a DB-5 column.

Analysis of the flower volatile constituents as shown in Figure 1(A) resulted in the identification of 47 components, in which fatty acids and their esters predominated with dodecanoic acid (22.84%), capric acid (21.12%), myristic acid (14.32%), and methyl caprate (7.90%) representing the most abundant components. The analysis of the leaf volatiles led to the identification of 42 compounds, in which spathulenol constituted the major compound accounting for 48.44% of the total oil composition. Moreover, dihydro-edulan I, cubenol and τ-cadinol, were found to exist in considerable quantities in the leaf oil representing 6.25, 6.00 and 5.90%, respectively as illustrated in Figure 1(B). The rhizome oil showed only three fatty acids, namely capric acid (46.14%), *n*-hexadecanoic acid (23.84%) and *n*-caprylic acid (17.11%) representing 87.09% of the total oil content as shown in Figure 1(C).

Table 1: Composition of the essential oils isolated from the flowers (DBF), leaves (DBL) and rhizomes (DBR) of *Dietes bicolor*.

	Component	RI_{exp} [a]	RI_{lit} [b]	Content (%) [c]			Identification [d]
				DBF	DBL	DBR	
1.	Ethylcyclohexane	826	827	tr	–	–	MS, RI
2.	2-Hexen-1-al	845	847	tr	–	–	MS, RI
3.	*p*-Xylene	861	862	tr	–	–	MS, RI
4.	3-Methyloctane	864	867	tr	–	–	MS, RI
5.	1-Methyl-(*E*)-4-ethylcyclohexane	880	882	tr	–	–	MS, RI
6.	*o*-Xylene	885	888	tr	0.55	–	MS, RI
7.	Heptanal	894	895	2.78	–	–	MS, RI
8.	Methyl caproate	920	922	tr	–	–	MS, RI
9.	*n*-Propylcyclohexane	924	924	tr	–	–	MS, RI
10.	3,5-Dimethyloctane	929	924	tr	–	–	MS, RI
11.	2,6-Dimethyloctane	930	933	tr	–	–	MS, RI
12.	Benzaldehyde	956	957	tr	–	–	MS, RI
13.	*m*-Ethyltoluene	958	959	tr	tr	–	MS, RI
14.	*o*-Ethyltoluene	973	974	–	tr		MS, RI
15.	6-Methyl-5-heptene-2-one	987	988	6.92	–	–	MS, RI
16.	1,2,4-Trimethylbenzene	988	990	–	tr	–	MS, RI
17.	2-Methylnonane	963	962	tr	–	–	MS, RI
18.	1,3,5-Trimethylbenzene	993	994	0.42	tr	–	MS, RI
19.	Decane	1001	1000	0.88	–	–	MS, RI
20.	(*E*,*E*)-2,4-Heptadienal	1012	1011	tr	–	–	MS, RI
21.	1,2,3-Trimethylbenzene	1023	1023	tr	tr	–	MS, RI
22.	3-Carene	1023	1025	–	tr	–	MS, RI
23.	*n*-Butylcyclohexane	1033	1033	tr	–	–	MS, RI
24.	1-Methyl-3-propylbenzene	1054	1058	tr	–	–	MS, RI
25.	(*E*)-Decahydronaphthalene	1056	1056	tr	–	–	MS, RI
26.	(*E*)-2-Octenal	1061	1060	tr	–	–	MS, RI
27.	Undecane	1103	1100	0.48	–	–	MS, RI
28.	Nonanal	1107	1107	1.61	–	–	MS, RI
29.	Methyl caprylate	1127	1125	0.90	–	–	MS, RI
30.	(*E*)-2-Nonenal	1161	1058	tr	–	–	MS, RI
31.	*n*-Caprylic acid	1175	1177	0.95	tr	17.11	MS, RI
32.	Myrtenol	1195	1195	–	tr	–	MS, RI
33.	Dodecane	1200	1200	tr	–	–	MS, RI
34.	Decanal	1206	1206	tr	–	–	MS, RI
35.	β-Cyclocitral	1218	1219	–	tr	–	MS, RI
36.	β-Citral	1244	1240	tr	–	–	MS, RI
37.	Citral	1274	1273	tr	–	–	MS, RI
38.	Dihydro-edulan I	1285	1286	–	6.25	–	MS, RI
39.	Thymol	1295	1295	tr	–	–	MS, RI
40.	(*E*,*E*)-2,4-Decadienal	1320	1320	tr	–	–	MS, RI
41.	Methyl caprate	1327	1327	7.90	–	–	MS, RI
42.	α-Longipinene	1347	1348	–	tr	–	MS, RI
43.	1,1,6-Trimethyl-1,2-dihydronaphthalene	1354	1352	–	0.50	–	MS, RI
44.	Capric acid	1369	1369	21.12	–	46.14	MS, RI
45.	α-Copaene	1377	1376	–	0.50	–	MS, RI
46.	Isolongifolene	1388	1386	–	tr	–	MS, RI
47.	β-Cubebene	1389	1388	–	2.06	–	MS, RI
48.	β-Elemen	1389	1390	–	2.69	–	MS, RI
49.	β-Bourbonene	1390	1390	–	4.06	–	MS, RI
50.	Ethyl caprate	1396	1397	tr	3.72	–	MS, RI
51.	Tetradecane	1400	1400	tr	–	–	MS, RI
52.	β-Caryophyllene	1419	1419	–	tr	–	MS, RI
53.	α-Ionone	1427	1430	–	tr	–	MS, RI
54.	β-Gurjunene	1433	1433	–	2.06	–	MS, RI
55.	Aromadendrene	1441	1441	–	0.30	–	MS, RI

Table 1 (continued)

	Component	RI_{exp} [a]	RI_{lit} [b]	Content (%) [c]			Identification [d]
				DBF	DBL	DBR	
56.	β-(*Z*) Guaiene	1451	1451	–	0.36	–	MS, RI
57.	Geranyl acetone	1455	1455	tr	–	–	MS, RI
58.	Allo-Aromadendrene	1460	1461	–	tr	–	MS, RI
59.	τ-Muurolene	1465	1466	–	1.88	–	MS, RI
60.	α-Amorphene	1485	1484	–	tr	–	MS, RI
61.	Germacrene D	1485	1485	–	1.57	–	MS, RI
62.	β-Selinene	1490	1490	–	tr	–	MS, RI
63.	Valencene	1496	1496	–	tr	–	MS, RI
64.	α-Selinene	1498	1494	–	tr	–	MS, RI
65.	α-Muurolene	1500	1500	–	tr	–	MS, RI
66.	τ-Cadinene	1510	1510	–	tr	–	MS, RI
67.	δ-Cadinene	1515	1514	–	0.52	–	MS, RI
68.	Calamenene	1524	1524	–	0.53	–	MS, RI
69.	Methyl dodecanoate	1527	1527	4.17	–	–	MS, RI
70.	Globulol	1556	1556	–	3.48	–	MS, RI
71.	Dodecanoic acid	1576	1578	22.84	–	–	MS, RI
72.	Spathulenol	1578	1578	–	48.44	–	MS, RI
73.	Viridiflorol	1591	1591	–	tr	–	MS, RI
74.	Cubenol	1618	1616	–	6.00	–	MS, RI
75.	τ-Cadinol	1635	1634	–	5.90	–	MS, RI
76.	β-Cadin-4-en-10-ol	1645	1646	–	tr	–	MS, RI
77.	Methyl myristate	1727	1727	2.26	–	–	MS, RI
78.	Myristic acid	1768	1768	14.32	–	–	MS, RI
79.	Methyl palmitate	1928	1927	2.12	–	–	MS, RI
80.	Chloropyrifos	1957	1967	–	4.26	–	MS, RI
81.	*n*-Hexadecanoic acid	1961	1961	4.33	–	23.84	MS, RI
82.	Methyl linoleate	2101	2099	1.05	–	–	MS, RI
83.	Oleic Acid	2114	2115	0.55	–	–	MS, RI
84.	Ethyl linoleate	2151	2155	–	tr	–	MS, RI
Monoterpene hydrocarbons				–	–	–	
Oxygenated monoterpenes				tr	tr	–	
Sesquiterpene hydrocarbons				–	16.53	–	
Oxygenated sesquiterpenes				–	70.07	–	
Others				94.65	9.03	87.09	
Total identified components				94.65	95.63	87.09	

[a] RI_{exp}, Retention index determined experimentally on a DB-5 column; [b] RI_{lit}, published Kovats retention indices [6, 8, 31]; [c] Content (%), an average of three analyses; tr, trace; –, not detected; [d] Identification, was based on comparison of the compounds' mass spectral data (MS) and retention indices (RI) with those of NIST Mass Spectral Library (December 2005), Wiley Registry of Mass Spectral Data 8th edition and literature [6, 8, 31].

The oxygenated sesquiterpene fraction of the leaf oil accounted for 70.07% and was mainly attributed to its high content of spathulenol. Furthermore, sesquiterpene hydrocarbons represented 16.53% of the total leaf oil with β-bourbonene being the most predominant compound. However, only traces of oxygenated monoterpenes were detected in the leaf oil, and monoterpene hydrocarbons were completely absent. Regarding the volatile constituents of flowers and rhizomes, fatty acids and their esters together with other aliphatic hydrocarbon compounds showed high prevalence representing nearly the total oil composition. However, monoterpenes, oxygenated monoterpenes, sesquiterpenes as well as oxygenated sesquiterpenes in these oils were hardly present or even absent. The remarkable chemical variations observed among the diverse components and their relative amounts in the volatile oil of the leaves, flowers and rhizomes can be used as a reliable marker for discriminating and ensuring the authenticity of the supplied oils [35].

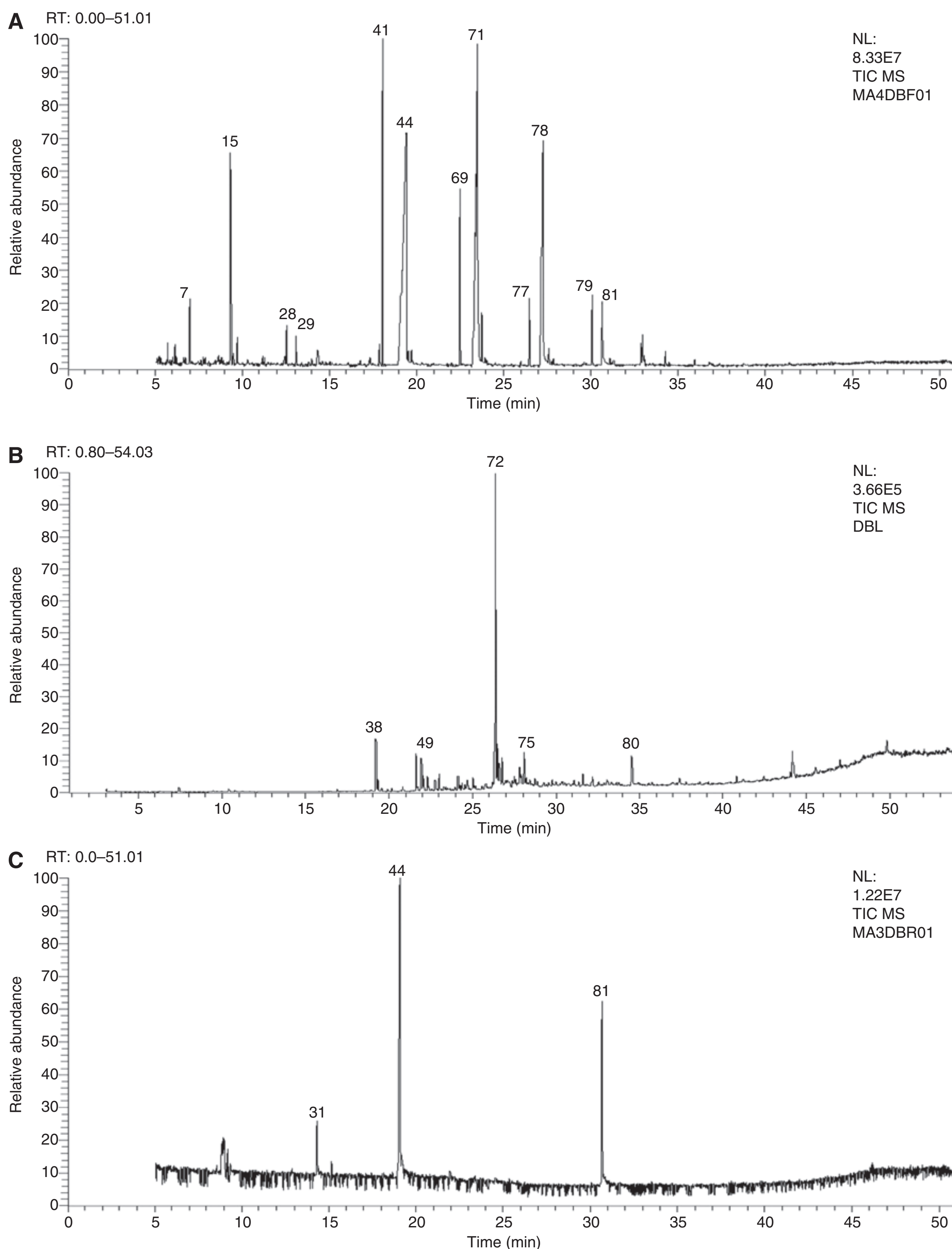

Figure 1: Gas chromatogram on a DB-5 column of the volatile oils from *D. bicolor* A) flowers, B) leaves and C) rhizomes.

The prevalence of fatty acids and their esters, as well as aliphatic hydrocarbons and their derivatives in the volatile constituents of flowers and rhizomes seems to be a common feature among members of Iridaceae [15, 22, 36]. Myristic acid was found to be the major component in the volatile constituents of the rhizomes of Syrian wild irises including, *I. germanica*, *I. barnumae*, *I. bostrensis* and *I. aurantiaca* [36]. In addition, lauric acid, capric

acid, palmitic acid and its methyl ester, octadecanoic acid methyl ester and elaidic acid methyl ester were obtained from Syrian wild *Iris* species [36]. Moreover, the main components characterized in *I. pseudacorus* flower oil include hexadecanoic acid, heptacosane and 6-methyl-5-hepten-2-one whereas *I. pseudacorus* rhizome oil contained nonacosane, triacontane, octacosane and pentacosane as major components [22].

The antimicrobial activity of essential oils from *D. bicolor* leaves and flowers was evaluated against four bacterial strains and four fungi. The results are documented in Table 2. The essential oils of the leaves and flowers exhibited notable antimicrobial activity against most of the tested organisms showing minimum inhibitory concentrations (MICs) ranging from 115 to 1860 μg/ml. The leaf oil showed higher activity against the tested Gram-positive bacteria exceeding that of the flower oil with MIC values of 115 and 460 μg/ml for *B. subtilis* and *S. pneumonia*, respectively. Gram-negative bacteria were generally less susceptible, where both oils produced similar effects on *E. coli* (MIC > 2000 μg/ml) and being completely ineffective against *P. aeruginosa*.

Concerning their antifungal activity, the leaf oil exerted a more pronounced effect than the flower oil on the tested fungi namely *G. candidum*, *S. racemosum* and *A. fumigatus* showing MIC values of 115, 920 and 1860 μg/ml, respectively except for *C. albicans* that is unsusceptible to the effect of both oils as shown in Table 2.

The use of multiple assays is generally recommended to evaluate the antimicrobial activity of herbal extracts and natural products with subsequent careful comparison of their results aiming to reach a solid conclusion [37]. In our study, a strong correlation was observed between the two screening methods employed, where lower IC_{50} values were correlated to lower MIC values, and this observation was consistent throughout the two experiments. Moreover, the results obtained in this study appear to be consistent with previous reports on the antimicrobial activity of essential oils of different species of Iridaceae including *Iris bulleyana* [38], *Iris pallida* [39], as well as *Gynandriris sisyrinchium* [40].

The antimicrobial activity exerted mainly by the leaf oil could be attributed to its high content of sesquiterpenes represented by spathulenol being its major constituent or as a result of the synergy between the major and minor constituents within the oil. Although spathulenol has been previously described in the literature as a potent antimicrobial agent [41, 42], its exact mechanism of action is still unknown [43]. Nevertheless, the leaf oil and its isolated components are lipophilic in nature. They can easily permeate and dissolve in the biomembrane of microbes resulting in a disturbance of permeability, leading to cell disruption, leakage of cell content and ultimately cell death [44, 45].

However, Gram-negative bacteria were not susceptible as compared to the Gram-positive bacteria, which is probably due to their different cell membrane structures among these two bacterial groups. Generally, Gram-negative organisms have an outer membrane enclosing the cell wall with a lipopolysaccharide coat that is crucial for the restriction of hydrophobic compounds. Its absence in the Gram-positive bacteria makes them more accessible to volatile oil components [42].

Table 2: The Half maximal inhibitory concentration (IC_{50}) and minimum inhibitory concentrations (MICs) of essential oils from leaves and flowers of *D. bicolor* against different microorganisms using the broth micro-dilution method.

Microorganisms	DBL[a]		DBF[b]		Ampicillin		Gentamicin		Amphotericin B	
	IC_{50}[c]	MIC[d]	IC_{50}	MIC	IC_{50}	MIC	IC_{50}	MIC	IC_{50}	MIC
Gram-positive bacteria										
Streptococcus pneumoniae (RCMB 000101)	3.55±0.37	460	>5	1860	0.72±0.08	0.25	NT[e]	NT	NT	NT
Bacillus subtilis (RCMB 000102)	0.88±0.11	115	>5	920	0.72±0.07	0.25	NT	NT	NT	NT
Gram-negative bacteria										
Escherichia coli (RCMB 000107)	>5	>2000	>5	>2000	NT	NT	8.85±0.9	1.95	NT	NT
Pseudomonas aeruginosa (RCMB 000104)	NI[f]	NI	NI	NI	NT	NT	115±10.4	15.63	NT	NT
Fungi										
Aspergillus fumigatus (RCMB 002007)	>5	1860	>5	>2000	NT	NT	NT	NT	0.61±0.05	0.25
Syncephalastrum racemosum (RCMB 005005)	4.87±0.67	920	>5	>2000	NT	NT	NT	NT	11.72±1.05	1.95
Candida albicans (RCMB 005008)	NI	NI	NI	NI	NT	NT	NT	NT	0.74±0.08	0.25
Geotrichum candidum (RCMB 052009)	0.94±0.09	115	>5	1860	NT	NT	NT	NT	0.61±0.07	0.25

[a]*Dietes bicolor* leaf volatile oil; [b]*D. bicolor* flower volatile constituents; [c]IC_{50} expressed in mg/ml for DBL and DBF but in μg/ml for antibiotics, data are measured in triplicates ($n = 3$) and presented as means ± SD; [d]MIC expressed in μg/ml; [e]not tested (NT); [f]no inhibition (NI).

4 Conclusions

The current investigation revealed qualitative and quantitative variations in the essential oil components of leaves, flowers and rhizomes from *D. bicolor*. Furthermore, it shed a light on the potential use of the leaf oil as an antimicrobial agent owing to its high content of oxygenated sesquiterpenes, represented by spathulenol. These findings should be supported by further *in vivo* studies to confirm the claimed activity.

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