

Article

Cladanthus scariosus Essential Oil and Its Principal Constituents with Cytotoxic Effects on Human Tumor Cell Lines

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Abstract: *Cladanthus* is a small genus of the Asteraceae family comprising just five species that, apart from *Cladanthus mixtus* (L.) Chevall., has a large distribution in all the Mediterranean countries, mainly in the North Africa area. Several ethnopharmacological uses have been reported for species of this genus. Notably, *Cladanthus scariosus* (Ball) Oberpr. & Vogt is endemic to Morocco. Seeking to delve deeper into the phytochemistry and pharmacological aspects of this species, in this work, we investigated the essential oil (EO) obtained from the aerial parts of a locally sourced accession, hitherto unexplored, growing wild near Tizi n'Ticha, Morocco. The chemical composition of the EO, obtained by the hydrodistillation method, was evaluated by GC and GC-MS. The most abundant EO constituent was germacrene D (13.2%), the principal representative of the sesquiterpene hydrocarbons class (27.2%). However, the major class of constituents was monoterpene hydrocarbons (43.0%), with α -pinene (11.9%), sabinene (10.2%), *p*-cymene (8.5%), and α -phellandrene (5.2%) as the most abundant. The EO and its main constituents have been tested for their possible cytotoxic activity against three human tumor cell lines (MDA-MB 231, A375, and CaCo2) using the MTT assay, with corresponding IC₅₀ values of 13.69, 13.21, and 22.71 μ g/mL, respectively. Germacrene D and terpinen-4-ol were found to be the most active constituents with IC₅₀ values between 3.21 and 9.53 μ g/mL. The results demonstrate remarkable cytotoxic activity against the three human tumor cell lines studied, and in the future, further analyses could demonstrate the excellent potential of *C. scariosus* EO as an antitumor agent.

Keywords: Asteraceae; breast adenocarcinoma cell; *Cladanthus scariosus*; colon adenocarcinoma cell; germacrene D; malignant melanoma cell



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1. Introduction

The genus *Cladanthus* Cass. is a genus with wide distribution in the Mediterranean basin [1]. *Cladanthus arabicus* (L.) Cass., which provides the type of *Cladanthus*, was enclosed in *Chamaemelum* sect. *Santolinopsis* Benedí. This section also included *Ch. eriolepis* (Maire) Benedí, *Ch. flahaultii* (Emb.) Benedí, *Ch. mixtum* (L.) All., and *Ch. scariosum* (Ball) Benedí, which showed morphological characters (floret and achene) very similar to *C. arabicus*. On the other hand, no phylogenetic relationship with the taxa of the *Chamaemelum* sect. *Chamaemelum* (consisting of *Ch. fuscatum* (Brot.) Vasc. and *Ch. nobile* (L.) All) were observed. Consequently, the genus *Cladanthus* (a name that has priority over *Ormenis* Cass., based on *Anthemis mixta* L.) was considered distinct from *Chamaemelum* [2].

Actually, five species are accepted in the genus *Cladanthus* [3] and their distribution as well as their synonymous variants are reported in Table 1.

Table 1. Distribution and synonymous of all the taxa of genus *Cladanthus*.

Taxa	Synonymous	Distribution
<i>Cladanthus arabicus</i> (L.) Cass.	<i>Anthemis arabica</i> L. <i>Chamaemelum cladanthus</i> E.H.L.Krause	Morocco, Algeria, Tunisia, Lybia, Malta, Spain
<i>Cladanthus eriolepis</i> (Maire) Oberpr. & Vogt	<i>Chamaemelum eriolepis</i> (Maire) Benedi <i>Ormenis eriolepis</i> Maire	Morocco
<i>Cladanthus flahaultii</i> (Emb.) Oberpr. & Vogt	<i>Chamaemelum flahaultii</i> (Emb.) Benedi <i>Ormenis flahaultii</i> Emb.	Morocco
<i>Cladanthus mixtus</i> (L.) Chevall.	<i>Anthemis mixta</i> L. <i>Chamaemelum mixtum</i> (L.) All. <i>Ormenis mixta</i> (L.) Dumort. <i>Ormenis aurea</i> Durieu	All Mediterranean countries
<i>Cladanthus scariosus</i> (Ball) Oberpr. & Vogt	<i>Ormenis multicaulis</i> Braun-Blanq. & Maire <i>Chamaemelum scariosum</i> (Ball) Benedi <i>Ormenis scariosa</i> (Ball) Litard. & Maire <i>Santolina scariosa</i> Ball	Morocco

Several ethnopharmacological uses have been reported for the species of this genus. *C. eriolepis* is endemic to Morocco and used by the local population against respiratory problems and as a poultice against bee stings [4,5]. *C. eriolepis* is also used to heal various health conditions including gastrointestinal disorders, stomach ulcers, hypertension, and helminthiasis [6]. *C. arabicus*, instead, is used in the treatment of diabetes in the High Atlas Central of Morocco [7] and of icterus [8]. *C. mixtus*, known as Moroccan chamomile, is very abundant in the Gharb region [9–11], and the collected plants are used for the extraction of an essential oil used in perfumery and cosmetics, with Morocco being the only supplier of this product on the international market [9]. Moroccan chamomile leaves and flowers have been largely used in traditional Moroccan medicine as an infusion to treat various ailments as an analgesic, antiallergic, anti-inflammatory, antispasmodic, carminative, digestive, febrifuge, fungicide, sudorific, vermifuge, and stimulant of leukocyte production [12–14]. Several ethnomedicinal surveys report its peculiar uses in different parts of Morocco. In the region of Rabat, it is utilized to treat metabolic diseases [15], in Agadir for diabetes [16], in Sidi-Boughaba as a stomachic, anthelmintic, antidiabetic, and anxiolytic medicine and as a treatment for nervous breakdowns and hepatic and gastric insufficiencies [13], and in Fez for digestive and neurological troubles [17].

C. scariosus (synonyms: *Chamaemelum scariosum* (Ball) Benedi; *Ormenis scariosa* (Ball) Litard & Maire, Basionym = *Santolina scariosa* Ball) is endemic to Morocco. It is a perennial plant with a strong aromatic odor, ranging from 30 to 60 cm in height; the stems are erect and ended by flower heads with orange-yellow ligules (Figure 1). This species is quite common in open areas on sandstone and is found in the Moroccan High Atlas [18].

**Figure 1.** *Cladanthus scariosus* (Ball) Oberpr. & Vogt plants collected in Morocco.

C. scariosus, known in Morocco by several vernacular names (“Irezghi, Irezgui, Itzghi, Ifskin’uarras, Gartûfa”), is generally used to treat all disorders where spasms are important symptoms; it has tonic, stomachic, analgesic, and antispasmodic properties [18]. Further-

more, the tea of its leaves and inflorescences is used for gastrointestinal, gynecological, and pediatric troubles and for the treatment of diabetes [19,20].

Phytochemical investigation of the non-volatile constituents of *Cladanthus* species indicated the occurrence of flavonoids and phenolic acids in the extracts of *C. arabicus* [21] and *C. mixtus* [22,23], poliacetylenes in *C. arabicus* [24], coumarins in *C. mixtus* [25], and sesquiterpenes in *C. mixtus* [26] and *C. arabicus* [27].

Apart from *C. flahaultii*, which is totally devoid of any investigation, all the other four taxa have been studied for the composition of their essential oils (EOs) and for some biological activities as reported in Table 2.

Table 2. Main constituents (>3%) and several biological properties of the EOs of all the taxa of *Cladanthus* studied so far.

Taxa	Origin	P.	Compounds (%)	Biological Properties Investigated	Ref.
<i>C. arabicus</i>	Ourika, Morocco	ap	sabinene (31.1), β -pinene (16.7), β -myrcene (12.3), α -pinene (5.3), <i>cis</i> -chrysanthenyl acetate (3.4)	antioxidant, antimicrobial, insecticidal	[28]
	Ouarzazate, Morocco	ap	sabinene (13.3), β -pinene (12.8), α -phellandrene (8.7), β -myrcene (6.9), α -pinene (8.3), <i>p</i> -cymene (4.4)	antibacterial	[29]
	Ouarzazate, Morocco	fl	β -pinene (31.9), sabinene (22.9), <i>p</i> -cymene (8.3), α -pinene (5.0), <i>epi</i> - α -cadinol (5.0), terpinen-4-ol (3.6)		
	Agadir, Morocco	ap	β -pinene (23.6), <i>t</i> -cadinol (9.5), diethyl phthalate (7.9), α -pinene (5.7)		[30]
<i>C. eriolepis</i>	Errachidia, Morocco	ap	camphor (37.0), sabinene (10.3), α -pinene (6.3), <i>p</i> -cymene (6.1), α -cadinol (5.6), tricyclene (3.8), artemisia ketone (3.4), α -pinene (13.0), isobutyl angelate (10.7), 2-methylbutyl angelate (9.5), germacrene D (7.1), sabinene (4.4)	antibacterial	[6]
	Errachidia, Morocco	ap	isobutyl angelate (23.9), 2-methylbutyl angelate (20.0), isobutyl isobutyrate (8.7), 2-methylbutyl isobutyrate (6.5), β -bisabolene (5.2), 2-methylbut-2-en-1-yl acetate (4.3), methyl allyl angelate (3.3)	antioxidant, antifungal	[4]
	Errachidia, Morocco	l	β -bisabolene (36.0), isobutyl angelate (12.2), isobutyl isobutyrate (7.6), 2-methylbutyl angelate (5.9), γ -curcumene (4.2), 2-methylbut-2-en-1-yl acetate (3.3), 2-methylbutyl isobutyrate (3.0)		
	Errachidia, Morocco	fl	isobutyl angelate (22.0), isobutyl isobutyrate (21.2), α -pinene (9.2), 2-methylbutyl angelate (7.7), 2-methylbutyl isobutyrate (5.7), 2-methylallyl isobutyrate (5.3), 2-methylallyl angelate (4.6)		[5]
	Jbal Zagora, Morocco	ap	isobutyl angelate (22.4), isobutyl isobutyrate (20.8), α -2-methylbutyl angelate (7.2), pinene (5.8), 2-methylbutyl isobutyrate (5.8), 2-methylallyl isobutyrate (5.5), 2-methylallyl angelate (4.9), β -bisabolene (4.0), (<i>E</i>)-2-methylbutenyl propionate (3.0)		[5]
	Ouled Ouchah, Morocco	ap			
<i>C. mixtus</i>	Algeria	ap	α -thujone (51.8), β -thujone (14.6), borneol (7.3), 3-hexenol (4.9)	antibacterial	[31]
	Temara, Morocco	ap	camphor (21.4), β -myrcene (13.8), santolinatriene (10.1), santolina alcohol (5.4), α -pinene (4.4), yomogi alcohol (4.1), camphinelone (3.8), 1,8-cineol (3.1), <i>cis</i> - α -bisabolene (3.0), (<i>E</i>)- β -farnesene (3.0)		[32]
	Tamensa, Morocco	fl	camphor (17.5), (<i>E</i>)- β -farnesene (13.8), (<i>E,E</i>)- α -farnesene (5.6), germacrene D (5.0), <i>cis</i> - β -ocimene (4.9), bornyl acetate (4.6), β -myrcene (4.4), santolinatriene (3.2)		[33]
	Tamensa, Morocco	l	camphor (19.9), bornyl acetate (5.5), (<i>E</i>)- β -farnesene (4.5), lavandulyl acetate (4.2), borneol (3.3), germacrene D (3.2)		[33]
	Sidi Allai Lbakraoui, Morocco	fl	(<i>E</i>)- β -farnesene (8.3), ledol (5.5), α -bisabolol (3.2)		[33]
	Sidi Allai Lbakraoui, Morocco	l	(<i>E</i>)-nerolidol (44.1), ledol (6.9), (<i>E</i>)- β -farnesene (5.1)		[33]
	Settat, Morocco	fl	camphor (29.0), β -myrcene (11.6), <i>cis</i> -chrysanthenyl acetate (4.6), camphene (4.1), (<i>E</i>)- β -farnesene (3.3), bicyclogermacrene (3.0)		[33]

Table 2. Cont.

Taxa	Origin	P.	Compounds (%)	Biological Properties Investigated	Ref.
<i>C. mixtus</i>	Settat, Morocco	l	β -myrcene (12.4), camphor (9.4), bicyclogermacrene (6.1), (<i>E</i>)- β -farnesene (3.6), bornyl acetate (3.0)		[33]
	Kenitra, Morocco	fl	camphor (14.4), neryl acetate (8.1), bornyl acetate (4.7), (<i>E</i>)- β -farnesene (4.5), <i>trans</i> - α -nerodyl acetate (3.1)		[33]
	Kenitra, Morocco	l	camphor (11.0), hexadecanoic acid (8.6), bornyl acetate (7.6), neryl acetate (6.0), spathulenol (3.6), (<i>E</i>)-nerolidol (3.2)		[33]
	Benguerir, Morocco	fl	2-tridecanone (21.5), camphor (17.8), <i>n</i> -pentacosane (9.5), (<i>E</i>)- α -bisabolene (6.7), bornyl acetate (3.4)		[33]
	Benguerir, Morocco	l	camphor (33.0), 2-tridecanone (7.8), 2-pentyl-1furan (4.4) santolina alcohol (37.7), α -pinene (4.8), camphenilone (4.8), yomogi alcohol (4.5), germacrene (3.3), 1,8-cineole (3.2), cubenol (3.2)	antibacterial	[34]
	Mamora, Morocco	ap	bornyl acetate (45.0), limonene (10.0), α -pinene (8.0)		[35]
	Morocco	ap	santolina alcohol (32.0), α -pinene (15.0), limonene (8.0), germacrene (5.0)		[36]
	Morocco	ap	santolina alcohol (37.0), α -pinene (16.0), limonene (6.0) camphor (26.8), santolina triene (9.1), camphene (8.1), 2-tridecanone (6.3), β -myrcene (5.1), α -thujene (5.1), β -pinene (4.3), palmitic acid (3.3)		[37]
	Benguir, Morocco	ap	camphor (25.8), β -myrcene (17.4), camphene (7.4), santolina triene (7.2), α -pinene (3.7), <i>trans</i> - β -farnesene (3.3), sabinene (3.0)		[38]
	Kenitra, Morocco	ap	camphor (18.8), β -myrcene (10.2), camphene (6.0), germacrene D (5.3), β -pinene (4.7), <i>trans</i> - β -farnesene (3.8) camphor (20.9), santolina triene (10.3), β -myrcene (9.1), <i>trans</i> - β -farnesene (7.9), camphene (5.0), <i>trans</i> - α -nerodyl acetate (4.1), α -pinene (3.3)		[38]
	Meknes, Morocco	ap	santolina triene (15.3), camphor (13.6), <i>trans</i> - β -farnesene (8.5), <i>trans</i> - α -nerodyl acetate (5.6), camphene (4.5), bornyl acetate (3.9)		[38]
	Tamesna, Morocco	ap	β -myrcene (26.5), <i>trans</i> - β -farnesene (17.9), 2-tridecanone (15.5), camphor (10.0), germacrene D (8.6)		[38,39]
	Chefchaouane, Morocco	ap	<i>trans</i> - β -farnesene (42.9), dendrolasin (3.0)		[38,39]
	Oujda, Morocco	ap	2-methyl-2- <i>trans</i> -butenyl methacrylate (34.0), <i>ar</i> -curcumene (13.6), pinocarvone (5.6), β -myrcene (3.0)		[38]
	Bouznika, Morocco	ap	santolina alcohol (17.4), 1,8-cineole (11.6), β -elemene (5.0), sabinene (3.9)		[38]
	Sidi Alal Ibrahroui, Morocco	ap	hexadecanoic acid (15.2), santolina alcohol (11.7), τ -cadinol (6.7), spathulenol (6.5), caryophyllene oxide (3.8), artemisia alcohol (3.8), τ -muurolol (3.0)	antibacterial	[40]
	Sicily, Italy	ap	hexadecanoic acid (52.0), 1,2-benzenedicarboxylic acid bis(2-methylpropyl) ester (12.3), tetradecanoic acid (4.8), dodecanoic acid (4.2), hexahydrofarnesylacetone (3.0)		[41]
	Commercial, Morocco	r	α -pinene (11.5), santolina alcohol (10.2), <i>trans</i> - β -farnesene (8.6), 1,8-cineole (7.4), germacrene D (6.3), δ -elemene (3.6) santolina alcohol (27.3), germacrene D (10.2), (<i>E</i>)- β -farnesene (4.5), α -pinene (4.5), <i>epi</i> - α -cadinol (4.4), α -cadinol (3.8), artemisia alcohol (3.6), caryophylladienol (3.1)		[9]
	Kenitra, Morocco	ap	santolina alcohol (40.7), germacrene D (8.9), α -pinene (5.7), artemesia alcohol (4.3), (<i>E</i>)- β -farnesene (4.0), limonene (3.1), yomogi alcohol (3.0)	antioxidant, antimicrobial	[42]
	Mezraoua, Morocco MWE	ap	santolina alcohol (46.2–39.8), (<i>Z</i>)-heptadeca-9,16-dien-7-one (12.7–12.3), (<i>E</i>)- β -farnesene (5.6–3.0), artemisia alcohol (4.7–3.4), α -pinene (3.5–0.6) yomogi alcohol (16.2–14.3), santolina alcohol (15.6–12.5), artemisia alcohol (13.2–12.0),	antimicrobial	[43]
Sardinia, Italy	ap	(<i>Z</i>)-heptadeca-9,16-dien-7-one (10.7–9.9), germacrene D (9.0–7.0), (<i>E</i>)- β -farnesene (6.8–5.2), (<i>E,E</i>)- α -farnesene (6.6–6.5)	antimicrobial	[43]	
Corsica, France Cluster 2	ap				

Table 2. Cont.

Taxa	Origin	P.	Compounds (%)	Biological Properties Investigated	Ref.
<i>C. mixtus</i>	Corsica, France Cluster 3	ap	germacrene D (28.6–13.3), santolina alcohol (26.2–15.4), (<i>E,E</i>)- α -farnesene (15.6–3.4), (<i>Z</i>)-heptadeca-9,16-dien-7-one (11.5–4.4), (<i>E</i>)- β -farnesene (11.3–1.9), yomogi alcohol (6.4–0.7), α -pinene (3.9–1.0)	antimicrobial	[43]
	Taounate, Morocco	ap	santolina alcohol (37.5), (<i>E</i>)- β -farnesene (6.2), <i>epi</i> - α -muurulol (5.0), α -cadinol (4.9), α -pinene (4.3), artemisia alcohol (4.1), germacrene D (3.8), 1,8-cineole (3.2), camphor (3.0)	antimicrobial	[44]
	Taounate, Morocco	ap	<i>p</i> -menthane-1,8-diol (18.2), α -pinene (10.8), germacrene D (9.2), (<i>Z</i>)- β -farnesene (7.6), δ -elemene (7.2), caryophyllene (3.6), β -elemene (3.3), β -pinene (3.2), α -muurolene (3.0)	antibacterial	[45]
	Taounate, Morocco	ap	germacrene D (11.5), 1,8-cineol (10.3), <i>cis</i> -methyl-eugenol (9.0), butyric acid (8.5), δ -elemene (5.5), <i>cis</i> -cadina-1(6),4-diene (3.2)	antioxidant, antibacterial	[46]
	Morocco	fl	α -pinene (24.9), santolina alcohol (15.2), 1,8-cineole (7.1), elemene (6.7), limonene (6.0), α -humulene (4.1), myrcene (3.7), sabinene (3.4)	insecticidal	[47]
<i>C. scariosus</i>	Clade 1 Morocco	ap	sabinene (17.8), α -pinene (14.3), germacrene D (9.5), dihydrochamazulene (7.5), chamazulene (4.4), α -phellandrene (4.2), terpinen-4-ol (3.9)		[18]
	Clade 2 Morocco	ap	sabinene (23.7), α -pinene (16.0), terpinen-4-ol (8.2), dihydrochamazulene (6.4), germacrene D (4.9), γ -terpinene (4.7), chamazulene (3.0)		[18]
	Clade 3 Morocco	ap	α -pinene (13.7), sabinene (11.0), terpinen-4-ol (7.5), germacrene D (5.6), camphor (4.8), α -phellandrene (3.5), chamazulene (3.2)		[18]
	Oukeimeden Morocco	ap	germacrene D (20.7), (<i>E</i>)-chrysanthenyl acetate (8.3), chamazulene (7.1), sabinene (6.9), α -pinene (4.8), <i>t</i> -muurolol (4.2), (<i>E,E</i>)-farnesyl acetate (3.9)		[48]
	Ait M'hamed Morocco	ap	<i>p</i> -cymene (11.3), caryophyllene oxide (9.9), γ -terpinene (8.3), sabinene (7.1), elemol (6.5), α -terpinolene (5.6), spathulenol (5.0), germacrene D (4.9), α -pinene (3.5), camphor (3.0), chamazulene (1.6)	antioxidant, antimicrobial	[49]

P. = parts; ap = aerial parts; fl = flowers; l = leaves; r = roots.

These findings suggest that EOs derived from the *Cladanthus* species possess diverse biological properties, making them potentially valuable in various medical and therapeutic applications. For this reason, the aim of this work was to investigate the chemical composition and to evaluate the antiproliferative effect of the *C. scariosus* EO and its main compounds against the human breast adenocarcinoma cell line, human malignant melanoma cell line, and human colon adenocarcinoma cell line, three of the most common cancers today.

2. Results and Discussion

2.1. Chemical Composition of *C. scariosus* EO

Hydrodistilled *C. scariosus* EO, obtained from fully flowering aerial parts of the plant, had a dark blue color. Overall, forty-seven different compounds were identified and listed in Table 3.

The EO was found to be very rich in monoterpene hydrocarbons (43.0%) with α -pinene (11.9%), sabinene (10.2%), *p*-cymene (8.5%), and α -phellandrene (5.2%) as the most abundant compounds. However, the major constituent was germacrene D (13.2%), the principal constituent of sesquiterpene hydrocarbons (27.2%), whereas terpinen-4-ol (8.8%) was the principal component among the oxygenated monoterpenes (11.7%). Chamazulene, responsible for the blue color of the EO, was present at 4.0%. Fortunately, the response factor obtained from the appropriate calibration lines confirmed the percentage of α -pinene, *p*-cymene, germacrene D, and terpinene-4-ol compounds from the integrals of the areas of the GC-TOF/MS chromatogram (Table 2).

Table 3. Chemical composition (%) of *Cladanthus scariosus* essential oil (EO) collected in Morocco.

No.	Compound ^a	LRI ^b	LRI ^c	A (%) ^d	Content ^e (w/w%)
1	α -Thujene	933	927	1.4	
2	α -Pinene	942	933	11.9	11.42
3	Camphene	958	953	0.1	
4	Thuja-2,4(10)-diene	961	953	0.1	
5	Benzaldehyde	970	960	0.1	
6	2-Methylbutyl-propanoate	973	968	0.2	
7	Sabinene	980	972	10.2	
8	β -Pinene	986	978	0.6	
9	Myrcene	992	991	0.2	
10	α -Phellandrene	1012	1007	5.2	
11	α -Terpinene	1022	1018	1.1	
12	<i>p</i> -Cymene	1030	1025	8.5	8.46
13	Limonene	1034	1030	0.5	
14	β -Phellandrene	1036	1031	0.3	
15	1,8-Cineole	1038	1032	0.4	
16	Isopentyl-butyrate	1056	1054	0.1	
17	γ -Terpinene	1062	1058	2.3	
18	<i>n</i> -Octanol	1071	1076	0.2	
19	Terpinolene	1090	1086	0.6	
20	Linalool	1101	1101	1.5	
21	Limona ketone	1135	1131	0.2	
22	Borneol	1178	1173	0.3	
23	Terpinen-4-ol	1187	1184	8.8	8.91
24	α -Terpineol	1199	1195	0.5	
25	δ -Elemene	1337	1335	1.0	
26	α -Cubebene	1352	1347	0.1	
27	α -Ylangene	1376	1371	tr	
28	α -Copaene	1383	1375	0.9	
29	β -Bourbonene	1391	1382	0.3	
30	β -Elemene	1394	1390	0.6	
31	Methyl eugenol	1401	1403	0.4	
32	β -Ylangene	1427	1422	2.2	
33	(<i>E</i>)-Caryophyllene	1429	1424	2.1	
34	β -Copaene	1438	1433	1.5	
35	Isogermaacrene D	1453	1447	0.6	
36	α -Humulene	1464	1454	0.4	
37	Germaacrene D	1490	1480	13.2	12.98
38	Viridiflorene	1500	1491	0.2	
39	γ -Amorphene	1500	1490	0.2	
40	Bicyclogermaacrene	1505	1497	1.1	
41	ϵ -Amorphene	1509	1502	0.7	
42	γ -Cadinene	1521	1512	0.7	
43	δ -Cadinene	1525	1518	1.4	
44	(<i>Z</i>)-Cadin-4-en-7-ol	1647	1638	3.2	
45	δ -Cadinol	1651	1641	1.2	
46	Cadin-4-en-10-ol	1666	1659	1.5	
47	Chamazulene	1742	1732	4.0	
Monoterpene Hydrocarbons				43.0	
Oxygenated Monoterpenes				11.7	
Sesquiterpene Hydrocarbons				27.2	
Oxygenated Sesquiterpenes				5.9	
Hydrocarbons				4.0	
Phenylpropanoids				0.4	
Other Compounds				0.6	
Total Composition				92.8	

^a Components listed in order of elution on a DB-5 MS column; ^b linear retention indices on a DB-5 MS non-polar column; ^c linear retention indices based on the literature (<https://webbook.nist.gov/>; accessed on 15 March 2024); ^d percentage amounts of the separated compounds calculated from integration of the peaks; ^e the content is the gravimetric percentage of α -pinene, terpinen-4-ol, germaacrene D, and *p*-cymene determined using appropriate calibration lines.

The data obtained in this work basically agreed with the compositions of the EOs from other accessions of *C. scariosus* previously published [18,48,49], although some differences should be noted. As can be seen from Table 2, the major compounds identified in *C. scariosus*, such as α -pinene, sabinene, chamazulene, and germaacrene D (Table 3), are present

in all the other accessions collected in different places in the Moroccan territory. They could be considered exactly as markers of the species in question.

The total composition detected also allows us to make comments about the chemical compositions of other *Cladanthus* ssp. EOs. Compounds such as sabinene, α -pinene, *p*-cymene, and terpinen-4-ol were similarly identified in *C. arabicus* EOs [28–30], but these did not present chamazulene nor germacrene D; differently, *C. eriolepis* EOs were rich in esters such as 2-methylbutyl angelate, 2-methylbutyl isobutyrate, and others [4,5], while the *C. mixtus* EOs present major compounds such as camphor, (*E*)- β -farnesene, and bornyl acetate [31–33,35].

2.2. Effects of *C. scariosus* EO on Tumor Cell Viability

In the literature, no data are available about the cytotoxic activity of *C. scariosus* EO. For the first time, we here report the effect of this EO against a panel of human cancer cells and a non-tumor cell line. Three human cancer cell lines, a human breast adenocarcinoma (MDA-MB 231), a human malignant melanoma (A375), and a human colon adenocarcinoma (CaCo2), and one human endothelial cell line (EA.hy926) were treated with different concentrations of EO and its main compounds such as germacrene D, α -pinene, terpinen-4-ol, and *p*-cymene for 72 h. An MTT assay was used to evaluate the cytotoxic activity of EO, and the results obtained are shown in Table 4 and Figure 2.

Table 4. In vitro cytotoxic activity of *C. scariosus* EO and its main constituents.

	Cell Line (IC ₅₀ μ g/mL) ^a			
	MDA-MB 231 ^b	A375 ^c	CaCo2 ^d	EA.hy926 ^e
Essential oil	13.69	13.21	22.71	24.51
95% C.I. ^f	12.48–13.99	11.98–15.65	20.06–25.72	20.92–28.70
Germacrene D	4.41	9.53	8.57	17.85
95% C.I.	3.75–5.18	8.32–10.92	7.25–10.12	16.28–19.57
α -Pinene	12.40	17.04	30.94	22.14
95% C.I.	8.79–17.49	14.88–19.52	25.68–37.28	18.07–27.13
Terpinen-4-ol	3.21	4.91	5.23	5.57
95% C.I.	2.79–3.70	4.34–5.54	4.66–5.89	5.13–6.07
<i>p</i> -Cymene	29.57	27.48	27.90	6.63
95% C.I.	26.91–32.49	23.53–32.09	26.64–29.21	5.53–7.98
Positive control				
Cisplatin	2.54	0.62	3.07	ND
95% C.I.	1.82–2.74	0.55–0.70	2.15–3.92	

^a IC₅₀ = The concentration of compound that affords a 50% reduction in cell growth (after 72 h of incubation). ^b Human breast adenocarcinoma cell line. ^c Human malignant melanoma cell line. ^d Human colon adenocarcinoma cell line. ^e Human vascular endothelial cells. ^f Confidence interval. ND: not determined.

C. scariosus EO showed antiproliferative activity against all three cell lines with IC₅₀ values of 13.69, 13.21, and 22.71 μ g/mL for MDA-MB 231, A375, and CaCo2, respectively. Based on the criteria of the American National Cancer Institute for considering a crude extract promising for further purification, the IC₅₀ value being lower than 30 μ g/mL [50] suggests that the EO shows excellent activity against tumor cells. Such a cytotoxic activity of EO could also be attributed to its main components. Indeed, in our experiments, germacrene D and terpinen-4-ol were the most active on all three cell lines, with IC₅₀ values ranging from 3.21 to 5.23 μ g/mL for terpinen-4-ol and from 4.41 to 8.57 μ g/mL for germacrene D on MDA-MB 231 and CaCo2, respectively.

Noteworthy, the cytotoxic activity of terpinen-4-ol is comparable with that of cisplatin, showing IC₅₀ values of 2.54 and 3.07 μ g/mL on MDA-MB 231 and CaCo2 cell lines, respectively. Germacrene D showed IC₅₀ values slightly higher than IC₅₀ values for terpinen-4-ol, and α -pinene showed remarkable activity on MDA-MD 231 (IC₅₀ value, 12.40 μ g/mL; 91 μ M) and on A375 (IC₅₀ value 17.04, 125 μ M). *p*-Cymene exerted lower cytotoxic activity. The data are in good agreement with the reported antiproliferative activity of terpinen-4-ol

against the human acute promyelotic leukemia cell line HL60 [51], human non-small-cell lung cancer cell line HNCCLC [52], and human leukemic MOLT-4 cell line [53], where terpinen-4-ol induced apoptotic and macrophagic cell death. Terpinen-4-ol was active on melanoma, as reported in the literature [54]; it potently induces cell cycle arrest, apoptosis, and necrotic cell death [55]. Germacrene D was reported to be active on human breast adenocarcinoma (MDA-MB 231 and MCF-7), human ductal carcinoma (Hs578T), and human hepatocellular carcinoma (HepG2) [56]. α -Pinene has also been reported to exhibit apoptotic and antimetastatic activity on melanoma cells [57]. In the literature, sabinene also exerts cytotoxic activity against liver (HepG2), colon (HCT116), and breast (MCF7) cancer cell lines [58]. From the data reported above on the antiproliferative activities of the main compounds of the EO, it could be hypothesized that the EO activity may involve the activation of apoptotic processes, a study project that could be explored further later.

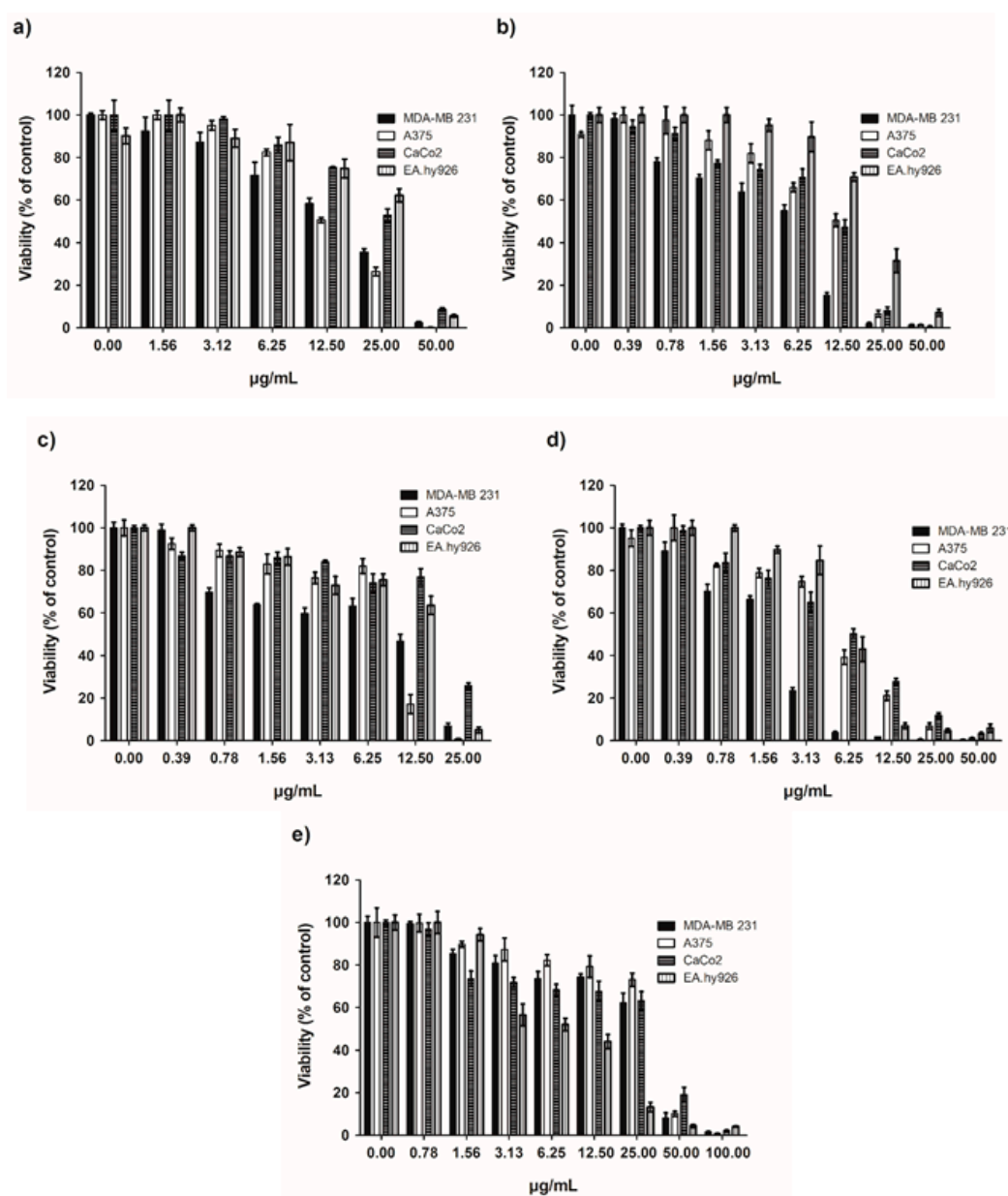


Figure 2. Cytotoxic effect of *C. scariosus* EO and its main compounds. Cell viability was determined in MDA-MB 231, A375, CaCo2, and EA.hy926 cell lines by MTT. Cells were treated for 72 h with different concentrations of (a) *C. scariosus* EO, (b) germacrene D, (c) α -pinene, (d) terpinen-4-ol, and (e) *p*-cymene. Data shown are expressed as mean \pm SE of three separate experiments.

Although the presence of germacrene D (13.2%), α -pinene (11.9%), sabinene (10.2%), terpinen-4-ol (8.8%), and *p*-cymene (8.5%) may explain the cytotoxic activity of *C. scariosus* EO, it cannot be excluded that even the presence of lower-concentration components might contribute to the final cytotoxic activity observed.

Although the cytotoxic activity of the essential oil is significantly relevant to tumor cell lines, its specificity is not very high. In fact, its cytotoxic activity on the normal vascular endothelial line EA.hy926 is only slightly lower than that of MDA-MB 231 and A375. Perhaps a better comparison can be made with a normal cell line of ectodermal derivation given that the three tumor lines tested are all of epithelial origin.

3. Materials and Methods

3.1. Plant Material

Aerial parts from several fresh individuals of *C. scariosus*, at the full flowering stage, were collected near Tizi n'Ticha, Morocco, at about 2170 m a.s.l., 31°17'18" longitude N and 7°22'55" latitude W, in May 2023. One of the samples, identified by Prof. Vincenzo Ilardi, has been stored in the University of Palermo Herbarium (No. PAL 109762).

3.2. Isolation of EO

Fresh aerial parts (660 g) were subjected to hydrodistillation for 3 h, according to the standard procedure described in European Pharmacopoeia [59]. The EO was dried over anhydrous Na₂SO₄ and preserved at 4 °C prior to further analysis (up to one month). Samples yielded 0.15% of EO. Hydrodistilled *C. scariosus* EO, obtained from fully flowering aerial parts of the plant, had a dark blue color (Figure 3).

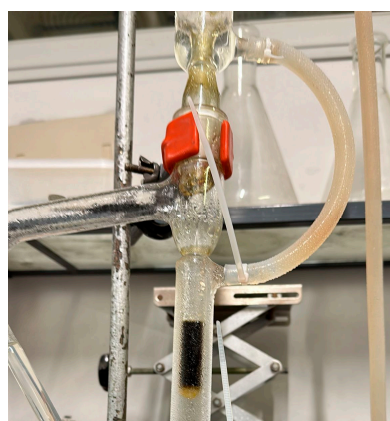


Figure 3. Close-up photo of *C. scariosus* EO isolated by the Clevenger apparatus.

3.3. GC-MS Analysis and Quantitative Determination

Analysis of EOs was carried out according to the procedure reported by Lauricella et al. [60]. The composition of the EO was determined by GC-MS analyses. They were achieved on an Agilent Technologies 7890 GC equipped with FID and mass spectrometer detectors using a DB-5MS (5% phenylmethylpolysiloxane) capillary column (30.00 m \times 0.25 mm, 0.25 μ m film thicknesses; J & W Scientific, Folsom, CA, USA). The carrier gas was helium at a flow rate of 0.8 mL/min. The initial column temperature was 60 °C and programmed to increase up to 280 °C at a rate of 4 °C/min. The split ratio was 40:1. The injector temperature was set at 300 °C. The acquisition range was 50–550 *m/z* in electron-impact (EI) mode using an ionization voltage of 70 eV. The EO was diluted 1:100 in *n*-hexane, and then 0.1 μ L was injected into the GC system.

Six calibration solutions containing α -pinene, terpinen-4-ol, germacrene D, and *p*-cymene were prepared in hexane at a concentration (*w/w* %) of 0.5, 1.0, 2.0, 5.0, 10.0, and 20.0%. These calibration solutions contained the compounds, except for the unavailable sabinene constituent, present in greater abundance in the EO sample. Linear retention

indices (LRIs) were calculated using a mixture of pure *n*-alkanes (C₈–C₄₀), and all the peaks' compounds were identified by comparison with MS and by comparison of their relative retention indices with WILEY275, NIST 17, ADAMS, and FFNSC2 libraries.

3.4. Pure Compounds

Pure compounds, such as α -pinene, terpinen-4-ol, and *p*-cymene, were purchased from Sigma-Aldrich (Sigma-Aldrich Chemie GmbH, Eschenstr. 5, 82,024 Taufkirchen, Germany), whereas germacrene D was isolated from the essential oil of *Kundmannia sicula* (L.) DC. [61].

3.5. Cell Culture

Cytotoxic studies were determined in A375 (human malignant melanoma cell line), MDA-MB 231 (human breast adenocarcinoma cell line), CaCo2 (human colon adenocarcinoma cell line), and EA.hy926 (human vascular endothelial cells). A375, MDA-MB 231, and EA.hy926 were grown in Dulbecco's Modified Eagle's Medium (DMEM) with 2 mM L-glutamine, 100 IU/mL penicillin, and 100 μ g/mL streptomycin and was supplemented with 10% heat-inactivated fetal bovine serum (HI-FBS). CaCo2 was cultured in DMEM medium with 2 mM L-glutamine, 100 IU/mL penicillin, 100 μ g/mL streptomycin, and 1% Non-Essential Amino Acid (NEAA) and was supplemented with 10% HI-FBS. Cells were maintained in a humidified atmosphere with 5% CO₂ at 37 °C.

3.6. MTT Assay

Cell viability was examined by the ability of the cells to cleave the tetrazolium salt MTT [3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazoliumbromide] using the mitochondrial enzyme succinate dehydrogenase following the procedure described earlier [62]. Briefly, cells were seeded at a density of 2×10^4 cells/mL. After 24 h, samples were exposed to different concentrations of essential oil and standards (0.78–200 μ g/mL) and incubated for 72 h in a humidified atmosphere of 5% CO₂ at 37 °C. The anticancer drug cisplatin (0.01–50 μ g/mL) was used as the positive control. At the end of incubation, each well received 10 μ L of MTT (5 mg/mL in phosphate-buffered saline, PBS) and the plates were incubated for 4 h at 37 °C. Then, the supernatant was removed, and DMSO (dimethyl sulfoxide) was added to dissolve the formazan crystals. The plates were placed on a shaker for 15 min and the optical density was determined at 540 nm using a microplate spectrophotometer FLUOstar Omega (BMG Labtech, Milan, Italy). Experiments were conducted in triplicate. The cell survival curves were calculated after comparing with the vehicle (Et-OH). The 50% cytotoxic concentration (IC₅₀) was defined as the compound concentration required to reduce the cell viability by half. The IC₅₀ values were determined with the GraphPad Prism 5 computer program (GraphPad Software, San Diego, CA, USA).

4. Conclusions

In this study, the chemical profile of *C. scariosus* EO's aerial parts was examined. The most abundant class was monoterpene hydrocarbons (43.0%), in which the main compounds were α -pinene (11.9%), sabinene (10.2%), *p*-cymene (8.5%), and α -phellandrene (5.2%). This composition was found to be very similar to the other five Moroccan *C. scariosus* accessions, not only in terms of the main components but also the different sub-divisions of metabolite classes. To explore the anticancer potential of the EO and its main components, their effects on several cancer cell lines were tested. The remarkable cytotoxic activity is promising for use in cancer prevention and treatment or in combination with conventional chemotherapy drugs to reduce the toxicity of the latter. Further in vitro and in vivo studies on the anticancer mechanisms exerted by EO and the components are required to validate these preliminary data. In addition, studies are necessary to demonstrate the non-toxicity of EO on non-tumor cells as well as the elucidation of the molecular mechanisms governing the anticancer properties of this EO and their major constituents.

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References

- Oberprieler, C. A phylogenetic analysis of *Chamaemelum* Miller (Compositae, Anthemideae) and related genera. *Bot. J. Linn. Soc.* **2002**, *138*, 255–273. [CrossRef]
- Oberprieler, C.; Vogt, R. *Cladanthus* Cass. In *Med-Checklist Notulae*, 21; Greuter, W., Raus, T., Eds.; Botanic Garden and Botanical Museum: Berlin, Germany, 2002; Volume 32, pp. 195–208.
- POWO. Plants of the World Online. Available online: <https://powo.science.kew.org/> (accessed on 24 February 2024).
- Chibane, E.M.; Ouknin, M.; Renucci, F.; Costa, J.; Majidi, L. Chemical profile, antioxidant and antifungal activity of essential oil from *Cladanthus eriolepis*. *J. Essent. Oil Bear Plants* **2020**, *23*, 1296–1305. [CrossRef]
- El Hafidi, S.; Bakhy, K.; Ouhssine, M.; Casanova, J.; Tomi, F.; Paoli, M. Essential oil composition of *Cladanthus eriolepis* (Coss. ex Maire) Oberpr. & Vogt, an endemic species to Morocco. *J. Essent. Oil Res.* **2021**, *33*, 369–375.
- Bammou, M.; Tariq Bouhlali, E.D.; Sellam, K.; Derouich, M.; El-Rhaffari, L.; Ibjibjen, J.; Nassiri, L. Chemical profile and antimicrobial properties of liquid and vapor phases of the essential oil of *Cladanthus eriolepis*: An endemic Asteraceae growing in the Moroccan oases. *J. Essent. Oil Bear Plants* **2020**, *23*, 1042–1053. [CrossRef]
- Belhaj, S.; Chaachouay, N.; Zidane, L. Ethnobotanical and toxicology study of medicinal plants used for the treatment of diabetes in the High Atlas Central of Morocco. *J. Pharm. Pharmacogn. Res.* **2021**, *9*, 619–662. [CrossRef]
- Benkhniguel, O.; Khamar, H.; Bussmann, R.W.; Chaachouay, N.; Zidane, L. Ethnobotanical and ethnopharmacological study of medicinal plants used in treating some liver diseases in the Al-Haouz Rehamna region (Morocco). *Ethnobot. Res. Appl.* **2023**, *25*, 34. [CrossRef]
- Zrira, S.; Menut, C.; Bessiere, J.M.; Benjilali, B. Chemical composition of the essential oils of Moroccan *Ormenis mixta* (L.) Dumort. ssp. *multicaulis*. *J. Essent. Oil Bear Plants* **2007**, *10*, 378–385. [CrossRef]
- Hajjaj, G.; Bahlouli, A.; Tajani, M.; Cherrah, Y.; Zellou, A. In vivo anti-nociceptive and anti-inflammatory properties of *Ormenis mixta* L. from Morocco. *Int. J. Pharm. Pharm. Sci.* **2016**, *8*, 49–52.
- Jahandiez, E.; Maire, R. *Catalogue des Plantes du Maroc*; Umprimerie Minerva: Algiers, Algeria, 1934; Tome III; p. 764.
- Bellakhdar, J. *La Pharmacopée Marocaine Traditionnelle*; Médecine Arabe Ancienne et Savoirs Populaires; Edition Ibis Press: Paris, France, 1997; p. 766.
- Merghoub, N.; Benbacer, L.; Amzazi, S.; Morjani, H.; El Mzibri, M. Cytotoxic Effect of some Moroccan medicinal plant extracts on human cervical cell lines. *J. Med. Plants Res.* **2009**, *3*, 1045–1050.
- Lahsissene, H.; Kahouadji, A.; Hseini, S. Catalogue des plantes médicinales utilisées dans la région de Zaër (Maroc Occidental). *Lejeunia Rev. De Bot.* **2009**, *186*, 1–26.
- Benkhaira, N.; Ech-Chibani, N.; Fikri-Benbrahim, K. Ethnobotanical survey on the medicinal usage of two common medicinal plants in Taounate Region: *Artemisia herba-alba* Asso and *Ormenis mixta* (L.) Dumort. *Ethnobot. Res. Appl.* **2021**, *22*, 1–19. [CrossRef]
- Ouhaddou, H.; Alaoui, A.; Laaribya, S.; Ayan, S. Ethnobotanical survey of medicinal plants used for treating diabetes in Agadir Ida Outanane region, Southwestern Morocco. *Arab. J. Med. Arom. Plants* **2020**, *6*, 72–86.
- Zeggwagh, A.A.; Lahlou, Y.; Bousliman, Y. Enquête sur les aspects toxicologiques de la phytothérapie utilisée par un herboriste à Fès, Maroc. *Pan Afr. Med. J.* **2013**, *14*, 125. [CrossRef]
- El Hafidi, S.; Bakhy, K.; Ouhssine, M.; Benzakour, A.; Khamar, H.; Casanova, J.; Paoli, M.; Tomi, F. Composition and chemical variability of the essential oil from aerial parts of *Cladanthus scariosus*, an endemic species to the Moroccan High Atlas. *Chem. Biodivers.* **2023**, *20*, e202201022. [CrossRef]

19. Eddouks, M.; Ouahidi, M.L.; Farid, O.; Moufid, A.; Khalidi, A.; Lemhadri, A. L'Utilisation des plantes médicinales dans le traitement du diabète au Maroc. *Phytotherapie* **2007**, *5*, 194–203. [[CrossRef](#)]
20. Teixidor-Toneu, I.; Martin, G.J.; Ouhammou, A.; Puri, R.K.; Hawkins, J.A. An ethnomedicinal survey of a Tashelhit-speaking community in the High Atlas, Morocco. *J. Ethnopharmacol.* **2016**, *188*, 96–110. [[CrossRef](#)]
21. Aghraz, A.; Gonçalves, S.; Rodríguez-Solana, R.; Dra, L.A.; Di Stefano, V.; Dugo, G.; Cicero, N.; Larhsini, M.; Markouk, M.; Romano, A. Antioxidant activity and enzymes inhibitory properties of several extracts from two Moroccan Asteraceae species. *S. Afr. J. Bot.* **2018**, *118*, 58–64. [[CrossRef](#)]
22. Benmerache, A.; Alabdul Magid, A.; Kabouche, A.; Harakat, D.; Voutquenne-Nazabadioko, L.; Kabouche, Z. 6'''-O-acetylisopinosin, a new C-glycosylflavone and known compounds from the aerial parts of *Cladanthus mixtus*. *Nat. Prod. Res.* **2020**, *34*, 2887–2893. [[CrossRef](#)]
23. El Mihyaoui, A.; Charfi, S.; Erbiai, E.H.; Pereira, M.; Duarte, D.; Vale, N.; Candela Castillo, M.E.; Badoc, A.; Lamarti, A.; Esteves da Silva, J.C.G.; et al. Phytochemical compounds and anticancer activity of *Cladanthus mixtus* extracts from Northern Morocco. *Cancers* **2023**, *15*, 152. [[CrossRef](#)]
24. Bohlmann, F.; Arndt, C.; Bornowski, H. Polyacetylenverbindungen, XXVIII. Äoer weitere Polyine aus dem *Tribus anthemideae* L. *Chem. Berichte* **1960**, *93*, 1937–1944. [[CrossRef](#)]
25. Engelmeier, D.; Hadacek, F.; Hofer, O.; Lutz-Kutschera, G.; Nagl, M.; Wurz, G.; Greger, H. Antifungal 3-butylisocoumarins from Asteraceae-Anthemideae. *J. Nat. Prod.* **2004**, *67*, 19–25. [[CrossRef](#)]
26. Benteldjoune, M.; Chini, M.G.; Iannuzzi, A.M.; Kabouche, A.; Kabouche, Z.; Dambola, M.; Marzocco, S.; Autore, G.; Bifulco, G.; De Tommasi, N. Guaianolides from *Ormenis mixta*: Structural insights and evaluation of their anti-inflammatory profile. *Planta Med.* **2019**, *85*, 947–956. [[CrossRef](#)]
27. Daniewski, W.M.; Danikiewicz, W.; Gumuka, M.; Pankowska, E.; Krajewski, J.; Grabarczyk, H.; Wichacz, M. Sesquiterpenes of *Cladanthus arabicus*. *Phytochemistry* **1993**, *34*, 1639–1641. [[CrossRef](#)]
28. Aghraz, A.; Wanner, J.; Schmidt, E.; Aitdra, L.; Aitsidibrahim, M.; Tabanca, N.; Ali, A.; Nafis, A.; Hassani, L.; Markouk, M.; et al. Chemical composition, in vitro antioxidant, antimicrobial and insecticidal activities of essential oil from *Cladanthus arabicus*. *J. Essent. Oil Bear Plants* **2017**, *20*, 601–609. [[CrossRef](#)]
29. El Hanbali, F.; Mellouki, F.; Akssira, M.; El Hassani, B.; Blázquez, M.A.; Boira, H. Composition and antibacterial activity of essential oils of *Cladanthus arabicus* Cass. (Asteraceae). *J. Essent. Oil Bear Plants* **2005**, *8*, 213–217. [[CrossRef](#)]
30. Mziouid, A.; Chebli, B.; Berrabah, M.; Chebli, H.; Heimeur, N.; Bounimi, S.; Mayad, E.H. Phytochemical screening and antioxidant activity of four Moroccan aromatic plant methanolic extracts and essential oils. *Arab. J. Med. Arom. Plants* **2022**, *8*, 117–132.
31. Tadrent, W.; Bachari, K.; Kabouche, A. Comparative compositions and antibacterial activity of the essential oils of *Anthemis nobilis* L. and *Anthemis mixta* L. (Asteraceae). *Int. J. Pharm. Pharm. Sci.* **2016**, *8*, 457–459.
32. Ainane, T.; Elkouali, M.; Ainane, A.; Talbi, M. Moroccan traditional fragrance based essential oils: Preparation, composition and chemical identification. *Der. Pharma Chem.* **2014**, *6*, 84–89.
33. Anass, E.; Amrani, A.; Eddine, J.J. Effect of the parts of plant material (flowers and leaves) on essential oil chemical composition of *Ormenis mixta* from Morocco. *J. Essent. Oil Bear Plants* **2015**, *18*, 398–408. [[CrossRef](#)]
34. Satrani, B.; Ghanmi, M.; Farah, A.; Aafi, A.; Fougrach, H.; Bourkhiss, B.; Bousta, D.; Talbi, M. Composition chimique et activité antimicrobienne de l'huile essentielle de *Cladanthus mixtus*. *Bull. Soc. Pharm. Bordx.* **2007**, *146*, 85–96.
35. Dulou, R.; Pourrat, H.; Janot, M.M. Essence of *Ormenis multicaulis* (Braun-Blanquet and Maire) grown in Morocco. *Ann. Pharm. Fr.* **1952**, *10*, 111–117.
36. Toulemonde, B.; Beauverd, D. Contribution à l'étude d'une camomille sauvage du Maroc: L'huile essentielle d'*Ormenis mixta* L. *Parfum. Cosmétiques Arômes* **1984**, *68*, 65–67.
37. Tantaoul-Elaraki, A.; Errifi, A.; Benjilali, B.; Lattaoui, N. Antimicrobial activity of four chemically different essential oils. *Riv. Ital. EPPOS* **1992**, *6*, 13–23.
38. Elouaddari, A.; El Amrani, A.; Eddine, J.J.; Correia, A.I.D.; Barroso, J.G.; Pedro, L.G.; Figueiredo, A.C. Yield and chemical composition of the essential oil of Moroccan chamomile [*Cladanthus mixtus* (L.) Chevall.] growing wild at different sites in Morocco. *Flav. Fragr. J.* **2013**, *28*, 360–366. [[CrossRef](#)]
39. Elouaddari, A.; El Amrani, A.; Eddine, J.J.; Barroso, J.G.; Pedro, L.G.; Figueiredo, A.C. Intraspecific variability of the essential oil of *Cladanthus mixtus* from Morocco. *Nat. Prod. Commun.* **2014**, *9*, 133–136. [[CrossRef](#)]
40. Formisano, C.; Rigano, D.; Senatore, F.; Raimondo, F.M.; Maggio, A.; Bruno, M. Essential oil composition and antibacterial activity of *Anthemis mixta* and *A. tomentosa* (Asteraceae). *Nat. Prod. Commun.* **2012**, *7*, 1379–1382. [[CrossRef](#)] [[PubMed](#)]
41. Wanner, J.; Schmidt, E.; Bail, S.; Jirovetz, L.; Buchbauer, G.; Gochev, V.; Girova, T.; Atanasova, T.; Stoyanova, A. Chemical composition and antibacterial activity of selected essential oils and some of their main compounds. *Nat. Prod. Commun.* **2010**, *5*, 1359–1364. [[CrossRef](#)] [[PubMed](#)]
42. Zeroual, A.; Sakar, E.H.; Eloutassi, N.; Mahjoubi, F.; Chaouch, M.; Chaqroune, A. Wild chamomile [*Cladanthus mixtus* (L.) Chevall.] collected from Central-Northern Morocco: Phytochemical profiling, antioxidant, and antimicrobial activities. *Bioint. Res. Appl. Chem.* **2021**, *11*, 11440–11457.
43. Darriet, F.; Bendahou, M.; Costa, J.; Muselli, A. Chemical compositions of the essential oils of the aerial parts of *Chamaemelum mixtum* (L.) Alloni. *J. Agric. Food Chem.* **2012**, *60*, 1494–1502. [[CrossRef](#)]

44. Chraibi, M.; Fadil, M.; Farah, A.; Lebrazi, S.; Fikri-Benbrahim, K. Antimicrobial combined action of *Mentha pulegium*, *Ormenis mixta* and *Mentha piperita* essential oils against *S. aureus*, *E. coli* and *C. tropicalis*: Application of mixture design methodology. *LWT* **2021**, *145*, 111352. [[CrossRef](#)]
45. Jeddi, M.; El Hachlafi, N.; Fadil, M.; Benkhaira, N.; Jeddi, S.; Benziane Ouaritini, Z.; Fikri-Benbrahim, K. Combination of chemically-characterized essential oils from *Eucalyptus polybractea*, *Ormenis mixta*, and *Lavandula burnatii*: Optimization of a new complete antibacterial formulation using simplex-centroid mixture design. *Adv. Pharm. Pharm. Sci.* **2023**, *2023*, 5593350. [[CrossRef](#)] [[PubMed](#)]
46. Ouedrhiri, W.; Balouiri, M.; Bouhdid, S.; Harki, E.H.; Moja, S.; Greche, H. Antioxidant and antibacterial activities of *Pelargonium asperum* and *Ormenis mixta* essential oils and their synergistic antibacterial effect. *Environ. Sci. Pollut. Res.* **2018**, *25*, 29860–29867. [[CrossRef](#)]
47. Seo, S.-M.; Kim, J.; Kang, J.; Koh, S.-H.; Ahn, Y.-J.; Kang, K.-S.; Park, I.-K. Fumigant toxicity and acetylcholinesterase inhibitory activity of 4 Asteraceae plant essential oils and their constituents against Japanese termite (*Reticulitermes speratus* Kolbe). *Pest. Biochem. Physiol.* **2014**, *113*, 55–61. [[CrossRef](#)] [[PubMed](#)]
48. Spadaro, V.; Raimondo, F.M.; Fennane, M.; Bruno, M.; Senatore, F. Chemical composition of the essential oil of *Cladanthus scariosus* (Asteraceae) wild grown in Morocco. In Proceedings of the Societa Botanica Italiana 109^o Congresso, International Plant Science Conference (IPSC) from Nature to Technological Exploitations, Florence, Italy, 2–5 September 2014; p. 142.
49. Bellioua, S.; Amari, S.; Warda, K.; Aghraz, A.; Dilagui, I.; Ouhaddou, S.; Sissi, S.; Bekkouche, K.; Larhsini, M.; Markouk, M. Chemical profile, antioxidant and antimicrobial effects of essential oil from the Moroccan endemic plant *Cladanthus scariosus* (L.). *J. Essent. Oil Res.* **2022**, *34*, 394–404. [[CrossRef](#)]
50. Suffness, M.; Pezzuto, J.M. Assay related to cancer drug discovery. In *Methods in Plant Biochemistry Assays for Bioactivity*; Hostettmann, K., Ed.; Academic Press: Cambridge, MA, USA, 1991; Volume 6.
51. Banjerdpongchai, R.; Khaw-On, P. Terpinen-4-ol induces autophagic and apoptotic cell death in human leukemic HL-60 cells. *Asian Pac. J. Cancer Prev.* **2013**, *14*, 7537–7542. [[CrossRef](#)] [[PubMed](#)]
52. Wu, C.S.; Chen, Y.J.; Chen, J.J.W.; Shieh, J.J.; Huang, C.H.; Lin, P.S.; Chang, G.C.; Chang, J.T.; Lin, C.C. Terpinen-4-ol induces apoptosis in human non-small cell lung cancer in vitro and in vivo. *Evid. Based Complement. Altern. Med.* **2012**, *2012*, 818261. [[CrossRef](#)] [[PubMed](#)]
53. Khawon, P.; Banjerdpongchai, R. Induction of intrinsic and extrinsic apoptosis pathways in the human leukemic MOLT-4 cell line by terpinen-4-ol. *Asian Pac. J. Cancer Prev.* **2012**, *13*, 3073–3076. [[CrossRef](#)] [[PubMed](#)]
54. Calcabrini, A.; Stringaro, A.; Toccaceli, L.; Meschini, S.; Marra, M.; Colone, M.; Salvatore, G.; Mondello, F.; Arancia, G.; Molinari, A. Terpinen-4-ol, the main component of *Melaleuca alternifolia* (tea tree) oil inhibits the in vitro growth of human melanoma cells. *J. Invest. Dermatol.* **2004**, *122*, 349–360. [[CrossRef](#)] [[PubMed](#)]
55. Greay, S.J.; Ireland, D.J.; Kissick, H.T.; Levy, A.; Beilharz, M.W.; Riley, T.V.; Carson, C.F. Induction of necrosis and cell cycle arrest in murine cancer cell lines by *Melaleuca alternifolia* (tea tree) oil and terpinen-4-ol. *Cancer Chemother. Pharmacol.* **2010**, *65*, 877–888. [[CrossRef](#)]
56. Setzer, W.N.; Schmidt, J.M.; Noletto, J.A.; Vogler, B. Leaf oil compositions and bioactivities of Abaco bush medicine. *Pharmacology-online* **2006**, *3*, 794–802.
57. Matsuo, A.L.; Figueiredo, C.R.; Arruda, D.C.; Pereira, F.V.; Borin Scutti, G.A.; Massaoka, M.H. α -Pinene isolated from *Schinus terebinthifolius* Raddi (Anacardiaceae) induces apoptosis and confers antimetastatic protection in a melanoma model. *Biochem. Biophys. Res. Commun.* **2011**, *411*, 449–454. [[CrossRef](#)] [[PubMed](#)]
58. Abdallah, H.M.; Ezzat, S.M. Effect of the method of preparation on the composition and cytotoxic activity of the essential oil of *Pituranthos tortuosus*. *Z. Naturforsch. C J. Biosci.* **2011**, *66*, 143–148. [[CrossRef](#)] [[PubMed](#)]
59. Council of Europe. Determination of Essential Oils in Herbal Drugs. In *European Pharmacopoeia*; Council of Europe: Strasbourg, France, 2008; pp. 251–252.
60. Lauricella, M.; D'Anneo, A.; Badalamenti, N.; Bruno, M.; D'Angelo, G.D.; Maggio, A. Essential oil of *Foeniculum vulgare* subsp. *piperitum* fruits exerts an anti-tumor effect in triple-negative breast cancer cells. *Mol. Med. Rep.* **2022**, *26*, 12759. [[CrossRef](#)] [[PubMed](#)]
61. Casiglia, S.; Bruno, M.; Bramucci, M.; Quassinti, L.; Lupidi, G.; Fiorini, D.; Maggi, F. *Kundmannia sicula* (L.) DC growing in Sicily (Italy) as a rich source of germacrene D. *J. Essent. Oil Res.* **2017**, *29*, 437–442. [[CrossRef](#)]
62. Mosmann, T. Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. *J. Immunol. Methods* **1983**, *65*, 55–63. [[CrossRef](#)]

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