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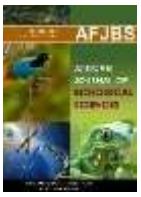

Effect of the Growth Stages on Essential Oils composition of Algerian *Ferula Communis* and their İmpact on Atiproliferative Activity

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Abstract:

Ferula communis of the apiaceae family is renowned for its richness in secondary metabolites, reputed for their biological activities is widely used in medicine for the treatment of many diseases, the objective of our study is the extraction by hydrodistillation of the essential oils of the aerial part of *F.communis* harvested during two vegetative stage before flower (F1) and in full flowering (F2), both oils were analyzed by GC-MS followed by the study the anti- peoliferative activity on line C6 (rat brain tumor) with the ELISA test assay and the xCELLigence test (RTCA) against HeLa cell lines, The results showed that the percentage of essential oil of the F1 is **99.99%** with majoritarian compounds the β-Cubebene with a percentages of 18.33% followed with the β-cadinene at a rate of 12.56 % whereas the rate of the plant essential oil in full flowering (F2) is 99.88 % with the majority compound the phytol (27.67%) followed by the 3.4,4a,5-Tetrahydrobenzo[gisoquinolin-10(2H)-one (15, 38%) following Naphthalene, 1.3-dimethyl- (11,56%) The anti-proliferative activity is proportionate to the concentration in essential oil, the F1 vegetative stage records the best antiproliferative activity against the HeLa line than the F2 (full flower) at different concentrations, while against the C6 line it is the F2 that records a high activity and remains higher than that of the standard the 5-FU

***Keywords*:** *Ferula communis*, essential oils, antiproliferative activity, ELISA test assay, xCELLigence test.

Introduction

The genus *Ferula* is one of the plants of the Apiaceae family, known for its richness in secondary metabolism. It includes about 130 species spread among the countries of the mediterranean and central Asia. In Algeria, there are 12 species, according to including *F. communis*, which is the most widespread species in northeastern Algeria, and it is a perennial plant with tall stems, 1-3m. Leaves 3-pinnatized. Yellow flowers. Large, smooth fruits. Chemically, this genus is characterized by its richness in sesquiterpenes, especially daucan type (Yoshino et al.2024), as well as coumarin sesquiterpenes (Dastan et al. 2014), flavonoids (Nouioura et al. 2024) and essential oils (Sahebkar et al. 2011). These substances are distributed throughout all parts of the plant, and the roots and fruits are considered among the richest parts. Although this species has received many studies, the difference in climate and soil has led to higher differences in the active compoundss, especially those related to essential oils, which are significantly and clearly affected by the growth stages. From a biological standpoint, the genus *Ferula* in general has shown great antibacterial, antifungal, antioxidant, anti-inflammatory and anti-cancer activities (Naji et al.2021). Cancer, which is considered the second most dangerous disease in the world, threatens the lives of many people (Pilleron et al.). Breast cancer may be the First, yet uterine cancer and brain cancer are considered dangerous types as well. In this context, we propose this work, which is based on the essential oils of the *Ferula communis* plant during two stages of growth and its effect on two types of cancer cells.

Materials and Methods

Extraction

The aerial parts of *Ferula communis* of two stage *have been* collected during january and april 2019 from constantine, Algeria. The two samples were dried at room temperature until they reached a constant weight.

The plants were identiFied by Pr.Dr.zellagui amar and A sample voucher was placed in the Laboratory of Biomolecules and Plant Breeding at the University of Larbi Ben Mhidi Oum El Bouaghi, Algeria (*Ferula communis* voucher number ZAGA 261). The dried plant material (100 g for two samples) were hydrodistilled in a Clevenger-type apparatus for 3 h. The extracts oil were stored under dry conditions at 4°C until analyzed.

GC-MS analysis

GC/MS analyses were obtained on Perkin Elmer mass spectrometer with built-in auto sampler using BPX-20 column (30 m × 0.25 mm × 0.25 μm F1lm). For GC/MS detection, an electron ionization system, ionization energy of 70 eV, was used. Helium was the carrier gas, at a flow rate of 1.3 mL/min. The column temperature was operated under the same conditions as described above. Identification of the individual components was based on (a) comparison of their GC retention indices (RI) with those of authentic compounds or literature data and (b) computer matching with a mass spectral library and commercial libraries (WILLEY and NIST database/ChemStation data system).

Antiproliferative activity

Antiproliferative activity was evaluated by estimation of the inhibitory effect of the essential oils on the growth of cells on C6 (rat brain tumor) using proliferation BrdU ELISA assay, and was tested for HeLa cell lines using a real-time cell analyzer (xCELLigence) (Yaglioglu *et al*, 2014).

Cell Culture

The cells were developed in Dulbecco's modified eagle's medium (DMEM, Sigma, Munich, Germany), complemented with 10% (v/v) fetal bovine serum (Sigma, Munich, Germany) and PenStrep solution (Sigma, Munich, Germany) at 37°C in a 5% CO_2 humidified atmosphere.

ELISA Assay

The cells were seeded into 96-well culture plates (COSTAR, Corning, USA) at a density of 30,000 cells per well. The samples were tested at concentrations of 250, 100, and 50 µg/mL. Following seeding, the cells were allowed to incubate overnight. Subsequently, the BrdU Cell Proliferation ELISA assay reagent (Roche, Germany) was applied to the cells according to the manufacturer's protocol.

The absorbance representing cell proliferation was measured at 450 nm using a microplate reader (Awareness Chromate, USA). The results were reported as the percentage of inhibition of cell proliferation, with the optical density from vehicle-treated cells considered as 100% proliferation. The stock solutions of the extracts were prepared in dimethyl sulfoxide (DMSO) and then diluted with DMEM, ensuring that the Final concentration of DMSO remained below 0.1% in all tests. 5-FU was utilized as the standard compound.

The percentage of inhibition of cell proliferation was calculated as follows: [1 - [$A_{\text{treatments}}$ /$A_{\text{vehicle control}}$]] x 100. The half maximal inhibitory concentration **(IC_{50})** is a measure of the effectiveness of a compound in inhibiting a biological function. In this paper, IC_{50} wes determined using **ED50** in addition to **V1.0**

XCELLigence Assay

A real-time cell analyzer–single plate (RTCA-SP) instrument (Roche Applied Science, Basel, Switzerland) was used to analyze the ability of extracts to induce cell growth of HeLa cell line. A newly developed electronic cell sensor array, the xCELLigence RTCA, was used with a recently published literature method at concentrations of 250, 100, and 50 _g/mL. All the measurements were done in 10 min intervals and triplicated (Kolda et al, 2015).

Results and discussion

GC-MS analyses

The compounds identified in *F. communis* essential oil during the two vegetative stages by GC-MS are presented in Table 1. For the F1, 22 compounds were identified representing 100% of the total compositions of the oil while for the sample F2 only 16 compounds were identified representing 99.98% of the overall oil.

The major F1 compounds (before flowering) are found to be: B-cubebene (18.33%) and beta-cadinene (12,56%) which are sesquiterpenes. in the oil of the full flowering phase (F2) the major compounds were phytol (27.67%), the 3,4,4a,5-Tetrahydrobenzo[g]isoquinolin-10(2H)-one (15.38%) and Naphthalene, 1,3-dimethyl- (11.56%)

It is noted that the composition of *F. communis* essential oil differs depending on the stage of growth. The results in both vegetative stages are different and have only two compounds in common which are Caryophyllene (1.71%, 1.60%) and Phytol, Acetate (2.42%, 3.89%)

The GC-MS analysis results reported in table 1 indicate that the majority of constituents of the essential oil are sesquiterpenes for example B- cubebene (18.33%). beta-cadinene (12.56%). α-muurolen (4.75%). γ-Muurolene (6.77%) Caryophyllene (1.71%) in the F1 and o beta-Cubeben (5.94%). gamma-elemene (3.54%) and caryophylene (1.60%) in F2. A number of studies reported the chemical composition of this plant. It seems that the essential oil composition of *F.communis* varies depending mainly on the collection region. The essential oil of different parts of *F.communis* growing spontaneously in Greece is characterized by its

richness in sesquiterpenes which represent (85,7- 88,7%) of the leaf oil composed mainly of α- and β-eudesmol (12.6%, 9.7%), δ-cardinene (10.8%) and germacrene β (10.1%) δ-cadinene (13.6) and γ-cadinene (12.5) **(Manolakou *et al,* 2013).** Another study conducted on the essential oil from the leaves of *F.Communis* de Corcica revealed 47 compounds representing 95.0% of the total oil and the major compound is the monoterpene myrcene (53.5%) and the sesquiterpene aristolene (8.5%) **(Ferrari *et al,* 2005).** Marongiu *et al* (2005) made a comparison between Hydrodistillation and supercritical fluid extraction of flowerheads from Sardinia and found α- and β-gurjunene (40.7 and 7.1%, respectively) as the major components in both essential oils **(Marongiu *et al,* 2005).** Results obtained by Kavaz and El faraj, (2023) on the ethanol extracts of the leaves of *F.communis* grown in Cyprus show that the major compound is phytol (35.63%). Maggi *et al* (2016) analyzed the essential oils of the different parts of *F. Communis* (flowers, fruits, leaves and roots) growing in central Italy. α-pinene (10.5%), γ-terpinene (7.6%) and hedycariol (8.4%) were the principal constituents in flowers; α-pinene (55.9%), β-pinene (16.8%) and myrcene (5.9%) in fruits; β-eudesmol (12.1%), α-eudesmol (12.1%) and hedycariol (10.3%) in leaves and (E)-β-farnesene (9.5%), β-cubebene (8.2%) and (E)- caryophyllene (7.2%) in roots. Rahali et al (2021) studies the essential oil composition of different parts of Tunisian *Ferula communis* and the results obtained are as follows: The major compounds of stem essential oil were isoshyobunone (18.3%), 6-tert-butyl-4-methylcoumarin (7.95%) and β-bisabolene (5.94%). The leaf essential oil was characterized by the predominance of α-eudesmol (12.3%), caryophyllene oxide (5.47%), ɣ-terpinene (5%), α-pinene (5%) and γ-cadinene (5%). The main essential oil in flower was caryophyllene (15.12%), myrcene (10.28%), α-eudesmol (9.42%) and α-pinene (8.26%). However, hexadecanoic acid (16.45%) and α-gurjunene (10.45%) were the main compounds of fruit essential oil. According to the studies of the literature carried out on the essential oils of the different parts of *F.communis* in different regions of the Mediterranean there is a great variability depending on geography and other factors.

Table I: Essential oils composition of F1 and F2 analysed by GC-MS

Pic	Compounds	Tr	F1	F2
1	α-Cubebene	27.021	1.1	-
2	γ-Muurolene	28.088	**6.8**	tr
3	Eremophilene	28.403	1.4	-
4	Caryophyllene	29.513	1.7	1.6
5	γ -Elemene	29.710	tr	3.5
6	Isoledene	30.105	3.1	-
7	Guaia-9,11-diene	30.569	2.3	-
8	α-amorphene	31.137	4.2	tr
9	β-Cubebene	31.432	**18.3**	**5.9**
10	α-Muurolene	31.825	4.7	2.7
11	Chamigren	32.015	1.5	-
12	γ-Cadinene	32.327	4.0	tr
13	delta-Cadinene	32.496	-	2.0
14	β-Cadinene	32.521	**12.6**	tr
15	cubedol	34.231	3.8	tr
16	ledene oxide	34.360	4.1	tr
15	Drimenol	34.481	**7.1**	-

17	Caryophyllene	34.569	-	1.5
18	Calarene epoxide	34.806	5.2	tr
19	Calarene epoxide	34.932	3.6	tr
20	Phytol	35.843	tr	**27.7**
21	tau-Muurolol	36.080	3.1	-
22	t-Cadinol	36.446	3.1	-
23	γ-Eudesmol	37.008	3.3	tr
24	Farnesol	37.868	2.5	-
25	Benzene, 1,2-bis(1-buten-3-yl)-	38.347	-	1.8
26	Naphthalene, 1,3-dimethyl-	39.949	tr	**11.6**
27	Phytol, acetate	40.768	2.4	3.9
28	Hexahydrofarnesyl acetone	40.955	-	1.1
29	2-Furanpropanoic acid	41.132	tr	5.2
31	3,4,4a,5-Tetrahydrobenzo[g]isoquinolin-10(2H)-one	42.231	tr	**15.4**
32	2H-Pyran, tetrahydro-2-[(1-methyl-4-phenyl-2-butynyl)oxy]-	43.401	tr	**10.2**
33	Bicyclo[4.1.0]heptane, 7-(phenylmethylene)-	44.839	tr	5.5
Total			99.9	99.8

Anti-proliferative activity of essential oils of *F. communis*

Antiproliferative activities of **F1, F2** and 5-FU were determined against C6 cells. The antiproliferative activities of all the essential oils were shown to increase of the activities depending to dose increasing against C6 cells (Fig 1). **F1, F2** essential oils were determined to have quite high antiproliferative activities than 5-FU against C6 cells at all concentrations (Fig 1). The potency of inhibitions (at 100 and 50 µg/mL) against C6 cells were: **F2** > **F1** > 5-FU**.**

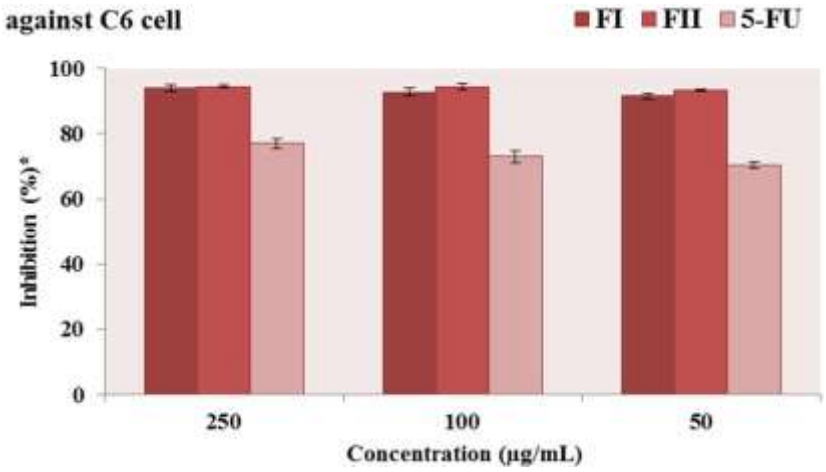

Fig 1. The antiproliferative activity of the essential oil against C6 cells. *Each substance was tested twice in triplicates against cell lines. Data show average of two individual experiments ($p<0.01$).

— 250 — 100 — 50 —Control —Medium

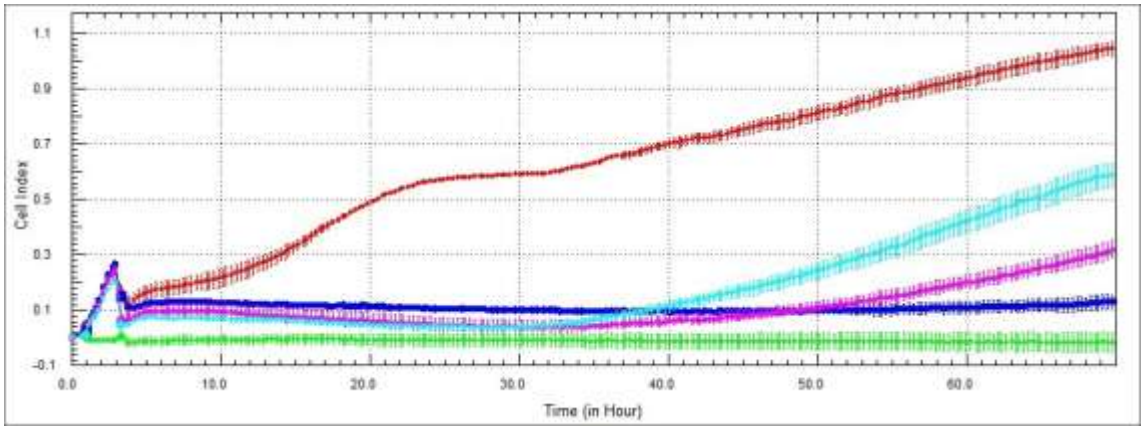

Fig 2 : Activité anti-proliférative contre la lignée HeLa de la F1

250 100 50 Control Medium

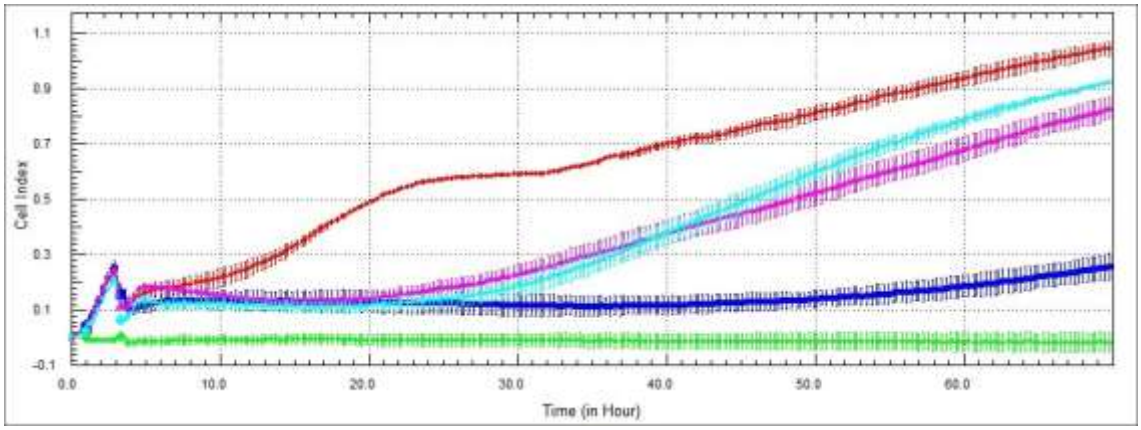

Fig 3: Activité anti-proliférative contre la lignée HeLa de la F2

Pre-flower (F1) plant essential oil (First growth phase) has improved anti-proliferative activity at 250 µg/ml and moderate activity at 100 µg / ml and 50 µ g / ml while the F2 phase is active only at 250 µg / ml. The potency of inhibitions (at 100 and 50 µg/mL) against HeLa cells were: **F1** > **F2** >**Control**

Discussion

Studies revealed that *F. communis* exhibits different biological activities, and contains various bioactive compounds. Although, antibacterial and cytotoxic activities are the two main pharmacological effects of this plant **(Akaberi *et al,* 2015).**

The antiproliferative activity of the essential oils of *F.communis* during both vegetative stages is carried out on C6 cell lines using the BrdU Elisa assay and angains the HeLa cell lines by using the RTCA instrument xcelligence test at 250µg/ml, 100µg /ml and 50 µg /ml.

Hela cells have always been used for cancer research, AIDS, the effects of radiation and toxic substances, gene mapping and countless other scientific activities. We note from our results that the anti-proliferative activity of *F. communis* essential oil increased with the

increase in concentration this can be explained by the number of bioactive compounds in the oil that increases with the increasing concentration. Maiuolo et al, (2023) reported that extract obtained from the plant *F. communis*, and precisely from the root, collected in Sardinia, Italy using the Cell proliferation was assessed by colorimetric assay. 3-(4,5-dimethyl(thiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) against The human breast cancer cell line (MCF-7), human cervical cancer cell (HeLa) and immortalized Human Mammary Epithelial Cells (HBL-100) induce cytotoxicity, at certain concentrations so the extract could be used for its potential role against uncontrolled cancer Growth**.** Another study conducted on the cytotoxic effect of *F. communis* leaf ethanol extract grown in Cyprus on MDA-MB-231 and MCF-7- breast cancer cell lines decrease the viability of both types of cancer cell (**Kavaz et El Faraj, 2023)**

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