International Journal of ChemTech Research
CODEN (USA): IJCRGG, ISSN: 0974-4290, ISSN(Online):2455-9555
Vol.11 No.07, pp 171-182, 2018

In vitro Propagation and Essential Oils Composition with Cytotoxicity of *Daucus capillifolius* Gilli (Apiaceae)

Wafaa H. B. Hassan[1], Mohamed Salaheldin. A. Abdelkader[2]*, Entesar El Senosy[1], Samihl. Eldahmy[1]

[1]Department of Pharmacognosy, Faculty of Pharmacy, Zagazig University, Zagazig, 44519, Egypt.
[2]Department of Pharmacognosy, Faculty of Pharmacy, Sohag University, Sohag, 82524, Egypt.

Abstract : Micropropagation and callus culture of *Daucus capillifolius* Gilli (Apiaceae) were successfully established in Murashige and Skoog(MS) solid medium enriched with different plant growth regulators. The combination of NAA (1mg/L) + BAP (0.1mg/L) was found to be the optimum for both organogenesis and embryogenesis. The compositions of essential oils obtained from wild fruits, cultivated fruits and callus culture were analyzed by GC–MS. The major identified constituents of the essential oils of wild and cultivated fruits were geranyl acetate (31.84%, 32.49%), α-azarone (21.69%), β-azarone (20.63 %), trans methyl isoeugenol (11.0%, 7.48%), α-humulene (7.25%, 4.35%), juniper camphor (4.71%, 4.26%) and α-pinene (3.26%, 3.63%), respectively. When screened, the essential oil of the wild fruits exhibited potent cytotoxic effect against Hep-G2 with IC5014.9 µg/mL and moderate activity against MCF-7 and HCT-116 cells with IC50 (29.1and 59.8 µg/mL), respectively. Meanwhile, the essential oil of the cultivated fruitsexhibited moderate activity against Hep-G2, MCF-7 and HCT-116 with IC50(36.7, 53.7 and 48.0 µg/mL), respectively.
Keywords : *Daucu scapillifolius*, essential oils,asarone, callus culture, micropropagation, cytotoxicity.

Introduction

The genus *Daucus* is a member of the family Umbelliferae (Apiaceae). It includes 60 species which are mainly distributed in the Mediterranean regions, extending to East Asia, tropical and South Africa and Australia[1]. In Libya there are ten species including *D. durieua, D. muricatus, D. carota, D.guttatus, D.capillifolius, D.jordanicus, D. littorals, D.syrticus* and *D. sahariensis*[1]. Chemically, the genus *Daucus*was reported as one of the rich sources in essential oil[2-5] , sesquiterpens[6,7], sterols and triterpenes[8], polyacetylenic compounds[9,10], sesquiterpene lactones[11,12], flavonoids and phenylpropanoids[13]. Biologically, the essential oils from many *Daucus* species were reported to possess a wide range of activities including cytotoxicity[8,12],

DOI= http://dx.doi.org/10.20902/IJCTR.2018.110721

antimicrobial[8,14], insecticidal[15], anti-inflammatory[16]and antioxidant[17]. The composition of the essential oils of fruits, leaves and stem of the wild plant of *D. capillifolius* have been studied by GC/MS[16]. In Libya, *D. capillifolius* is suffering from the threat of extinction due to the expansion in construction which destroys its environment. Micropropagation is now adopted as an efficient method of saving such endangered plant specie[17]. However, studies on the tissue culture establishment of *D. capillifolius*haven't been reported. Recently, there is an increasing interest towards the use of essential oils in the treatment and prevention of cancers[19,20].The different mechanisms by whichessential oils exhibitedsuch activityhave been recently reviewed[21]; theyinclude antioxidant, antimutagenic and antiproliferative, enhancement of immune system, enhancing detoxification, and modulation of multidrug resistance and synergistic effects.Also, individual constituents of essential oils such as carvacrol, α-humulene, and caryophyllenedisplayed cytotoxic and antitumor effects on different cancer cell lines and in*in vivo* studies[19]. Moreover,perillyl alcohol as a constituent of some essential oils has gone through phase I[22] and phase II clinical trials for ovarian and breast cancers[23,24]. The aim of the current study is to induce *in vitro* germination, callus production, somatic embryogenesis and micropropagation of seeds of *D. capillifolius* which may be used as conservation for the plant from extinction. Additionally, essential oils composition of wild, cultivated and callus tissues was determined by GC-MS and the cytotoxicity of different essential oils of wild and cultivated fruits against different cell lines was evaluated.

Experimental

Plant Material

The plant material of *Daucus capillifoliu s*Gilli (wild), was collected in March 2013 from Libya (25 Km from Al Assa, on road to Ragdalin Zwara) during the fruiting stages. The identification of the plant was verified by Assistant Prof. Mohamed Saddiki, Faculty of Pharmacy, Tripoli University. The seeds were cultivated in the experimental farm and a voucher specimen (D.C 2013/12) was deposited in the Department of Pharmacognosy Herbarium, Faculty of Pharmacy, Zagazig University, Egypt.

In vitro Seeds [Fruits] Germination of *D. capillifolius*

The seeds were soaked in both of 70% ethanol for 5 minutes, and then in 5% hypochlorite solution (Clorox®) for 10 minutes with shaking and subsequently rinsed with sterile distilled water.The sterilized seeds were transferred to 250-mL jars with solidified hormone-free media (4.4gm/L MS media, 30 gm/L sucrose and 8gm/L agar). The jars were incubated at 25±1°C and 16/8 hours light/darkphotoperiod. The percentage of seed germination was calculated as follow.

$$\textbf{Rate of } \boldsymbol{germination} = \frac{Germinated\ seeds\ number\ X\ 100}{\text{Total number of cultured seeds}}$$

Callus Culture Establishment

Seeds of *D. capillifolius* Gilli (wild), were used for establishment of plant tissue cultures. Commercially available MS medium[25] (Duchefa); 6-Benzylaminopurine (BAP), Kinetin (K), 2,4-Dichlorophenoxyacetic acid (2,4-D), Naphthalene acetic acid (NAA) and Thidiazuron (TDZ) (Sigma Chemical Co, U.S.A) as plant growth regulators; Sucrose (Adwic, A.R.E.); Agar (purified agar for plant tissue culture, Bioworld, USA).

Using aseptic technique, equal sized explants (0.5-1 cm) from different parts, leaf, stem and root, of germinated seedlings were cultured on MSmedia with 3% sucrose and 0.8% agar supplemented with different plant growth regulators combinations. Medium **A**: NAA (1 mg/L), Medium **B**: NAA (1mg/L) + BAP (0.1mg/L), Medium **C**: TDZ (0.5 mg/L) + 2, 4 D (1 mg/L) + BAP (0.1 mg/L), Medium **D**: 2, 4 D (1 mg/L), Medium **E**: Kinetin (0.5 mg/L), Medium **F**: 2, 4 D (2mg/L) + Kinetin (1 mg/L). The PH was adjusted at 5.6-5.8 and the cultures were incubated at 25 ºC under a white fluorescent lamp with light intensity (1000 µmol·m-^{2}·sec-^{1}), 16/8 hours light/dark periods for 5 weeks. Callus induction percentages (callusing capacity) and the morphological characters of induced callus of six different media was determined after 40 days of cultivation. The callus capacity was calculated by the following equation.

$$\boldsymbol{Callus\ induction\ percentage} = \frac{Number\ of\ callus\ produced}{Total\ number\ of\ explants}\ x\ 100$$

Growth Parameters

One gram of 40 days old callus grown on media **B**, **C** and **F** were subcultured on media with the same combination. The fresh weight in each case was recorded at 10, 20, 30 and 40 days after inoculation. The growth dynamic was determined as follow:

Growth Index (GI)

The growth index of callus culture was calculated based on reported method[26]; $\boldsymbol{GI} = \frac{Ge-G\ start}{G\ start}$in which **Ge**= final weight of callus at the end of generation, **G start** = Initial weight of callus the beginning.

Specific Growth rate (μ)

The specific growth rate of callus culture was calculated based onthe reported method[27]. $\boldsymbol{\mu} = (lnx - lnxo)/t.$

Where xo is the starting dry callus mass and x is the dry callus mass at 20 days (t).

Doubling Time

The doubling time of callus culture was calculated adopting a reported method[27].

$\boldsymbol{dt} = ln\ (2)/\mu$

Micropropagation of *D. capillifolius*

In vitro propagation of *D. capillifolius* from seedlings was established by different methods including direct and indirect propagation through different types of hormonal combinations using MS solid media at 25 and 16 hours photoperiod with sucrose containing media

Direct Micropropagation of *D. capillifolius* via Organogenesis

30 days old seedlings were cut aseptically into leaf, stem and root explants. The different explants were cultured in jars containing MS solid medium containing either NAA (1mg/L) + BAP (0.1mg/L) or TDZ (0.5 mg/L) + 2, 4 D (1 mg/L) + BAP (0.1 mg/L) and incubated at temperature 25 ± 1 ºC and 16 h light/8 h dark photoperiods. Also, the multi-shooted buds were cut, then transferred individually to jars containing rooting medium which consist of MS with 3% sucrose, 0.6% agar and 1 mg/L NAA. After 15-20 days, when roots appeared they transferred to MS hormone-free media containing 3% sucrose and 0.6 % agar to continue their growth.

Indirect Micropropagation of *D. capillifolius*

The callus induced first using media either NAA (1mg/L) + BAP (0.1mg/L) or TDZ (0.5 mg/L) + 2, 4 D (1 mg/L) + BAP (0.1 mg/L); after 60 days, the callus transferred to hormonal free media where the callus become more nodular and axillary shoot buds began to emerge. The 90-100 days old regenerated plantlets were transferred to liquid medium with 30 % agar and 1 mg/L NAA for 90 days for root hardening. The plantlets with good root and shoot system were carefully cleaned with sterile distilled water to remove the adhered growth medium from the roots. The plantlets were transferred into sterile soil and hardening mixtures consists of sand + peat moss (2:1), sand + soil + peat moss (1:1:1) and sand+ soil + compost (1:1:1) and covered with colorless perforated plastic bags which were removed carefully after 7 days, then maintained the pots in greenhouse.

Essential oils Isolation

200 g of the air-dried powdered fruits of each of wild as well as cultivated *D. capillifolius* Gilli plant were subjected to hydro distillation for 8 hours using Clevenger apparatus and following the method reported in Egyptian Pharmacopoeia. The oils samples were collected and completely dried by passing through anhydrous sodium sulfate. Also, 50 g of 100 days old callus grown on media (B, C and F) were extracted with *n*-hexane

(200 mL × 3), filtered and concentrated under a steam of nitrogen. All samples were kept in a refrigerator at 4ºC till use.

Essential Oils Analysis

GC-MS was carried out using Instrument: Agilent 6890 gas chromatograph, USA. Column: fused silica capillary column PAS-5 ms (30 m × 0.25 um film thickness).Carrier gas: Helium**,** Flow rate: 1 mL/ min, Injection size: 1 µL**,** oven temp. Program: started at 55^{o}C then subsequently programmed at 8 ^{o}C/ min up to 280 ^{o}C. Injector temp.: 250 ^{o}C**,** Detector temp.: 280 ^{o}C, Detector: Mass spectrophotometric, scanning from *m/z* 50 to 500**,** ionization mode: EI 70 ev. The individual components of the essential oils were identified by matching of their mass spectrawith those stored in the spectrometer database using NIST 05 mass spectral library and literature. Further confirmation was made by comparison ofretention time with standards where available and comparing their retention indices using C8-C26 *n*-alkane series[28].

Cytotoxic Assay

Cytotoxicity of the different essential oils samples of fruits of *D. capillifolius* was measured against hepatocellular carcinoma cells (Hep-G2), colon carcinoma cells (HCT-116) and breast adenocarcinoma cells (MCF-7) according to the MTT assay which previously reported[29]method using cisplatin as standard. Serial dilutions of 500, 250, 125, 62.5, 31.25, 15.6, 7.8, 3.9 and 2 µg/mL of each essential oil were used. The percentages of cell viability were measured at a wavelength of 490 nm. All results were corrected for background absorbance detected in wells without added stain. Treated samples were compared with the cell control in the absence of the tested essential oils. The effect on cell growth was calculated as the difference in absorbance percentage in presence and absence of the tested oils and illustrated in a dose-response curve. IC_{50} was obtained from the dose-response curve. Each value in the results represents the mean ± S.D. of three samples[30].

Results and Discussion

In vitro*Germination of Seeds of *D. capillifolius

The best sterilization condition was achieved by immersing the seeds into 70% ethanol for 5 minutes then in 5% sodium hypochlorite for 5 minutes. Sterilized seeds were inoculated on several types of MS media for seed germination. The results in table 1 showed that the MSand MSwith 1mg/L NAA and 0.1mg/L BAP media showed the highest germination percentage of 90 % and 60 %, respectively. No difference was noticed in the seedling length after 30 days between them. In addition small radical formation was formed after 7 days and reached up to 2 cm on solid MShormonal free. Complete shoot formation developed after 20 days and complete root formation after 25 days. The whole plant is obtained after 30 days (Figure 1). Germination percentages and seedling length after 30 days of seeds cultivation using different media composition are reported in table1**.**

Table 1.Germination percentage and seedling length after 30 days of seeds cultivation of *D. capillifolius* using different media composition.

Media composition	Germination percentage	Seedling length (30 days)
M&S	90%	6-8 cm
M&Swith 2mg/2,4 D	6%	0.5-1 cm
M&S with 2mg/2,4 D:1mg /Kinetin	20%	2-2.5 cm
M&S with 1mg/ Kinetin	3%	0.3-0.8 cm
M&S with 1mg/NAA:0.1mg/BAP	60%	5-8 cm

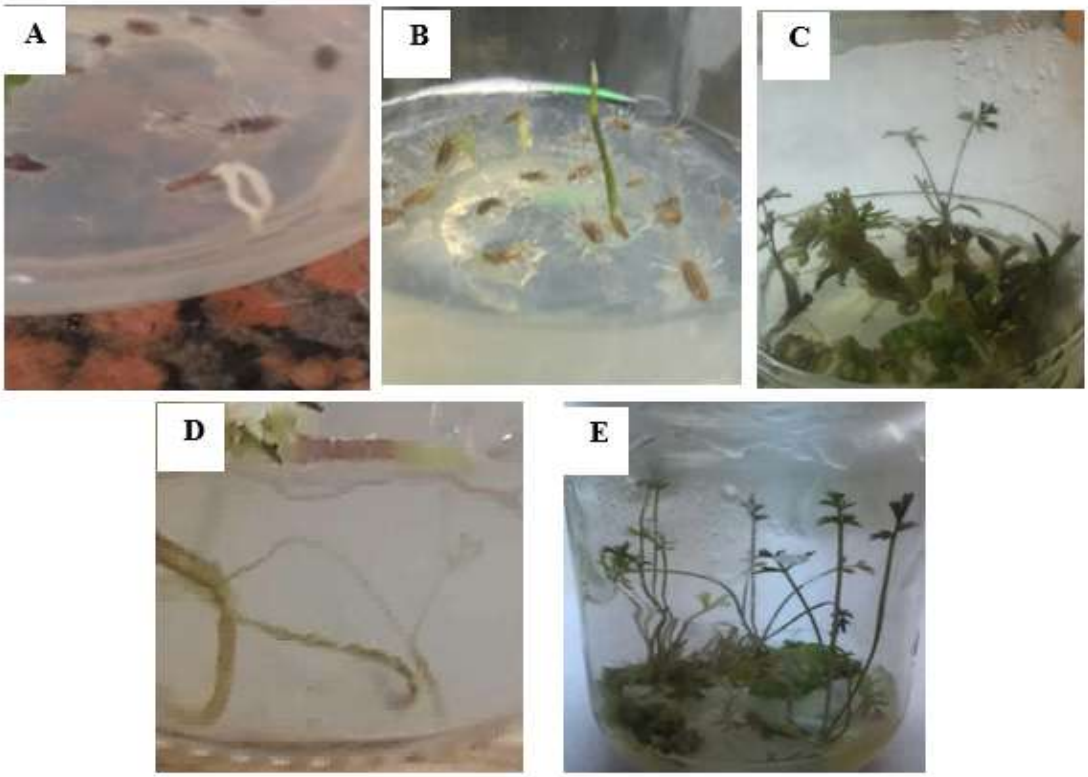

Figure 1.Stages of germination of *D. capillifolius* seeds at different time intervals on solid M&S hormonal free medium: A: Small radical formation after 7 days; B: Increase in the length of the radical up to 2 cm after 10 days; C: Complete shoot formation after 20 days; D: Complete root formation after 25 days; E: Whole plant formation after 30 days.

Induction and Growth Dynamics of Callus Culture

Callus was initiated fromleaf explants of *D. capillifolius* seedling. Excellent growth of callus with friable bright yellow, yellowish white, compact or friable greenish white were obtained in media B, C and F. It is noteworthy that medium B showed the highest, 90%, callusing capacity, followed by medium F, 85%, then medium C, with 75% as shown in table 2 and figure 2. The results in table 3 showed values of *D. capillifolius* callus growth dynamics grown on three different media (medium B, C and F)**.** Callus grown on medium B showed the fastest growth rate with the greatest value of the specific growth rate (μ = 0.047) and the shortest time to reach double its initial weight (dt= 14.7 days), whilecallus on medium C showed the slowest growth rate with the smallest specific growth rate (μ = 0.029) and the longest time to reach double its initial weight (dt= 23.9 days). According to these results, callus grown on medium B could be considered as the best medium for large biomass callus production of *D. capillifolius* plant.

Media	**(14 days)**	**(28 days)**	**(40 days)**
MediumB: [M&S +NAA (1mg/L) + BAP (0.1mg/L)]			

Medium C: [M&S + TDZ (0.5 mg/L) + 2, 4 D (1 mg/L) +BAP (0.1 mg/L)]			
Medium F: [M&S +2, 4 D (2mg/L) +K(1 mg/L)			

Figure.2 Morphological characters of callus produced from leaf explants of *D. capillifolius* seedling on three different culture media after 14, 28 and 40 days of culture

Table2. Callus induction percentages (callusing capacity) of in vitro cultivated seedlings of *D. capillifolius* on six culture media after 4 weeks of cultivation.

Media	**Callus induction percentages**		
	Seed 15 seeds/Jar	**Seedling 4 Explants/Jar**	**Mean**
Medium A: [M&S + NAA (1 mg/L)]	50	70	60
Medium B: [M&S +NAA (1mg/L) + BAP (0.1mg/L)]	100	100	100
Medium C: [M&S + TDZ (0.5 mg/L) + (2, 4 D) (1 mg/L) + (BAP) (0.1 mg/L)]	100	100	100
Medium D: [M&S +2, 4 D (1 mg/L)]	80	80	80
Medium E: [M&S +K (0.5 mg/L)]	20	60	40
Medium F: [M&S +(2, 4 D) (2mg/L) + K (1 mg/L)]	100	100	100

Table 3.Growth dynamics of *D. capillifolius* callus grown on three different media.

Type of media	**Growth dynamics**		
	GI	**M**	**dt (days)**
Medium B	2.8	0.047	14.7
Medium C	2.0	0.029	23.9
Medium F	2.9	0.043	16.1

GI: Growth index, **μ:** Specific growth rate, **dt:** Doubling time

In vitro* Micropropagation of *D. capillifolius.

Different *in vitro* culture techniques have been tested for propagation of *D. capillifolius*. The leaf explants after 30 days of cultivation on MShormonal free medium were able to differentiate into a whole plant. Meanwhile, stem and root explants were not able to differentiate. The shoot number per explant ranged from 2 to 5 shoots with length range from 4-10 cm. Embryogenic callus was obtained from callus using either medium NAA (1mg/L) + BAP (0.1mg/L) or medium TDZ (0.5 mg/L) + 2, 4 D (1 mg/L) + BAP (0.1 mg/L). The 60 days old callus grown on the above media showed pre-embryogenic masses and globular embryos (Figure 3). When the callus transferred to hormonal free media, the callus become more nodular and axillary shoot buds began to

emerge with many red spots. Different forms of somatic embryos were formed (globular, heart shape, torpedo shaped and cotyledonary), the whole plantlets were regenerated after 90-100 days of growth on hormonal free MS media. Also, the multi-shooted buds developed whole plantlets when transferred individually to jars containing rooting medium which consist of MSwith 3% sucrose, 0.6% agar and 1mg/L NAA.The 90-100 days old regenerated plantlets were transferred to liquid medium with 30 % agar and 1mg/L NAA for 90 days for root hardening; only 25% of plants were survived to 60-65 days after transplantation as shown in figure 3. This might due to the inadequate control of the environmental condition from temperaturesand humidity inside the green house or to the root weakness.

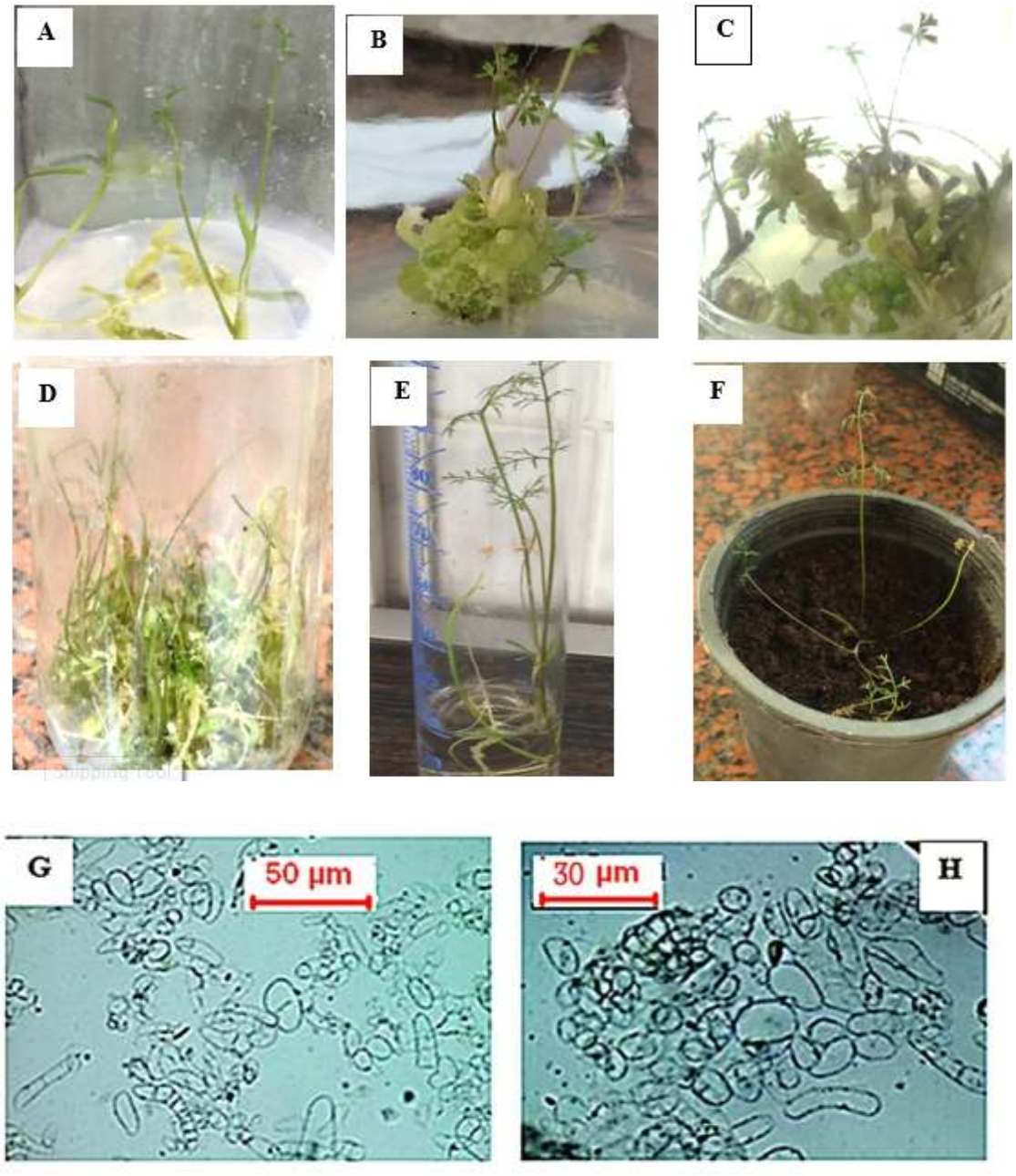

Figure 3.Maturation of somatic embryos and plantlets regeneration of *D. capillifolius* A: Direct micropropagation from leaf explant after 30 day of cultivation on medium C B: 90 days old multi-shooted callus on medium C C: Differentiated plantlets after 60 days B: Developed plantlets after 90 days on hormonal free medium E: 60 days old regenerated plantlets in liquid rooting medium F: 60 days old regenerated plant on soil; G and H: Different forms of somatic embryos including globular, heart shape, torpedo shaped on MS hormonal free medium.

Essential Oils Composition of Wild and Cultivated Fruits

Table 4 showed the yield, the compositions and the percentage of the essential oils samples obtained by hydro-distillation of wild and cultivated fruits of *D. capillifolius.* The oil yields of wild fruits (2% v/w) were higher than those obtained from cultivated fruits (1.5% v/w). Twenty-six components (93.20%) and twenty-five components (89.47%) were identified in the oil of wild and cultivated fruits, respectively. Apart from α- and β-azarones, profiles of the oil samples were qualitatively similar with minor quantitative difference. β-Azarone isomer (21.69%) was detected as one of the major components in the oil samples of wild fruits while α-azarone isomer (20.63%) was detected in the oil of cultivated fruits only. The other main components were geranyl acetate (31.84, 32.49%), *trans*-methyl-isoeugenol (11.00, 7.48%), *β*-bisabolene (6.08, 6.74%) and α-humulene (7.25, 4.35%) in the oils of wild and cultivated fruits, respectively. It was observed that *D*-limonene (0.12%), *cis*-verbenol (0.17%), *β*-elemen (0.04%), methylvanillin (0.04%) were detected in wild fruits oil and completely absent in cultivated fruits oil, while α-terpineol (0.02%), myrenyl acetate (0.06%), *α*-terpenyl acetate (0.13%), germacrene B (0.79%) were detected in cultivated fruits oil and completely absent in wild fruits oil. Azarones, are previously detected as components of essential oils of the genus *Acorus*[31]and of the genus *Daucus*[32]. Despite, α-asarone and β-asarone were exhibited genotoxicity and hepatocarcinogenicity in rodents[33]; many pharmacological activities including anti-epileptic, hypolipidemic and treatment of cerebral ischemia and Alzheimer's disease have been reported for asarones[33].

Table 4. Percentage compositions of essential oils isolated from the cultivated (C) and wild fruits (W) of *D. capillifolius*.

Compound	RI	Relative %		Identification
		W	C	
α- Thujene	936	0.03	0.05	RI, MS
α- Pinene	942	3.26	3.63	RI, MS
Camphene	955	0.24	0.25	RI, MS
Sabinene	977	0.68	2.17	RI, MS
β- Myrcene	993	0.19	0.31	RI, MS
α- Terpinene	1018	0.03	0.03	RI, MS
O- Cymene	1027	0.51	1.17	RI, MS
D-Limonene	1031	0.12	-	RI, MS
γ-Terpinene	1063	0.62	1.05	RI, MS
Linalool	1105	0.88	0.17	RI, MS
Nonanal	1109	0.01	-	RI, MS
Cis-Verbenol	1150	0.17	-	RI, MS
Terpinen-4-ol	1182	0.24	0.21	RI, MS
α-Terpineol	1194	-	0.02	RI, MS
Geraniol	1259	2.23	1.74	RI, MS
Bornyl acetate	1293	0.92	0.62	RI, MS
Myrenyl acetate	1332	-	0.06	RI, MS
α-Terpinenyl acetate	1355	-	0.13	RI, MS
Neryl acetate	1367	0.14	0.19	RI, MS
Geranyl acetate	1390	31.84	32.49	RI, MS
β-Elmene	1399	0.04	-	RI, MS
Methyleugenol	1408	0.21	0.26	RI, MS
Trans-α-Bergamotene	1444	0.06	0.07	RI, MS
α-Humulene	1467	7.25	4.35	RI, MS
Methyl vanillin	1489	0.05	-	RI, MS
Trans-methyl isoeugenol	1506	11.00	7.48	RI, MS
β-Bisabolene	1518	6.08	6.74	RI, MS
Germacrene B	1571	--	0.79	RI, MS
β-Asarone	1619	21.69	-	RI, MS
α-Asarone	1684	--	20.63	RI, MS

Juniper Camphor	1623	4.71	4.26	RI, MS
Total identified %		93.2	89.87	
Yield		2.0%	1.5%	

RI: Retention index relative to n-alkanes series MS: Mass spectroscopy

Essential Oil Components of Callus Culture

The *n*-hexane extract of the callus culture grown separately on MS media with different hormonal composition including media B, C and F were subjected to GC/MS analysis. The extraction resulted in the isolation of pale greenish yellow extracts (2% volume/dry weight) with no characteristic odor. Table 5 showed the major components of extract from different media which revealed the presence of minor components of essential oil (0.73–1.02%; collectively in the different media) with minor qualitative and quantitative differences. Instead of essential oil accumulation in the callus culture, the major components were the fatty acids linoleic (77.81-81.43%) and palmitic (7.4-9.13 %) acids. Although, thymoquinone was detected in variable quantities in all cultures, no thymoquinone was detected in the essential oils of the parent wild or cultivated fruits.Production of essential oils by plant cell cultures is widely studied; however, the accumulation was usually at very low levels[34]. The reason for such non-accumulation of terpenoids was attributed to the lack of specialized structure such as trichomes or oil glandsin the callus culture which is essential for production and storage of essential oils[35].

Table 5. Essential oil components of ether extract of the callus grown separately on three different media B, C and F.

Compound	RI	% Relative /Medium			Identification
		B	C	F	
α-Thujene	936	0.09	0.12	0.10	RI,MS
α-Pinene	942	0.02	0.03	0.02	RI,MS
β-Pinene	890	0.03	0.04	0.03	RI,MS
O-Cymene	1027	0.18	0.25	0.20	RI,MS
Thymoquinone	1256	0.22	0.39	0.27	RI,MS
Anethole *E*	1293	0.05	-	0.11	RI,MS
α-Copaene	1384	0.05	0.08	0.06	RI, MS
γ-Himachalene	1491	0.04	-	-	RI,MS
α-Muurolene	1511	0.05	0.04	0.05	RI,MS
Palmitinic acid	1677	9.13	7.4	8.42	RI,MS
Linoleic acid	2130	81.43	77.88	81.06	RI,MS
Total identified %		91.29	86.23	90.32%	

RI: Retention index relative to n-alkanes seriesMS: Mass spectroscopy

Cytotoxic Activity

The cytotoxic activity of the essential oils of wild and cultivated fruits of *D. capillifolius* against Hep-G2, HCT-116 and MCF-7 cells atvarious concentrations (500, 250, 125, 62.5, 31.25, 15.6, 7.8, 3.9, 2, 0 µg/mL) are showed in figure 4. The essential oil of wild fruits showed potent cytotoxic activity against Hep-G2 with IC_{50} 14.9 µg/mL and moderate activity against MCF-7 and HCT-116 cells with IC_{50} 29.1 and 59.8 µg/mL, respectively, followed by the essential oil of the cultivated fruits, where it showed moderate activity against Hep-G2, MCF-7 and HCT-116 with IC_{50} 36.7, 53.7 and 48.0 µg/mL respectively. None of the tested oils was active as cisplatinwhich showed strong activity against HCT-116,Hep-G2and MCF-7 cells with IC_{50}2.43, 3.67 and 5.71 µg/mLrespectively.The higher cytotoxic activity of wild essential oil especially against Hep-G2 and MCF-7 cell lines could be attributed to the presence of higher content of α-humulene, a sesquiterpene that reported to possess potent antitumor activity[36]. Also, the cytotoxicity of α-asarone and β-asarone was investigated in Hep-G2 cells; α-asarone was found to be more cytotoxic than β-asarone[37]. The National Cancer Institute (NCI) stated that plant extracts with cytotoxic activity of IC_{50} values ≤ 30µg/mL are considered promising and active[38]. Accordingly, the essential oil of wild fruits is considered a potential new natural remedy for liver and breast cancers that need further *in vivo* and clinical investigations to confirm this efficacy and

safety due to its contents of β-asarone.The presence of α-asarone and β-asarone in the essential oils of *D. capillifolius* could limit its use as herbal product because of their reported genotoxic and hepatocarcinogenic properties. Asarones content of herbal medicinal products was the subject of public statement announcementin 2005 from the committee of herbal medicinal products of European Medicines Agency. The statement limited the intake of β-asarone from different herbal products of up to 115 µg/day or about 2 µg/kg body weight/day until a full benefit to risk assessment to be carried out[39].

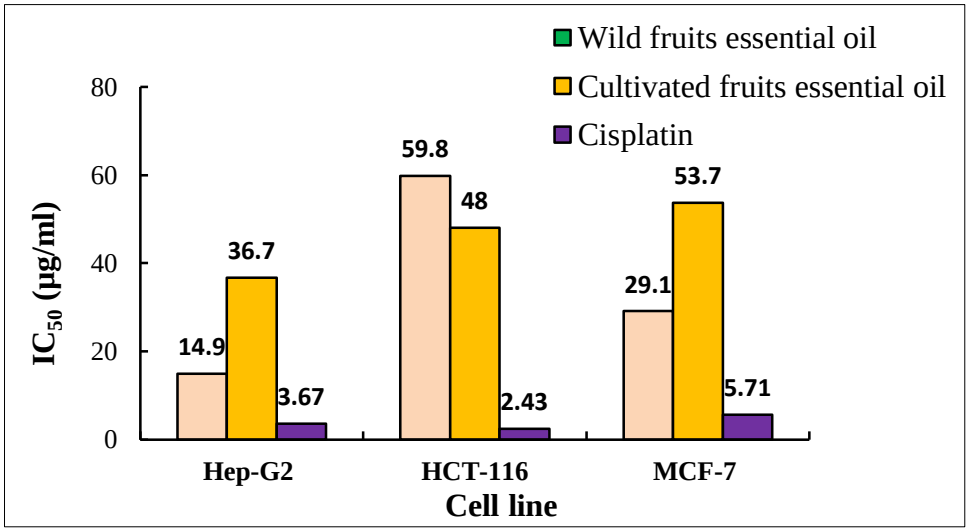

Figure 4. IC_{50} of cytotoxic effect of essential oils of the wild and cultivated fruits of *D. capillifolius* against Human hepato carcinoma cell line (Hep-G2), Colon carcinoma cell line (HCT-116) and breast adenocarcinoma cell line (MCF-7) using Cisplatin standard

Conclusion

In this study, micropropagation and callus culture of the endangered species*D. capillifolius*, were successfully established from seedlings in MSsolid medium with different hormonal combination. Composition and cytotoxicscreening of the essential oils from the wild and cultivatedfruitshave been studied. Monoterpene ester, geranyl acetate, was the major component of the essential oils of wild and cultivated fruits while it was completely absent in callus culture. Low accumulation of essential oils in callus culture was obtained; the major components were linoleic acid (77.81-81.43%) and palmitic acid (7.4-9.13%). The essential oil of wild fruits displayed higher cytotoxicity than the essential oil of the cultivated fruits against Hep-G2, MCF-7 and HCT-116 cells lines. Further *in vitro* experiments need to be carried out to enhance the micropropagation of *D. capillifolius* and compare the essential oils of the *in vivo* and *in vitro* plants.

Disclosure Statement

No potential conflict of interest was reported by the authors. This research is self-funding and not for profit sectors.

References

1. Jafri S.M.H, and El-Gadi A., Flora of Libya. Tripoli: Dept. of Botany, Al Faateh University,1987.
2. Gonny M. Bradesi P. and Casanova J., Identification of the components of the essential oil from wild Corsican *Daucus carota* L. using 13C-NMR spectroscopy. Flav Fragr J., 2004, 19:424-433.
3. Flamini G. Cioni P.L. Maccioni S. and Baldini R., Composition of the essential oil of *Daucus gingidium* L. ssp. gingidium. Food Chem., 2007, 103:1237-1240.
4. Chizzola R., Essential oil composition of wild growing Apiaceae from Europe and the Mediterranean. Nat Prod Commun., 2010, 5:1477-1492.
5. Aćimović M. Stanković J. Cvetković M. Ignjatov M.and Lj N., Chemical characterization of essential oil from seeds of wild and cultivated carrots from Serbia. Bot Serb., 2016, 40:55-60.

6. Fu H-w. Zhang L. Yi T. Feng Y-l.and Tian J-k., Guaiane type sesquiterpenes and other constituents from *Daucus carota* L. Biochem Syst Ecol., 2010, 38:309-312.
7. Fu Z-., Han H-T. Liu N. Xu X-B. Zhu W. Gong M-H. Zhang L. and Tian J-K., Two new eudesmane sesquiterpenoids from *Daucus carota* L. Phytochem Lett., 2015, 14:35-38.
8. Yousefbeyk F. Gohari A.R. Hashemighahderijani Z. Ostad S.N, Salehi S. Amini M. Golfakhrabadi F. Jamalifar H. and Amin G., Bioactive Terpenoids and Flavonoids from *Daucus littoralis* Smith subsp. hyrcanicus Rech.f, an Endemic Species of Iran. DARU J Pharm Sci., 2014, 22:12.
9. Dawid C. Dunemann F.Schwab W. Nothnagel T. and Hofmann T., Bioactive C17-Polyacetylenes in Carrots (*Daucus carota* L.): Current Knowledge and Future Perspectives. J Agric Food Chem., 2015, 63:9211-9222.
10. El-Houri R.B. Kotowska D. Christensen K.B. Bhattacharya S. Oksbjerg N. Wolber G. Kristiansen K.and Christensen L.P., Polyacetylenes from carrots (*Daucus carota*) improve glucose uptake in vitro in adipocytes and myotubes. Food Funct., 2015, 6:2135-2144.
11. Sallam A. Hitotsuyanagi Y. Mansour E-S.S. Ahmed A.F. Gedara S. Fukaya H.and Takeya K., Phenylpropanoid triesters from *Daucus glaber*. Phytochem Lett., 2009, 2:188-191.
12. Sallam A. Hitotsuyanagi Y. Mansour E-S.S. Ahmed A.F. Gedara S. Fukaya H. and Takeya K (2010) Sesquiterpene Lactones from *Daucus glaber*. HeIv. Chim. Act., 2010, 93:48-57.
13. Harborne J.B.and Williams C.A., Flavonoid patterns in the fruits of the umbelliferae. Phytochemistry., 1972, 11:1741-1750.
14. Bendiabdellah A. El Amine D.M. Djabou N. Allali H. Tabti B. Muselli A.and Costa J., Biological activities and volatile constituents of *Daucus muricatus* L. from Algeria. Chem Cent J., 2012 6:48.
15. Sharma R. Deshpande S. Tungikar V. and Joseph M., Toxicity of natural essential oils to mosquitoes, *Aedes aegypti* and *Culex fatigans*. Geobios., 1994, 21:162-165.
16. Taha E. Samih I. Mahmoud K. Afaf S.and Saad E., Study of the essential oil of *Daucus syrticus*. Bull Facualty of Pharmacy Cairo University Journal., 1994, 32:76-81.
17. Zhang D. and Hamauzu Y. Phenolic compounds and their antioxidant properties in different tissues of carrots (*Daucus carota* L.). J Food Agricul Environ., 2004, 2:95-100.
18. Liao Z. Chen M. Sun X.and Tang K., Micropropagation of Endangered Plant Species. In: Loyola-Vargas VM, Vázquez-Flota F, editors. Plant Cell Culture Protocols. Totowa, NJ: Humana Press, 2006, 179-185.
19. Gautam N. Mantha A.K.and Mittal S. Essential Oils and Their Constituents as Anticancer Agents: A Mechanistic View. Biomed Res Int. 2014, 154106.
20. Lesgards J-F. Baldovini N. Vidal N. and Pietri S., Anticancer Activities of Essential Oils Constituents and Synergy with Conventional Therapies: A Review. Phytother Res., 2014, 28:1423-1446.
21. Bhalla Y. Gupta V.K.and Jaitak V., Anticancer activity of essential oils: a review. J. Sci. Food Agric., 2013, 93:3643-3653.
22. Ripple G.H. Gould M.N. Stewart J.A. Tutsch K.D. Arzoomanian R.Z. Alberti D. Feierabend C. Pomplun M. Wilding G. and Bailey H.H., Phase I clinical trial of perillyl alcohol administered daily. Clin Cancer Res., 1998, 4:1159-1164.
23. Bailey H.H. Levy D. Harris L.S. Schink J.C. Foss F. Beatty P. and Wadler S. A Phase II Trial of Daily Perillyl Alcohol in Patients with Advanced Ovarian Cancer: Eastern Cooperative Oncology Group Study E2E96. Gynecol Oncol., 2002, 85:464-468.
24. Bailey H.H. Attia S. Love R.R. Fass T. Chappell R. Tutsch K. Harris L. Jumonville A. Hansen R. and Shapiro G.R., Phase II trial of daily oral perillyl alcohol (NSC 641066) in treatment-refractory metastatic breast cancer. Cancer Chemother Pharmacol., 2008, 62:149-157.
25. Murashige T. and Skoog F., A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol Plant., 1962, 15: 473-497.
26. Verpoorte R, Van Derheijden R. Ten H. and Memelink J., Metabolic engineering for the improvement of plant secondary metabolite production. Plant Tissue Cult Biotechnol.,1998, 4:3-20.
27. Godoy-Hernández G. and Vázquez-Flota F.A., Growth Measurements:Estimation of Cell Division and Cell Expansion. In: Loyola-Vargas VM, Vázquez-Flota F, editors. Plant Cell Culture Protocols. Totowa, NJ: Humana Press, 2006, 51-58.
28. Adams R.P., Identification of essential oil components by gas chromatography/mass spectrometry. Carol Stream, Ill.: Allured Pub. Corp., 2007.
29. Mosmann T., Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. J Immunol Meth.,1983, 65:55-63.

30. Elaasser M.M. Abdel-Aziz M.M.and El-Kassas R.A., Antioxidant, antimicrobial, antiviral and antitumor activities of pyranone derivative obtained from *Aspergillus candidus*. J Microbiol biotechnol Res., 2017, 1:5-17.
31. Raal A. Orav A. and Gretchushnikova T. β-Asarone content and essential oil composition of *Acorus calamus* L. rhizomes from Estonia. J Essent Oil Res., 2016, 28:299-304.
32. Khalil N. Ashour M. Singab A.N.and Salama O., Bioassay guided fractionation and cytotoxic activity of *Daucus carota* var. boissieri. Future J Pharm Sci., 2017, doi.org/10.1016/j.fjps.2017.07.002
33. Patel D.N. Ho H.K. Tan L.L. Tan M-M.B. Zhang Q. Low M-Y. Chan C-L.and Koh H-L., Hepatotoxic potential of asarones: in vitro evaluation of hepatotoxicity and quantitative determination in herbal products. Front Pharmacol., 2015, 6:25.
34. Susan C.R., Production and engineering of terpenoids in plant cell culture, Nat Chem Biol.,2007, 3:387-395.
35. Tisserat B. and Vaughn S.F., Growth, Morphogenesis, and Essential Oil Production in *Mentha spicata* L. Plantlets in vitro. . In Vitro Cell. Dev. Biol.Plant., 2008, 44:40-50.
36. Legault J. Dahl W. Debiton E. Pichette A. and Madelmont J-C., Antitumor Activity of Balsam Fir Oil: Production of Reactive Oxygen Species Induced by α-Humulene as Possible Mechanism of Action. Planta Med., 2003, 69:402-407.
37. Unger P.and Melzig M.F., Comparative Study of the Cytotoxicity and Genotoxicity of Alpha- and Beta-Asarone. Sci Pharm.,2012, 80:663-668.
38. Karuppiah V. Zhang F. and Li Z., Natural Products with Anticancer Activity from Marine Fungi. In: Kim S-K, editor. Handbook of Anticancer Drugs from Marine Origin. Cham: Springer International Publishing, 2015, 253-267.
39. European Medicines Agency (EMEA), Committee on Herbal Medicinal Products, Evaluation of Medicines for Human Use, Public Statement on the Use of Herbal Medicinal Products Containing Asarone (DocRef: EMEA/HMPC/139215/2005), London 2005.

*****