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Cachrys ferulacea (L.) Calest. extracts as natural photosensitizers: an in vitro photobiological study

Mariangela Marrelli *, Maria Rosaria Perri, Valentina Amodeo, Filomena Conforti, Francesca Giordano, Maria Luisa Panno and Giancarlo Statti

Department of Pharmacy, Health and Nutritional Sciences, University of Calabria, 87036 Rende (CS), Italy

* Corresponding author: mariangela.marrelli@unical.it

UNIVERSITÀ DELLA CALABRIA

Abstract: The *Cachrys* genus (Apiaceae) is widely distributed in the Mediterranean Basin. Previous studies highlighted the photobiological properties of different *Cachrys* species, such as *C. pungens* Jan, *C. libanotis* L. and *C. sicula* L. Based on these promising previous results, and in order to continue exploring such interesting genus, the aim of this study was to evaluate the photocytotoxic activity of extracts from *Cachrys ferulacea* (L.) Calest. Aerial parts were collected in Calabria (Southern Italy) and extracted through three different techniques: traditional maceration, pressurized cyclic solid-liquid (PCSL) extraction using Naviglio extractor® and supercritical CO_2. The phytochemical composition was assessed with gas chromatography-mass spectrometry (GC-MS) and the photocytotoxic potential of samples was evaluated on UVA-irradiated C32 melanoma cell line. The apoptotic responses on treated cells were also assessed. Furthermore, the phenolic and flavonoid content and the in vitro antioxidant activity were also estimated. Different coumarins were identified and quantified. All the extracts affected cell viability in a concentration-dependent manner after irradiation with UVA light for 1 hour at a dose of 1.08 J/cm^2. Sample obtained through supercritical CO_2 extraction showed the highest activity, with an IC_{50} value equal to 4.91 μg/mL. This study could provide a starting point for further researches focusing on new photosensitizing agents useful in cancer photochemotherapy.

Keywords: Apiaceae; *Cachrys*; melanoma; photosensitizing agents; plant extracts.

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Photochemotherapy

➢ Photodynamic therapy (PDT)

600-800 nm

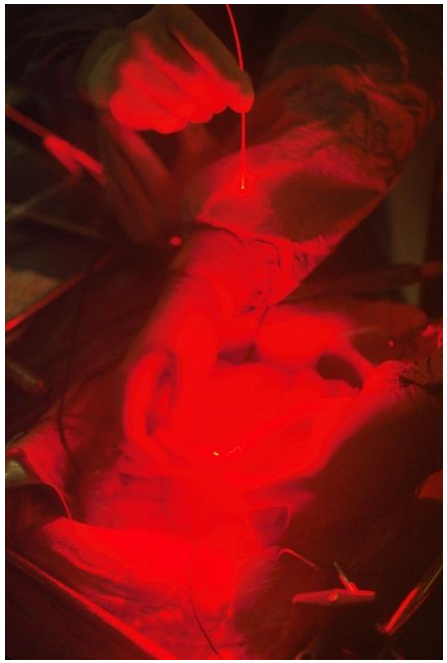
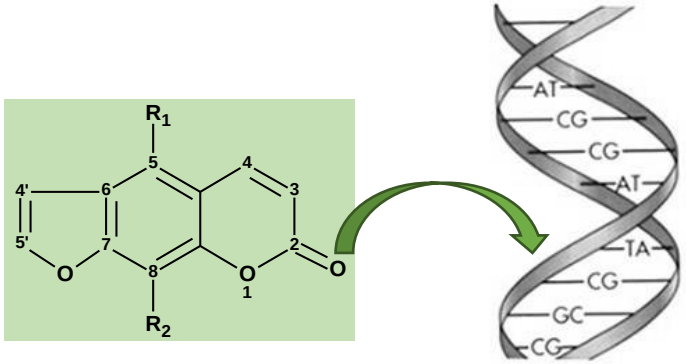


Photo from https://commons.wikimedia.org/

➢PUVA therapy (Psoralens + UVA)

(320-400 nm)

- Cutaneous T-cell lymphoma



Marrelli et al. *Curr. Med. Chem.* **2014**, *21*, 1371-1390.

Via, L., Magno, S. Photochemotherapy in the treatment of cancer. *Curr. Med. Chem* **2001**., *8*, 1405-1418.

Trautinger, F. Phototherapy of cutaneous T-cell lymphomas. *Photochem. Photobiol. Sci*. **2018**, *17*, 1904-1912.

Tarabadkar, E.S.; Shinohara, M.M. Skin directed therapy in cutaneous T-cell lymphoma. *Front. Oncol*. **2019**, *9*, 260.

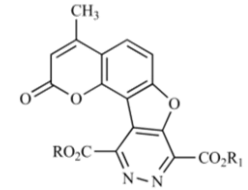
Furanocoumarins

Apiaceae

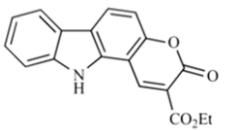
Fabaceae

Moraceae

Rutaceae



T1 R= R_1= Me
T2 R= OMe; R_1= $N(CH_2)_4$
T3 R= R_1= $N(CH_2)_4$
T4 R= OMe; R_1= $NH(CH_2)_2NMe_2$
T5 R= R_1= $NH(CH_2)_2NMe_2$
T6 R= $NH(CH_2)_2NMe_2$; R_1= $N(CH_2)_4$



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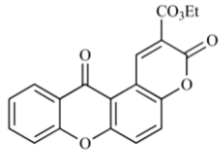
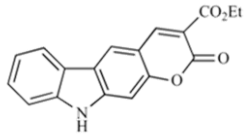


T9



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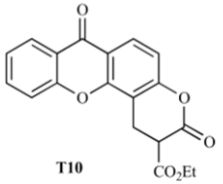
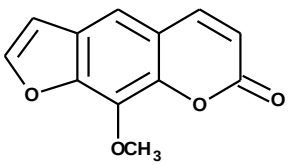
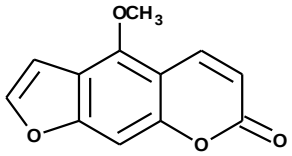


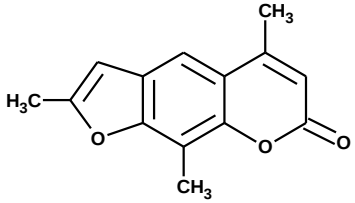
T10



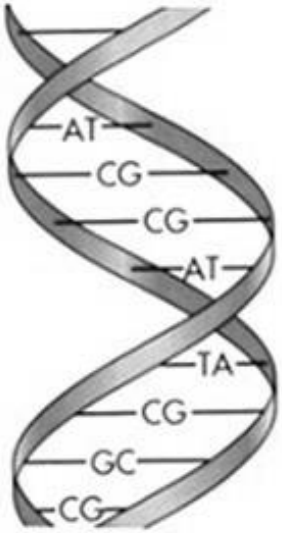
8-MOP



5-MOP



TMP



Diffey B. *Physics in medicine and biology*. **2006**, *51*, R229–R244.
Caffieri S. *Photochem. Photobiol. Sci*. **2001**, *1*, 149-157.
Conforti F. et al. Natural and synthetic furanocoumarins a treatment for vitiligo and psoriasis. *Curr. Med. Chem.* **2009**, *4*, 38-58.
Figures from: https: // publicdomainpictures. net - Petr Kratochvil

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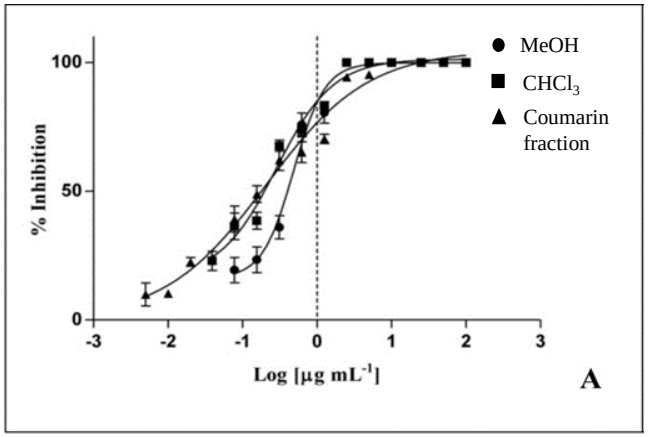
Previous works

Cachrys pungens Jan



Cell Proliferation

Cell Prolif., 2012, **45**, 39–47 doi: 10.1111/j.1365-2184.2011.00791.x

Cachrys pungens Jan inhibits human melanoma cell proliferation through photo-induced cytotoxic activity

G. Menichini*, C. Alfano*, E. Provenzano†, M. Marrelli‡, G. A. Statti‡, F. Menichini‡ and F. Conforti‡

Sample	IC_{50} (µg/mL)	
	Irradiated cells	Unirradiated cells
Methanol	0.487 ± 0.037	49.950 ± 0.018
Chloroform	0.286 ± 0.067	34.280 ± 0.022
Coumarin fraction	0.209 ± 0.033	31.620 ± 0.018

Data were expressed as mean ± S. E. M. (n=6).

Photo from Saxifraga-Willem van Kruijsbergen

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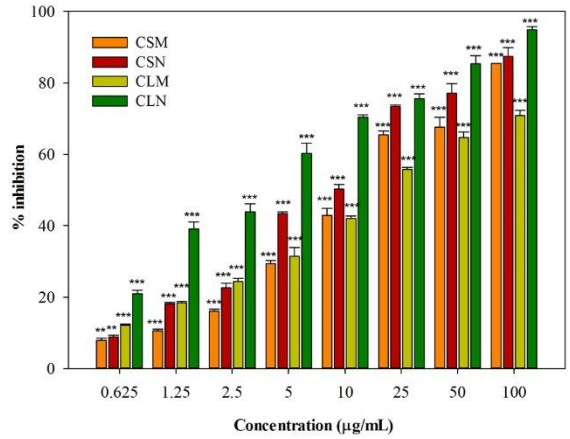
Article

Assessment of Photo-Induced Cytotoxic Activity of _Cachrys sicula_ and _Cachrys libanotis_ Enriched-Coumarin Extracts against Human Melanoma Cells

Mariangela Marrelli *, Maria Rosaria Perri, Valentina Amodeo, Francesca Giordano, Giancarlo A. Statti *, Maria Luisa Panno † and Filomena Conforti *,†



Cachrys libanotis L.

Photo from Saxifraga--Ed Stikvoort

Cachrys sicula L.

Cachrys ferulacea (L.) Calest.

- Synonym of *Prangos ferulacea* (L). Lindl.
- The *Cachrys* group (Apiaceae) is divided into several genera: *Cachrys*, *Prangos*, *Alocacarpum*, *Bilacunaria*, *Ferulago*, *Diplotaenia*, *Eriocycla* and *Azilia*.
- *C. ferulacea* is an orophilous species of the eastern Mediterranean and western Asia.
- Species rich in coumarins, the main class of secondary metabolites detected so far. In addition, the aerial parts also contain flavonoid glycosides.
- Antioxidant, antimicrobial, hypoglycemic activities; analgesic effects.

Aim of the research

Photo from Saxifraga--Ed Stikvoort

- **Photocytotoxic potential of aerial parts extracts**
  - **Traditional maceration (TM)**
  - **Pressurized cyclic solid-liquid (PCSL, Naviglio® extractor)**
  - **Supercritical CO_2 (S-CO_2)**
- Phytochemical composition
- Photocytotoxic effects on UVA-irradiated C32 melanoma cell line
- Apoptotic responses
- Antioxidant potential

Bruno, M. et al. *Planta Med.* **2019**, *85*, 815-824.
Kafash-Farkhad, N. et al. *Life Sci*. **2013**, *10*, 360-367.
Naviglio D. *Anal Lett* ***2003***, *36*, 1647-1659.
http://www.worldfloraonline.org/taxon/wfo-0000577534. Accessed on: 17 Nov 2021

Plant material and extraction procedure

Photo from Saxifraga--Ed Stikvoort

Extraction technique	Abbreviation	Yield (%)	Total phenolic content (mg/g)	Total flavonoid content (mg/g)
Maceration	TM	14.7	17.99 ± 0.50 [a]	0.63 ± 0.06 [a]
Naviglio®	PCSL	3.6	4.14 ± 0.24 [b]	0.18 ± 0.02 [b]
Supercritical CO_2	S-CO_2	2.4	2.32 ± 0.09 [c]	0.0073 ± 0.0006 [c]

Data are expressed as mean SD (n = 3). Results expressed as mg of chlorogenic acid (for phenolics) or quercetin equivalent (for flavonoids) per g of dry plant material. Letters along columns indicate statistically significant differences at $p < 0.05$ (Bonferroni post-hoc test).

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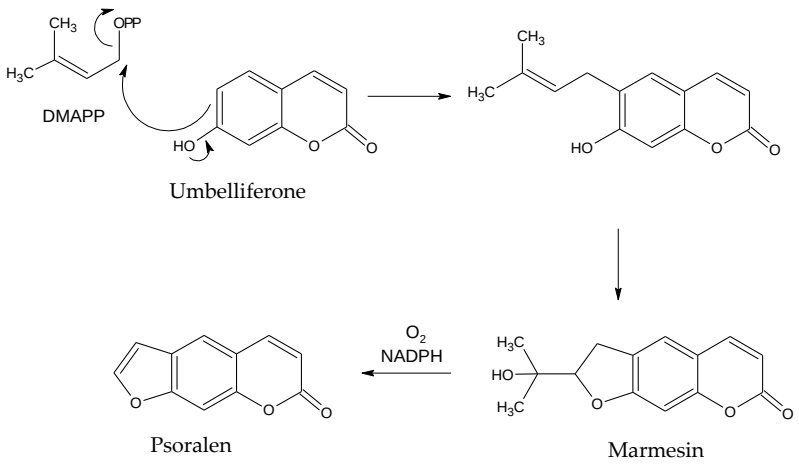
Chemical composition: GC-MS

Compound	Rt	Relative peak area percentage		
		TM	PCSL	S-CO_2
Furanocoumarins				
Psoralen	17.645	-	-	2.93 ± 0.25
Xanthotoxin	19.251	1.91 ± 0.04	2.73 ± 0.17	3.14 ± 0.11
Bergapten	19.411	-	2.83 ± 0.13	4.30 ± 0.09
Isopimpinellin	20.645	1.13 ± 0.05	0.89 ± 0.07	2.17 ± 0.14
Marmesin	21.223	-	-	3.96 ± 0.19
Coumarins				
Citropten	18.782	-	-	2.48 ± 0.26
Osthole	19.891	2.42 ± 0.12	2.03 ± 0.19	3.82 ± 0.19
Isomeranzin	20.582	-	-	1.90 ± 0.11
Terpenes				
Estragole	11.192	-	-	0.15 ± 0.02
trans-Caryophyllene	13.827	0.81 ± 0.03	-	-
Cadinene	14.816	0.54 ± 0.03	-	-
Neophytadiene	17.450	0.78 ± 0.04	-	0.71 ± 0.06



Compound	Rt	Relative peak area percentage		
		TM	PCSL	S-CO_2
Fatty acids				
Lauric acid	15.039	-	-	0.10 ± 0.01
Myristic acid	16.496	3.24 ± 0.20	0.25 ± 0.03	2.04 ± 0.09
Pentadecanoic acid	17.336	0.44 ± 0.03	-	-
7,10,13-hexadecatrienoic acid	17.959	0.97 ± 0.04	-	-
Isopalmitic acid	18.009	-	-	0.55 ± 0.04
Palmitic acid	18.113	8.49 ± 0.49	1.15 ± 0.10	0.14 ± 0.02
Margaric acid	18.891	0.33 ± 0.03	-	-
Oleic acid	19.091	-	0.36 ± 0.03	-
8,11- Octadecadienoic acid	19.371	-	1.08 ± 0.04	-
7,10,13-hexadecatrienoic acid	19.479	4.62 ± 0.41	-	-
Stearic acid	19.617	0.82 ± 0.03	-	-
Linoleic acid	19.702	1.69 ± 0.13	-	0.20 ± 0.02
Arachidic acid	20.988	1.13 ± 0.08	0.44 ± 0.04	-
Behenic acid	22.263	2.72 ± 0.22	1.33 ± 0.14	-
Tricosylic acid	22.954	1.13 ± 0.07	-	-
Lignoceric acid	23.760	4.39 ± 0.33	0.86 ± 0.04	1.00 ± 0.10
Cerotic acid	25.829	1.40 ± 0.10	-	-

Photo from Saxifraga--Ed Stikvoort

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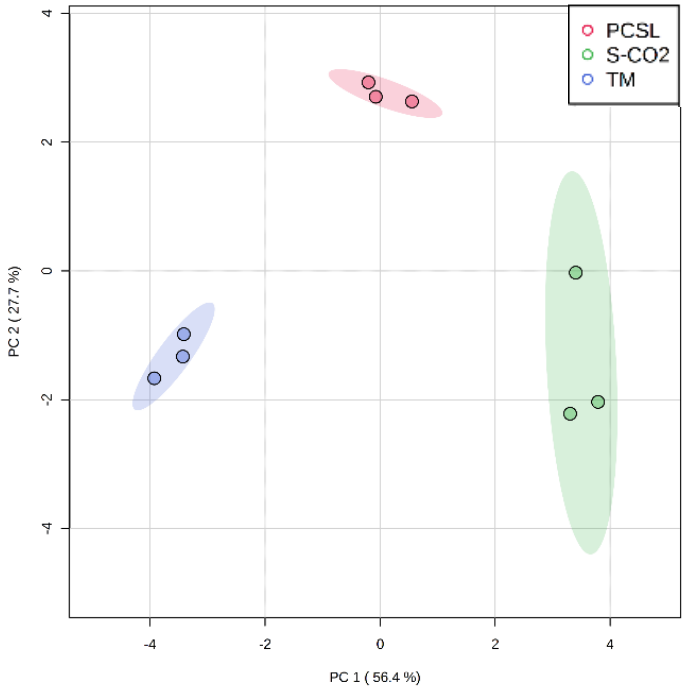
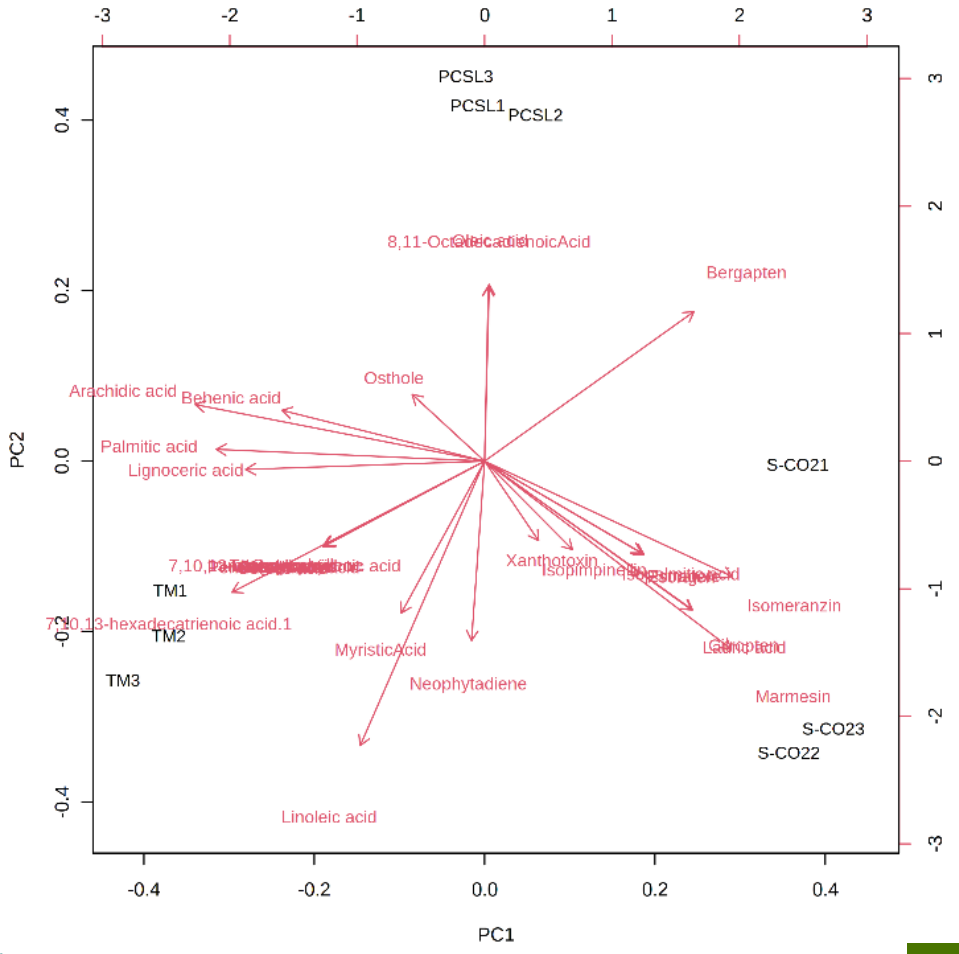
Multivariate Data Analysis





TM = Traditional maceration
PCSL = Pressurized cyclic solid-liquid extraction
S-CO_2 = Supercritical CO_2 extraction

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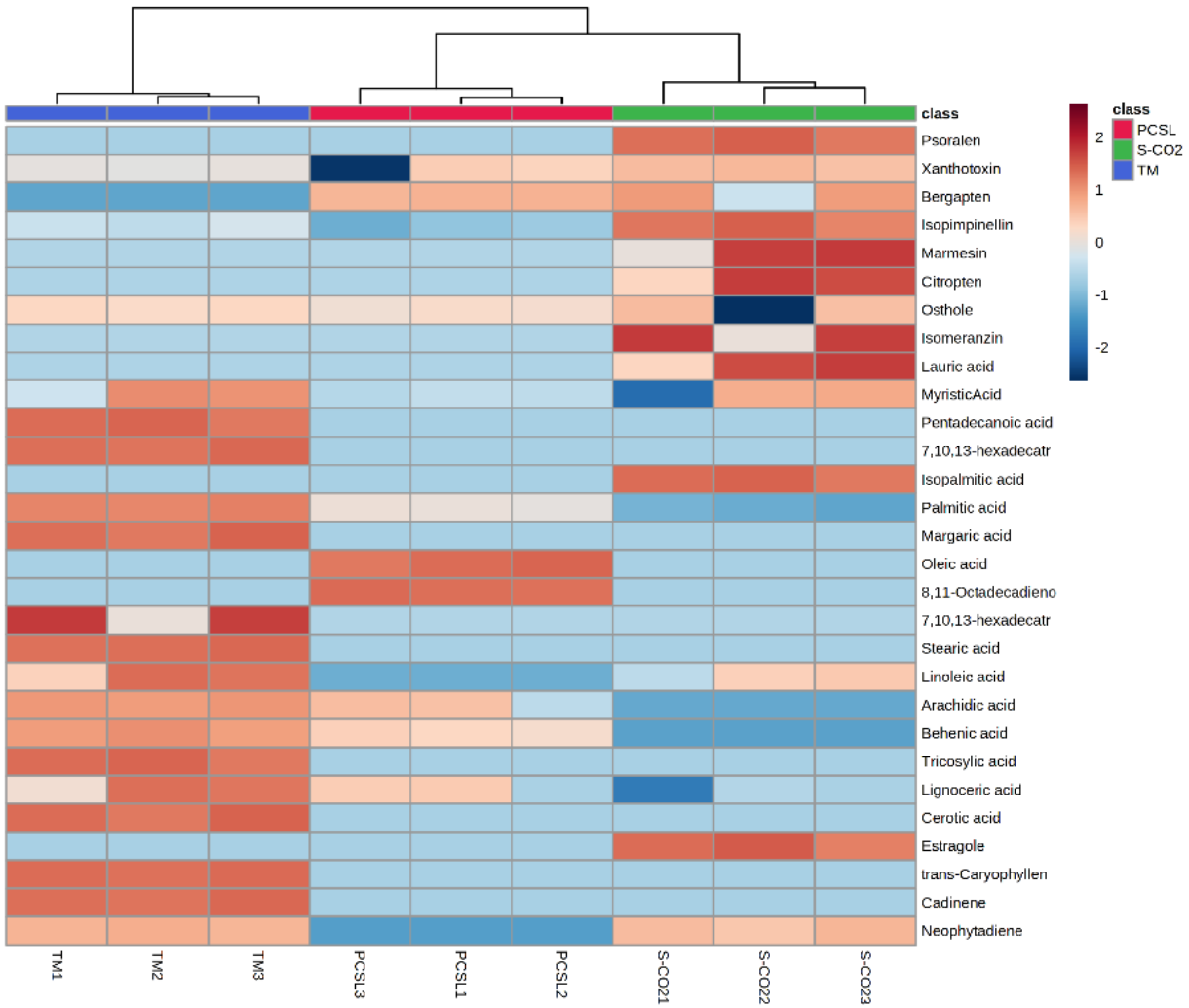
Cluster analysis



TM = Traditional maceration
PCSL = Pressurized cyclic solid-liquid extraction
S-CO_2 = Supercritical CO_2 extraction

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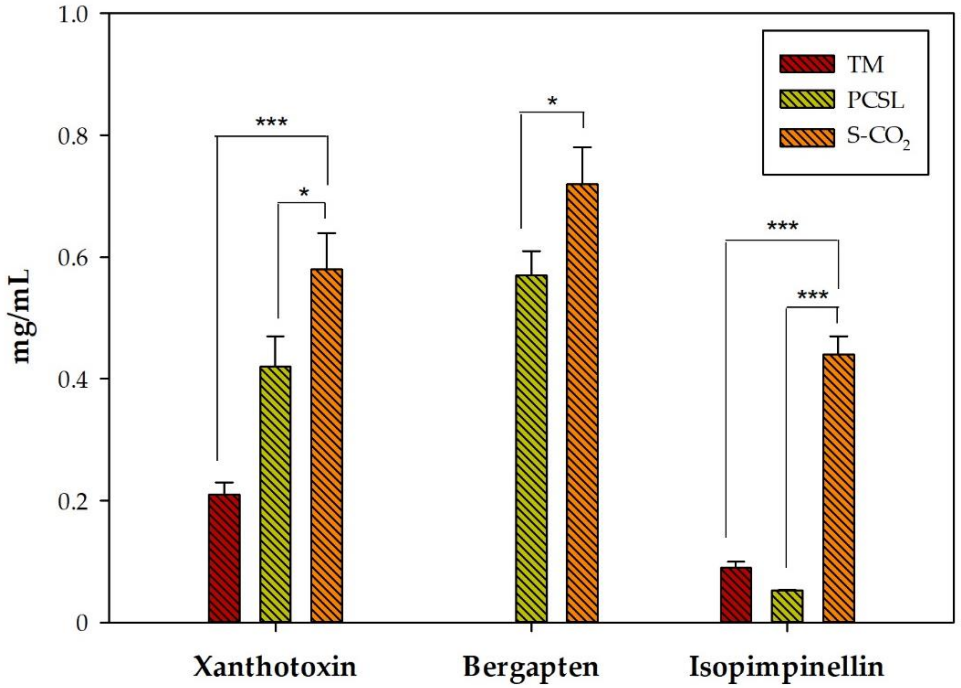
Quantitative analyses

Compound	TM	PCSL	S-CO_2
		mg/mL ± SD	
Xanthotoxin	**0.21 ± 0.02**	**0.42 ± 0.05**	**0.58 ± 0.06**
Bergapten	**-**	**0.57 ± 0.04**	**0.72 ± 0.06**
Isopimpinellin	**0.09 ± 0.01**	**0.053 ± 0.001**	**0.44 ± 0.03**

Data were expressed as mean ± SD (n=3).



*** $p < 0.001$, * $p < 0.05$ (Students' t test)

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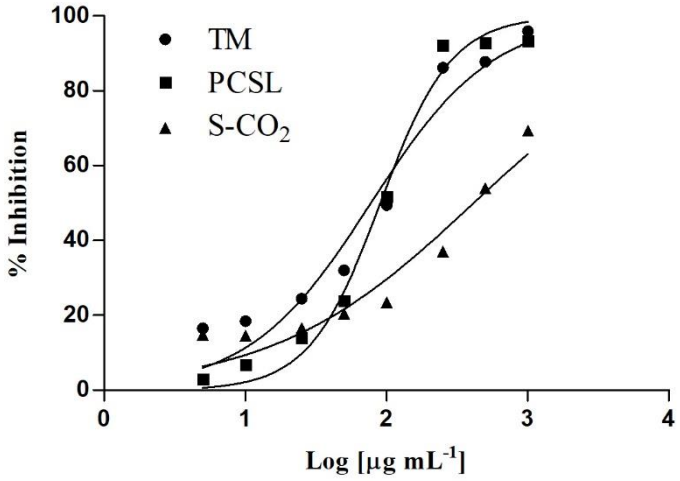
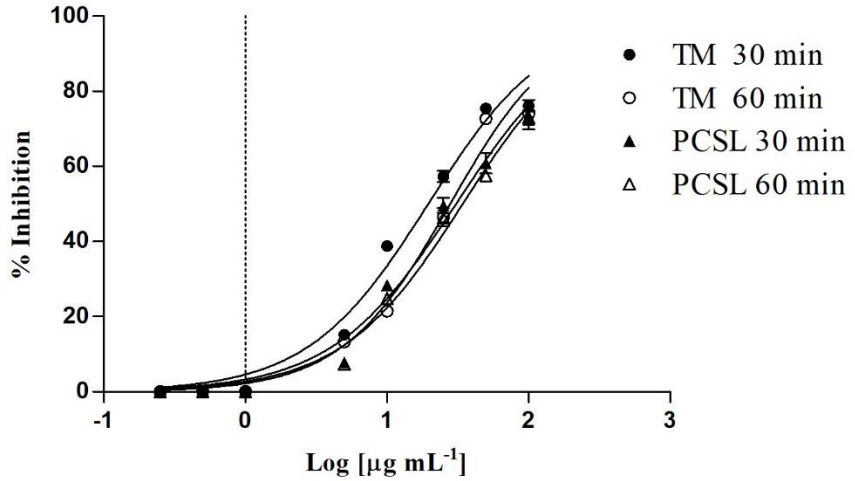
Antioxidant activity

Sample	IC_{50} (μg/mL)		
	DPPH	β-Carotene	
		30 min	60 min
TM	77.37 ± 1.58 [b]	19.57 ± 0.67 [b]	27.94 ± 0.48 [c]
PCSL	90.27 ± 1.45 [c]	30.75 ± 1.11 [c]	34.27 ± 0.35 [d]
S-CO_2	413.10 ± 1.79 [d]	n.a.	n.a.
Ascorbic acid*	2.00 ± 0.01 [a]	-	-
Propyl gallate*	-	1.00 ± 0.02 [a]	1.00 ± 0.02 [a]

Data were expressed as mean ± S. E. M. (n=3). Different letters along column (DPPH) or between columns (β-carotene bleaching test) indicate statistically significant differences at $P < 0.05$ (Bonferroni post-hoc test). N.a. = not active. * Positive controls.

Photo from Saxifraga--Ed Stikvoort





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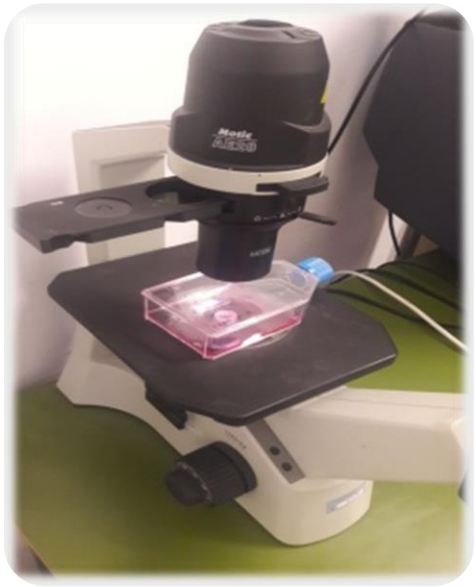
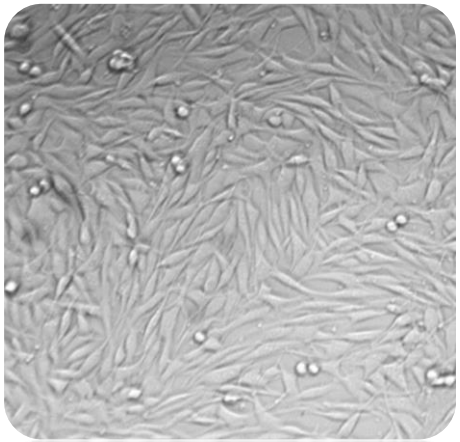
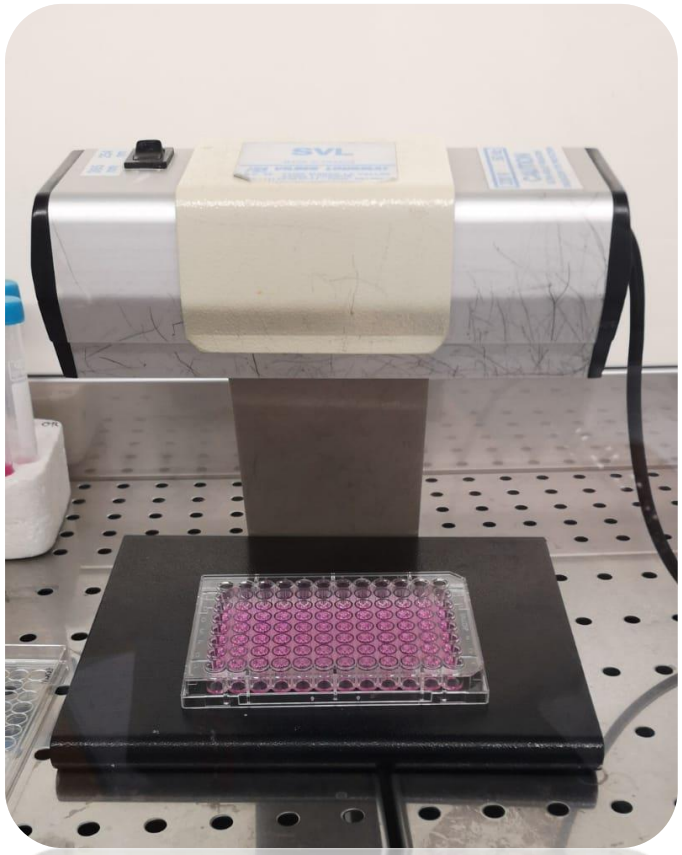
Cellular phototoxicity





Human melanoma C32 cell line



- Samples in *Hanks' Balanced Salt Solution* (HBSS, pH 7.2)
- 30 min incubation
- Irradiation at 365 nm – 1 h, 1.08 J/cm^2
- Cell viability 48h later: MTT test
- Unirradiated microtiter plates
- Positive control: Bergapten

Marrelli M. *et al*. *Food Chem Toxicol* **2012**, *50*, 726-733.
Marrelli M. *et al*. *Pharm Biol* **2014**, *52* 909-918.

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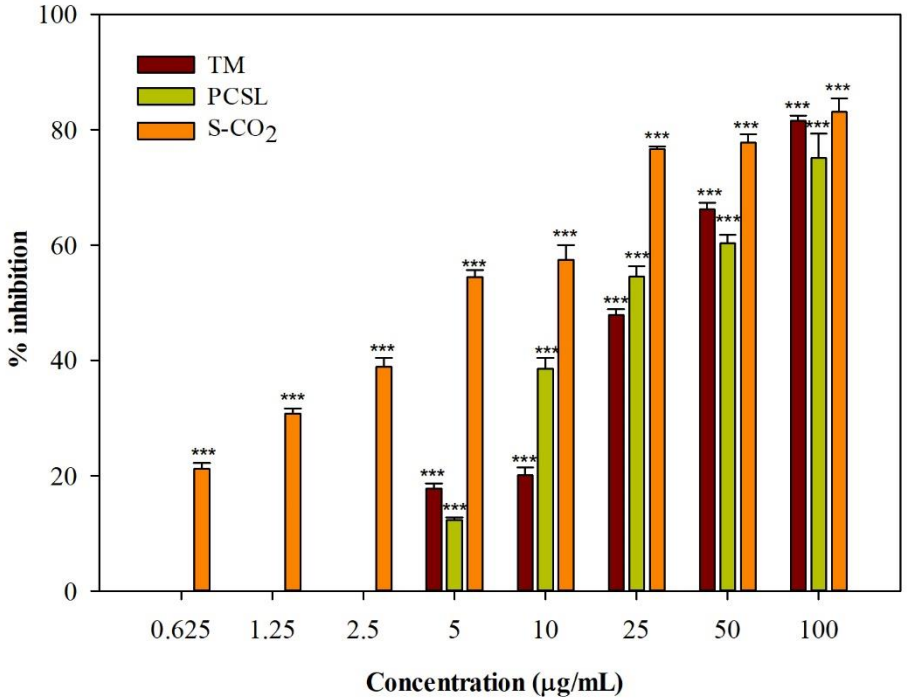
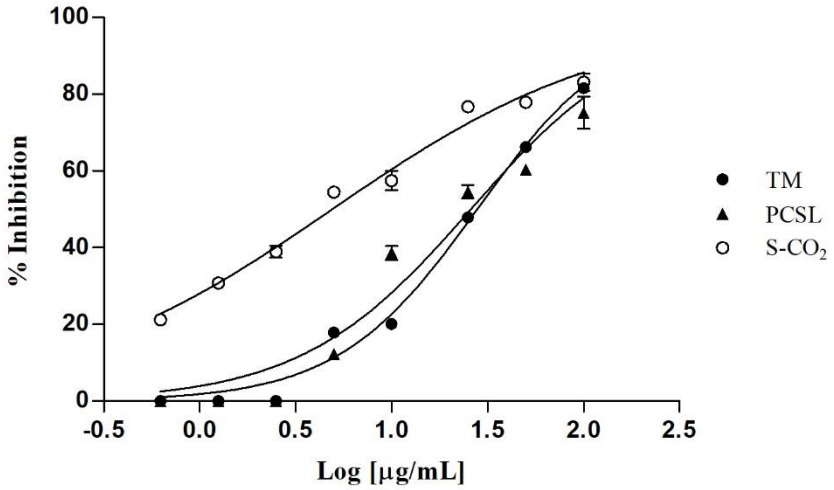
Photocytotoxic activity

Sample	IC_{50} (μg/mL)	
	Irradiated	Unirradiated
TM	27.95 ± 0.67 [c]	> 100
PCSL	25.90 ± 1.23 [c]	> 100
S-CO_2	4.91 ± 0.15 [b]	> 100
Bergapten*	0.191 ± 0.012 [a]	n.d.

Data were expressed as mean ± S. E. M. (n=4). Different letters indicate statistically significant differences at $P < 0.05$ (Bonferroni post-hoc test). n.d.: not detectable.
* Positive control.



Concentration-dependent photocytotoxic effects.
*** P < 0.001 compared to control (Dunnett's test).



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Photocytotoxic activity

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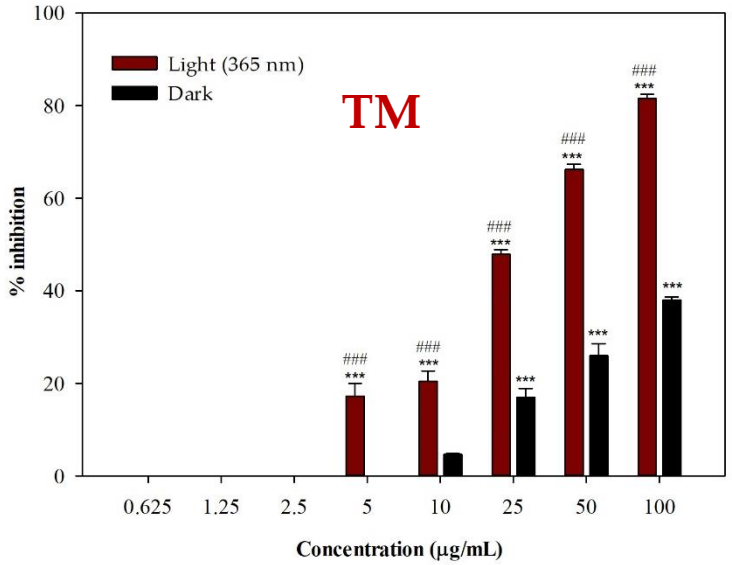
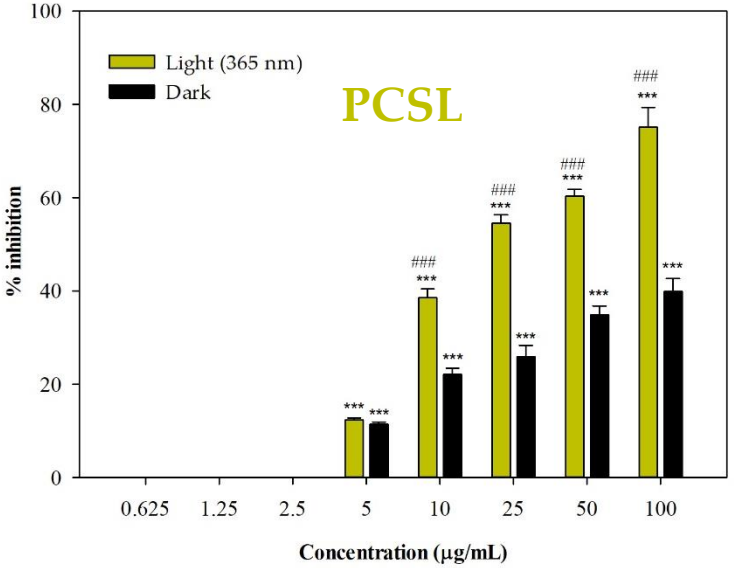
Morphological changes in C32 cells induced by photocytotoxic *C. ferulacea* (L.) extracts





Photocytotoxicity and dark toxicity.
*** $p < 0.001$ compared to control (Dunnett's test).
compared to cytotoxic effects in the dark

Photocytotoxic activity

Sample	IC_{50} (μg/mL)	
	Irradiated	Unirradiated
TM	27.95 ± 0.67 ^{c}	> 100
PCSL	25.90 ± 1.23 ^{c}	> 100
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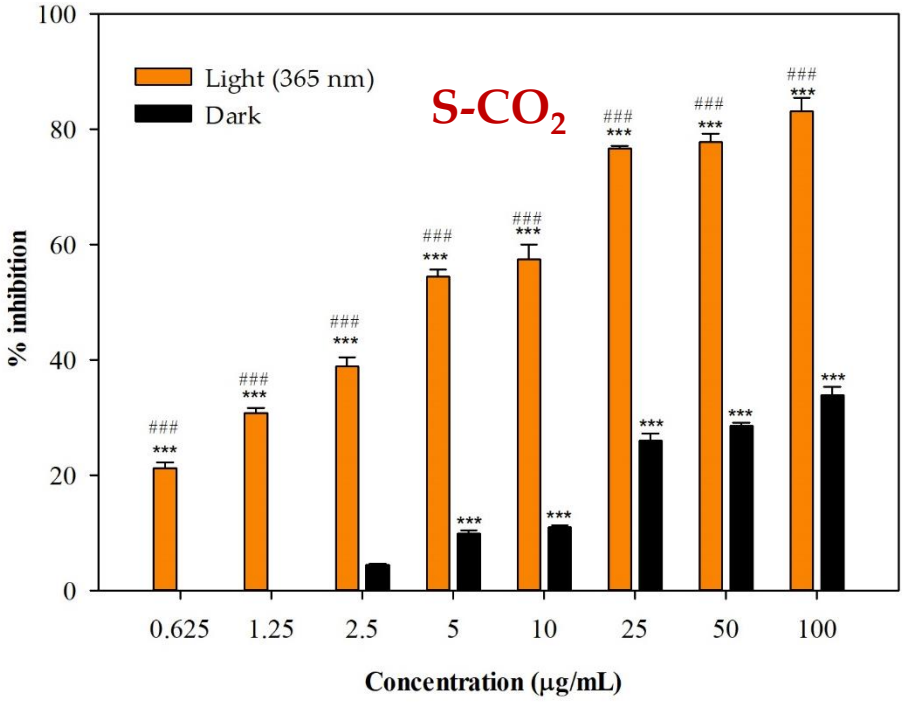

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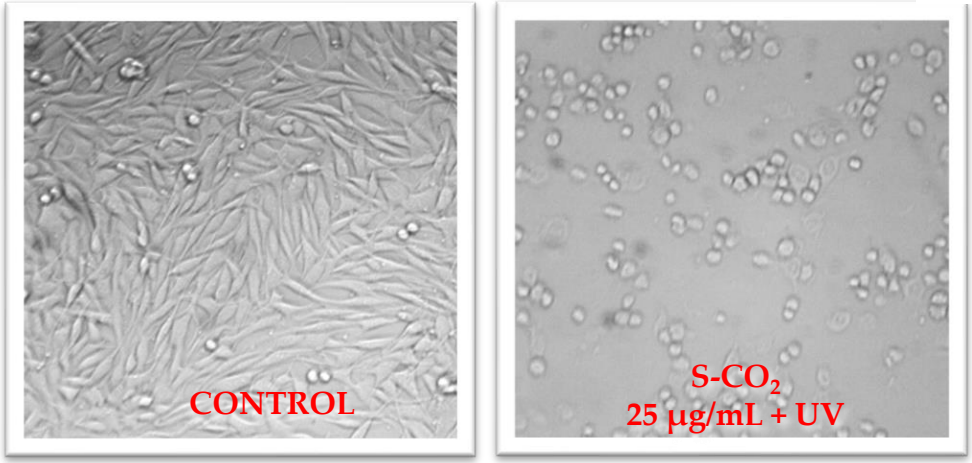
*** $p < 0.001$ compared to control (Dunnett's test).
compared to cytotoxic effects in the dark



Morphological changes in C32 cells induced by photocytotoxic *C. ferulacea* (L.) Calest. extract

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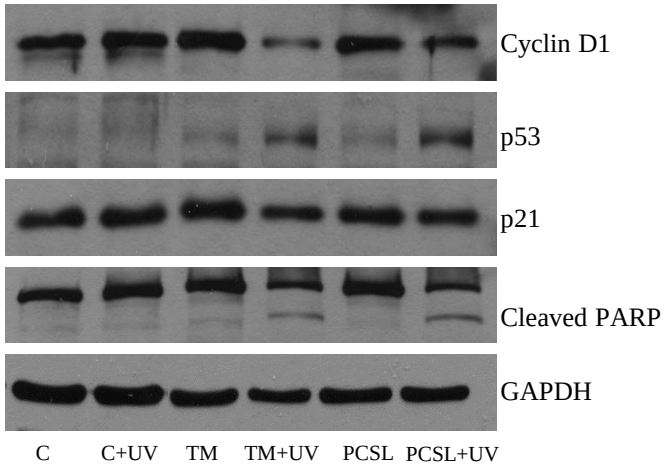
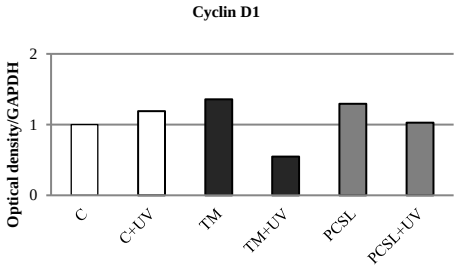
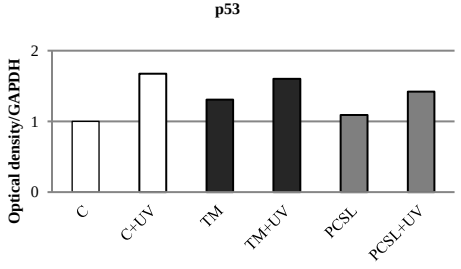
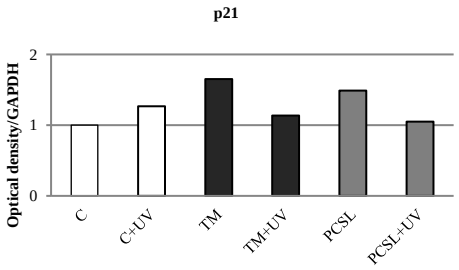
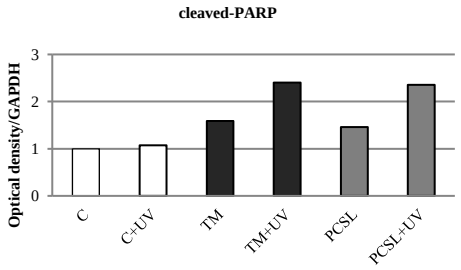
Immunoblotting Analysis: TM and PCSL





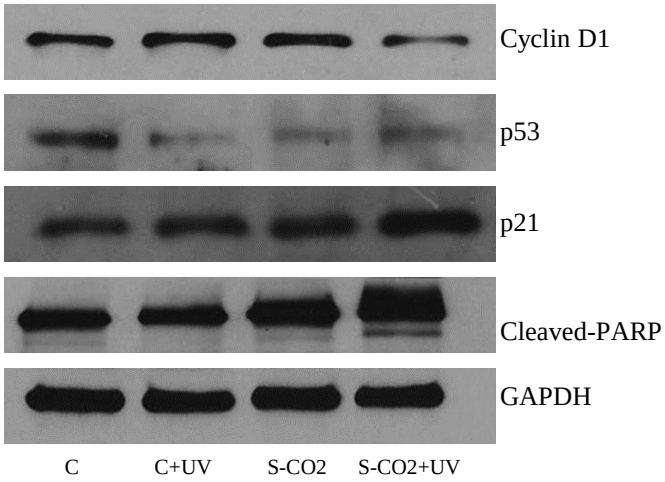


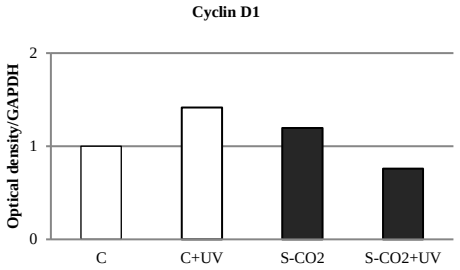


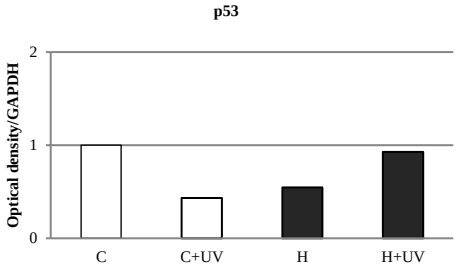


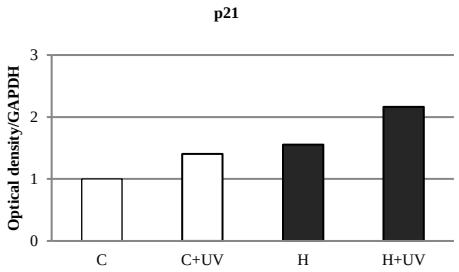
Western Blot analysis of Cyclin D1, p53, p21 and PARP (poly ADP-ribose polymerase) protein levels in C32 cells treated or not with TM and PCSL extracts, both in the presence and absence of UV. The histograms refer to the densitometric analysis (OD) of the Western blot shown in the figure.

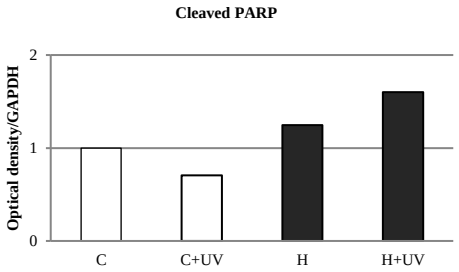
Immunoblotting Analysis: S-CO_2











Western Blot analysis of Cyclin D1, p53, p21 and PARP (poly ADP-ribose polymerase) protein levels in C32 cells treated or not S-CO2 extracts, both in the presence and absence of UV. The histograms refer to the densitometric analysis (OD) of the Western blot shown in the figure.

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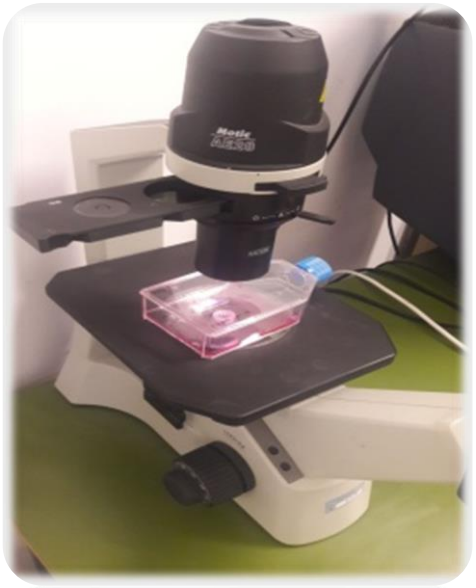
Conclusions

- *C. ferulacea* extracts, mainly the S-CO_2 sample, contain important photoactive constituents responsible for their photocytotoxic activity.
- Extracts induced cytotoxic effects on melanoma cells upon irradiation with UVA light.

Future perspectives

- Future studies could be useful to further optimize the extraction method and to continue investigating the interesting photobiological properties of this species.



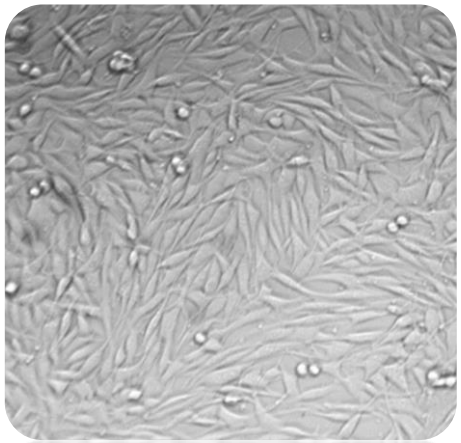


Photo from Saxifraga--Ed Stikvoort

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Research group

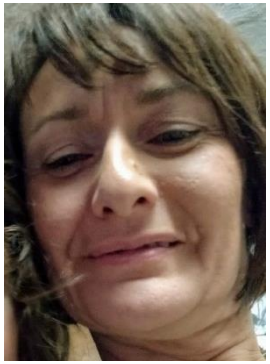
Prof. G. Statti

Prof. F. Conforti

Prof. M.L. Panno

UNIVERSITÀ DELLA CALABRIA

Dr. M. Marrelli



Dr. F. Giordano

Dr. M. R. Perri

Dr. V. Amodeo

Thanks for your attention!

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