IECPS 2020
1st International Electronic Conference on Plant Science
01–15 DECEMBER 2020 | ONLINE
plants MDPI

Cachrys libanotis L. extracts: photocytotoxic effects on UVA-irradiated human melanoma cells

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

Department of Pharmacy, Health and Nutritional Sciences, University of Calabria, 87036 Rende (CS), Italy
* Corresponding author: mariangela.marrelli@unical.it

UNIVERSITÀ DELLA CALABRIA

Abstract: Melanoma is the most aggressive form of skin cancer. Photochemotherapy, combining the action of a light source and a chemical photosensitizer, is one of the most interesting current therapeutic approaches. Plants represent a rich source of photoactive compounds, and furanocoumarins are some of the most important naturally occurring phytoconstituents. The aim of this study was to evaluate the photocytotoxic potential of *Cachrys libanotis* L. (Apiaceae) from Southern Italy. This species belongs to a genus rich in furanocoumarins and widely distributed in Europe. The aerial parts were extracted through both traditional maceration and pressurized cyclic solid-liquid (PCSL) extraction using Naviglio extractor®. Qualitative and quantitative analyses were performed to detect the coumarins content using GC-MS, and the photocytotoxic effects of the extracts were assessed on UVA-irradiated C32 melanoma cells. The apoptotic responses were also evaluated. Furthermore, phenolic content and the in vitro antioxidant potential were also estimated. Xanthotoxin, bergapten and isopimpinellin were identified and quantified. Both extracts affected cell viability in a concentration-dependent manner after irradiation for 1 hour at a dose of 1.08 J/cm^2. Sample obtained through PCSL extraction was the most effective, with an IC_{50} equal to 3.16 μg/mL, a very interesting value if compared with the positive control bergapten. This extract induced up-regulation of apoptotic signals such as BAX and PARP cleavage and, in the presence of UVA radiation, it caused a greater upregulation of p21 protein. Obtained results suggest that investigated species could be a good candidate for further studies aimed to find new drugs with photocytotoxic potential.

Keywords: Apiaceae; furanocoumarins; plant extracts; photochemotherapy; skin cancer.

Background

Photochemotherapy

Treatment which combines the action of a light source and a chemical photosensitizer

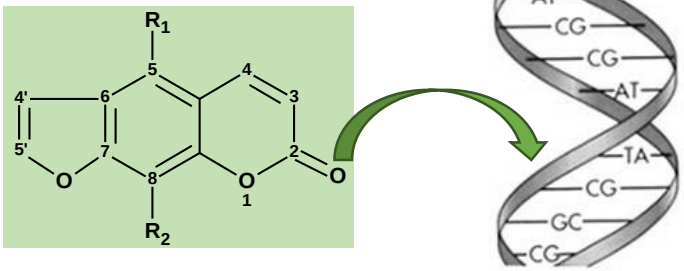
- PUVA therapy (Psoralens + UVA)
- Photodynamic therapy (PDT)

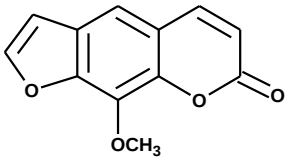
Jones L.J. *The Science of Phototherapy: An Introduction*. Springer: Netherlands, **2005**.
Roelandts R. *J Am Acad Dermatol*. **2002**, *46*, 926-930.
Figures from: https: // publicdomainpictures. net - Petr Kratochvil

Background

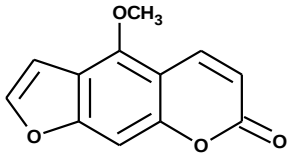
Psoralens + UVA ⟶ PUVA (320-400 nm)

- Cutaneous T-cell lymphoma
- Vitiligo, psoriasis

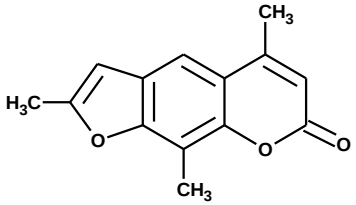




8-MOP



5-MOP



TMP

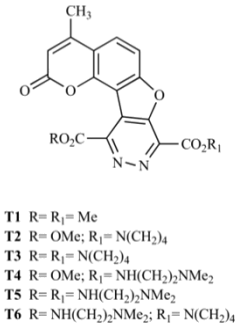
Furanocoumarins

Apiaceae

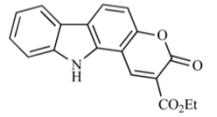
Fabaceae

Moraceae

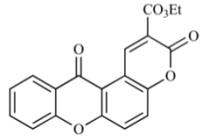
Rutaceae



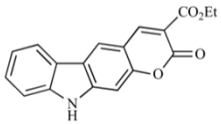
T1 R= R_1= Me
T2 R= OMe; R_1= $N(CH_2)_4$
T3 R= R_1= $N(CH_2)_4$
T4 R= OMe; R_1= $NH(CH_2)_2NMe_2$
T5 R= R_1= $NH(CH_2)_2NMe_2$
T6 R= $NH(CH_2)_2NMe_2$; R_1= $N(CH_2)_4$



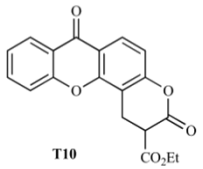
T7



T9



T8



T10

38 *Current Drug Therapy*, 2009, *4*, 38-58

Natural and Synthetic Furanocoumarins as Treatment for Vitiligo and Psoriasis

Filomena Conforti[1,*], Mariangela Marrelli[1], Federica Menichini[1], Marco Bonesi[1], Giancarlo Statti[1], Eugenio Provenzano[2] and Francesco Menichini[1]

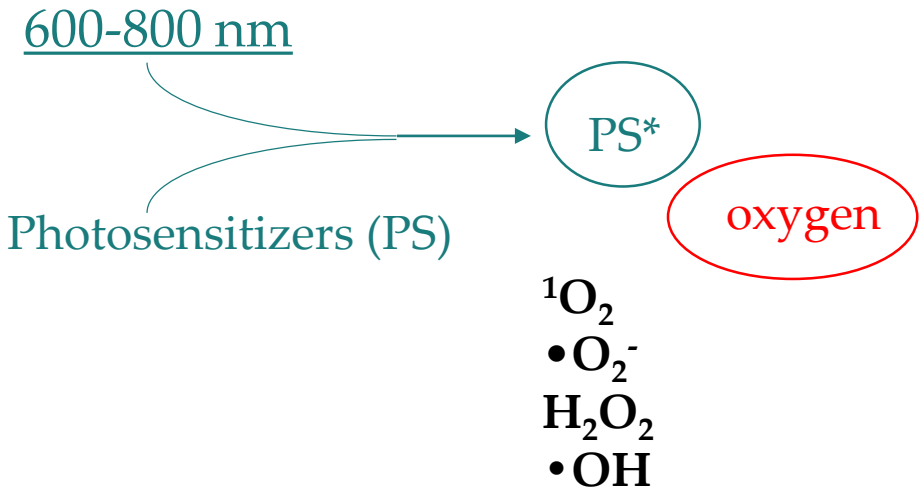
[1]*Department of Pharmaceutical Sciences, University of Calabria, Italy;* [2]*Operative Unit of Dermatology, A.O. of Cosenza, Italy*

Diffey B. *Physics in medicine and biology*. **2006**, *51*, R229–R244.
Caffieri S. *Photochem. Photobiol. Sci*. **2001**, *1*, 149-157.
Figures from: https: // publicdomainpictures. net - Petr Kratochvil

Background

Photodynamic therapy (PDT)

Local or systemic administration of photosensitizing molecules that exert a cytotoxic action when excited at appropriate wavelengths



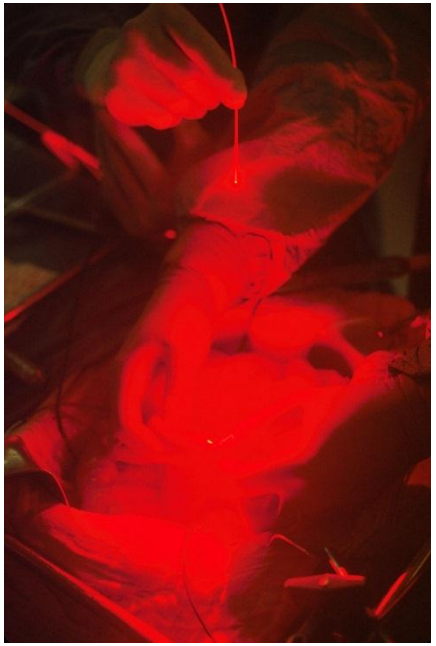


Photo from https://commons.wikimedia.org/

Current Medicinal Chemistry, 2014, *21*, 1371-1390 1371

Applications of Natural Compounds in the Photodynamic Therapy of Skin Cancer

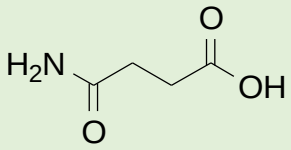
M. Marrelli[1], G. Menichini[2], E. Provenzano[3] and F. Conforti[1,*]

Castano A.P. *et al. Photodiagnosis Photodyn Ther*. **2005**, *2*, 1-23.
Castano, A.P. *et al. Photodiagnosis Photodyn Ther*. **2005**, *2*, 91-106.
Fukuda, H. *et al. Int. J. Biochem. Cell Biol*. **2005**, *37*, 272-276.

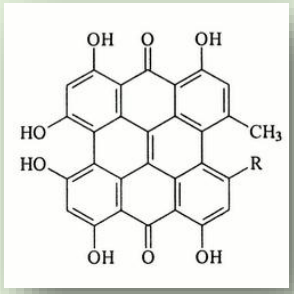
Background

Natural Photosensitizers

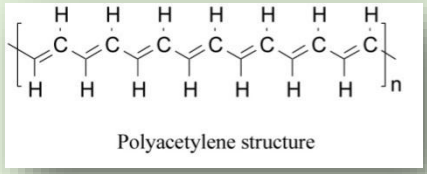
Porfimer sodium



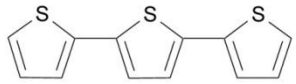
aminolevulinic acid, ALA



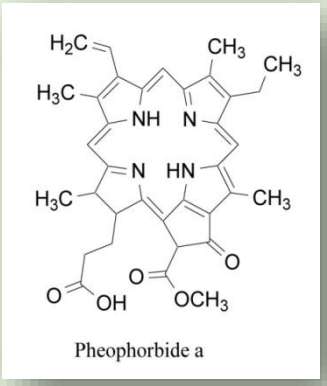
Hypericin



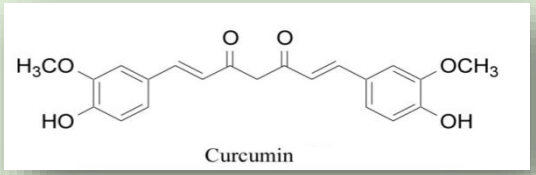
Polyacetylene structure



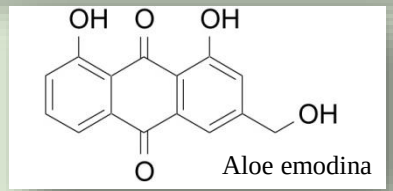
Thiophenes structure (α-terthienyl)



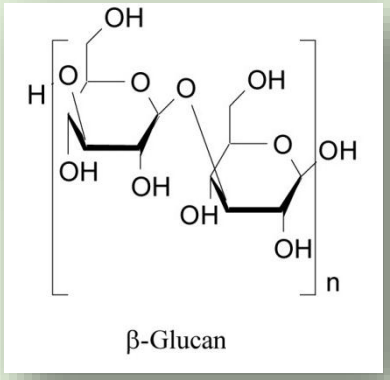
Pheophorbide a



Curcumin



Aloe emodina



β-Glucan

Skalkos, D. *et al.* Photochem. Photobiol. B: Biol., 2006, 82: 146-151
Guillet, G. *et al.* Phytochemistry, 1997, 45: 695-699.
Ebermann, R. *et al.* J. Photochem. Photobiol. B: Biol., 1996, 36: 95-97.
Lee, W.Y. *et al.* J. Photochem. Photobiol. B: Biol., 2004, 75: 119-126.
Koon, H. *et al.* J. Environ. Pathol., Toxicol. Oncol., 2006, 25: 205.
Akramiene, D. *et al.* Medicina (Kaunas), 2009, 45: 480.
Wamer, W.G. *et al.* Free Rad. Bio. Med., 2003, 34: 233-242.

Previous works

Anti-Cancer Agents in Medicinal Chemistry, 2012, *12*, 959-965 959

Fig Latex (*Ficus carica* L. cultivar Dottato) in Combination with UV Irradiation Decreases the Viability of A375 Melanoma Cells *In Vitro*

Giulio Menichini[a], Carmine Alfano[a], Eugenio Provenzano[b], Mariangela Marrelli[c], Giancarlo A. Statti[c], Francesco Somma[a], Francesco Menichini[c] and Filomena Conforti[c,*]

Pharmaceutical Biology
http://informahealthcare.com/phb
ISSN 1388-0209 print/ISSN 1744-5116 online
Editor-in-Chief: John M. Pezzuto
Pharm Biol, 2014; 52(7): 909–918
© 2014 Informa Healthcare USA, Inc. DOI: 10.3109/13880209.2013.872675
informa healthcare

ORIGINAL ARTICLE

***Hypericum perforatum*: Influences of the habitat on chemical composition, photo-induced cytotoxicity, and antiradical activity**

Mariangela Marrelli[1], Filomena Conforti[1], Chiara Toniolo[2], Marcello Nicoletti[2], Giancarlo Statti[1], and Francesco Menichini[1]

Cell Proliferation
Cell Prolif., 2013, **46**, 193–202 doi: 10.1111/cpr.12020

***Hypericum perforatum* L. subsp. *perforatum* induces inhibition of free radicals and enhanced phototoxicity in human melanoma cells under ultraviolet light**

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

Contents lists available at SciVerse ScienceDirect
Food and Chemical Toxicology
journal homepage: www.elsevier.com/locate/foodchemtox
ELSEVIER

Changes in the phenolic and lipophilic composition, in the enzyme inhibition and antiproliferative activity of *Ficus carica* L. cultivar Dottato fruits during maturation

Mariangela Marrelli [a], Federica Menichini [b], Giancarlo A. Statti [a], Marco Bonesi [a], Pierre Duez [c], Francesco Menichini [a], Filomena Conforti [a,*]

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

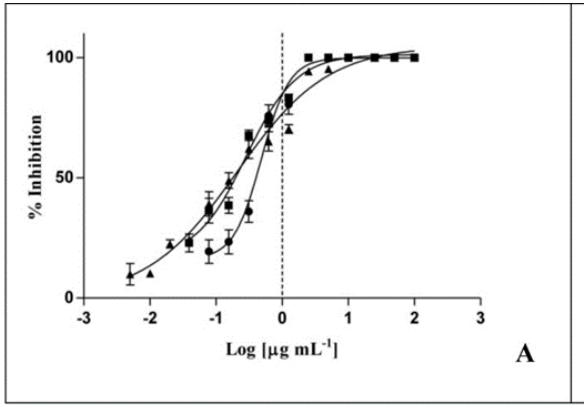
***Cachrys pungens* Jan inhibits human melanoma cell proliferation through photo-induced cytotoxic activity**

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

Cachrys pungens Jan

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).



Cachrys libanotis L.

A survey of the literature

- Widely distributed around the Mediterranean basin.
- Aerial parts essential oil: germacrene-D, γ-terpinene, p-cymene, caryophyllene oxide and limonene.
- Alcoholic extract: 5-methoxy-, 8-methoxy-and 5,8-dmiethoxypsoralen.
- Root extracts: antioxidant and antibacterial activities; xanthine oxidoreductase inhibitory potential.

Photo from Saxifraga-Willem van Kruijsbergen

Aim of the research

- **Photocytotoxic potential of aerial parts extracts**
  - **Traditional maceration (TM)**
  - **Pressurized cyclic solid-liquid (PCSL) (Naviglio® extractor)**
- Phytochemical composition
- Photocytotoxic effects on UVA-irradiated C32 melanoma cell line
- Apoptotic responses
- Phenolic content and antioxidant potential

Naviglio D. *Anal Lett* **2003**, *36*, 1647-1659.
Aouachria, S. *et al*. *J Drug Deliv Ther* **2020**, *10*, 71-79.
Bouderdara, N. *et al*. *Nat Prod Commun* **2011**, *6*, 115-117.
Ena, P. *et al*. *Contact derm* **1991**, *24*, 1-5.
Aouachria, S. *et al*. *J Drug Deliv Ther* **2020**, *10*, 71-79.

Plant material and extraction procedure

Photo from Saxifraga-Willem van Kruijsbergen

Extraction technique	Abbreviation	Yield (%)	Total phenolic content (mg/g)	Total flavonoid content (mg/g)
Maceration	TM	17.8	25.0 ± 0.2	1.29 ± 0.04
Naviglio®	PCSL	12.6	12.8 ± 0.1	0.09 ± 0.01

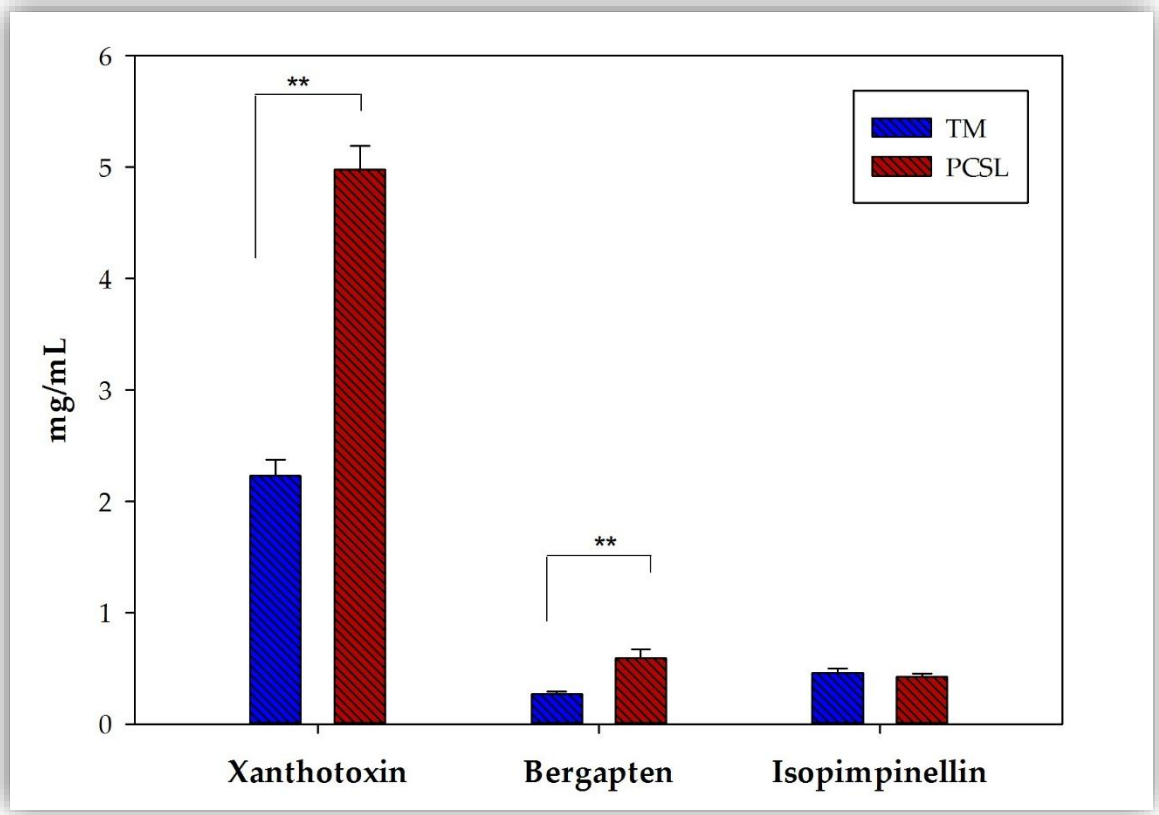
Chemical composition: GC-MS

Compound	Rt	Relative peak area percentage	
		TM	PCSL
Furanocoumarins		0.11	0.30
Xanthotoxin	19.154	9.1	14.8
Bergapten	19.354	2.8	2.5
Isopimpinellin	20.571	3.4	3.0
Pyranocoumarins			
Seselin	19.462	0.6	-
2-Methyl-2-butenoic acid 9,10-dihydro-8,8-dimethyl-2-oxo-2H,8H-benzo[1,2-b:3,4-b']dipyran-9-yl ester	24.423	-	9.7
Coumarins			
Osthol	19.822	2.8	-
Suberosin	20.388	2.7	-
Isogeijerin	21.154	1.2	5.6
Fatty acids		-	
Myristic acid	16.496	-	0.2
Palmitic acid	18.085	2.2	1.8
α-Linolenic acid	19.897	0.7	-
Terpenes		-	
Estragole	11.141	0.1	0.7

Quantitative analyses

Compound	TM	PCSL
	mg/mL ± SD	
Xanthotoxin	**2.23 ± 0.14**	**4.98 ± 0.21**
Bergapten	**0.27 ± 0.02**	**0.59 ± 0.08**
Isopimpinellin	**0.46 ± 0.04**	**0.42 ± 0.03**

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

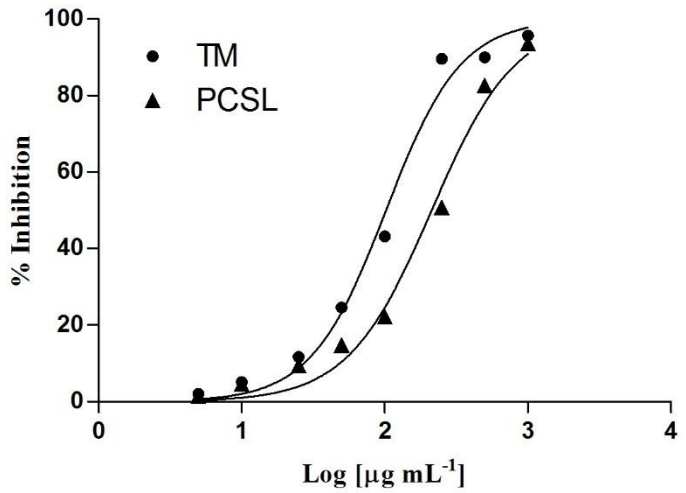


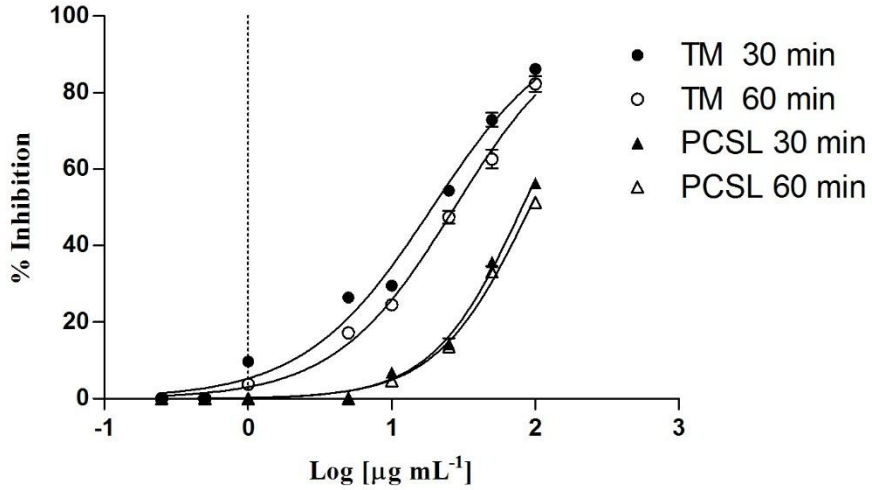
** $P < 0.01$ (Students' t test)

Antioxidant activity

Sample	IC_{50} (μg/mL)		
	DPPH	β-Carotene	
		30 min	60 min
TM	102.13 ± 0.79 [b]	19.22 ± 1.07 [b]	27.52 ± 1.73 [c]
PCSL	212.80 ± 6.91 [c]	81.20 ± 1.52 [d]	92.44 ± 1.08 [e]
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). * Positive controls.



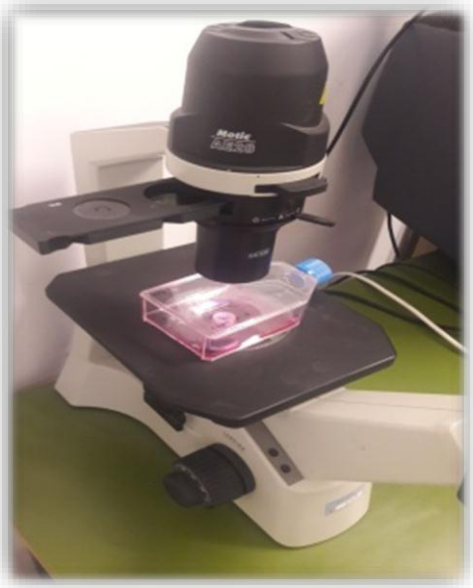


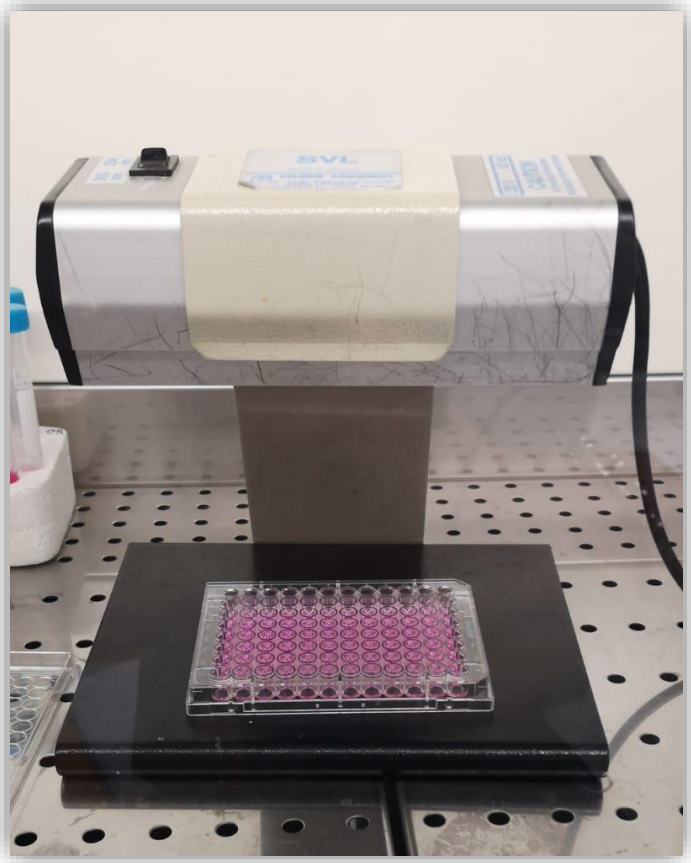
IECPS 2020

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



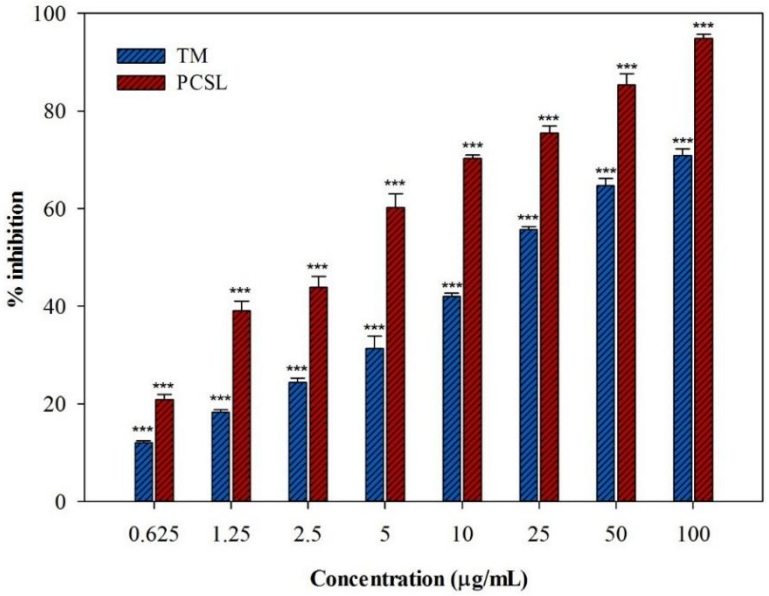


Marrelli M. *et al*. *Food Chem Toxicol* **2012**, *50*, 726-733.
Marrelli M. *et al*. *Pharm Biol* **2014**, *52* 909-918.

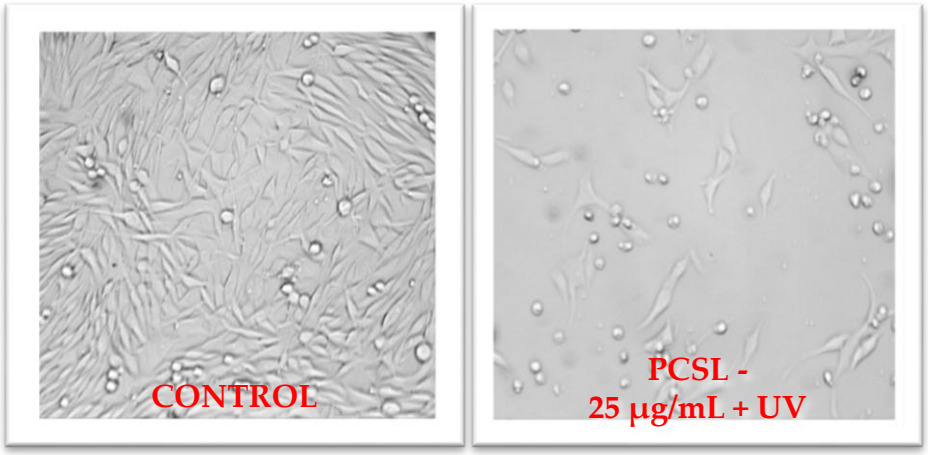
Photocytotoxic activity

Sample	IC_{50} (µg/mL)	
	Irradiated	Unirradiated
TM	18.18 ± 1.33 [b]	> 100
PCSL	3.16 ± 0.21 [a]	55.20 ± 1.65 [c]
Bergapten*	0.191 ± 0.012 [a]	n.d.

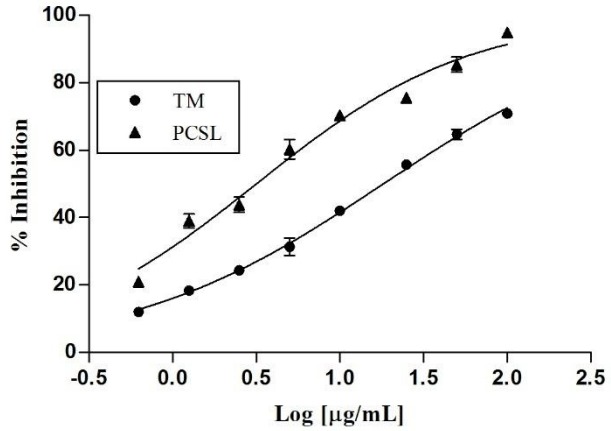
Data were expressed as mean ± S. E. M. (n=4). Different letters indicate statistically significant differences at $P < 0.05$ (Bonferroni post-hoc test). * Positive contol. n.d.: not detectable.



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

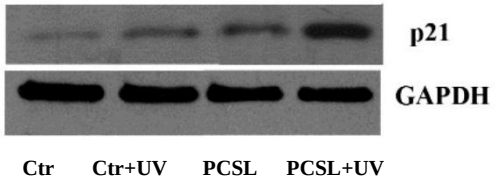


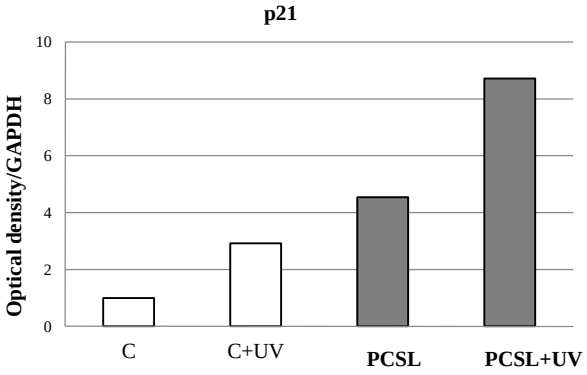
Morphological changes in C32 cells induced by photocytotoxic *C. libanotis* L. extract

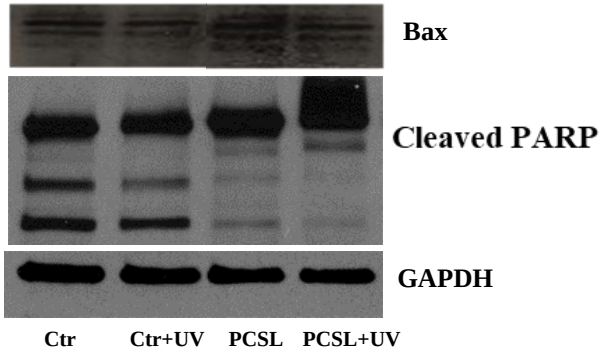


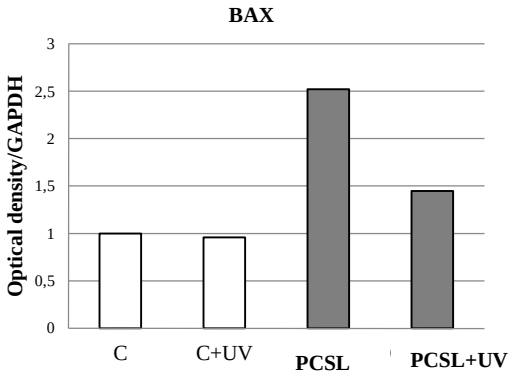
IECPS 2020

Immunoblotting Analysis: PCSL extract

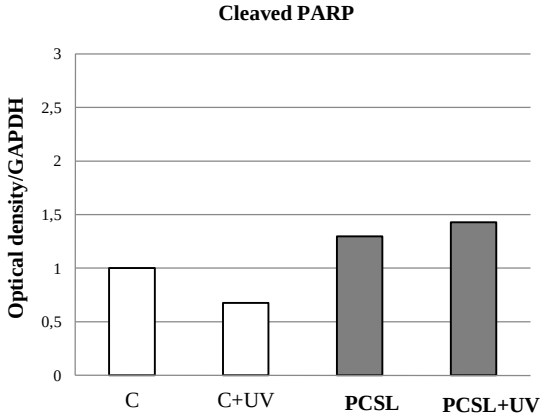




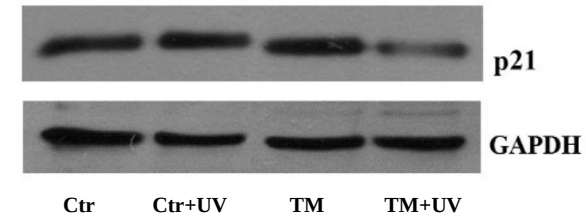


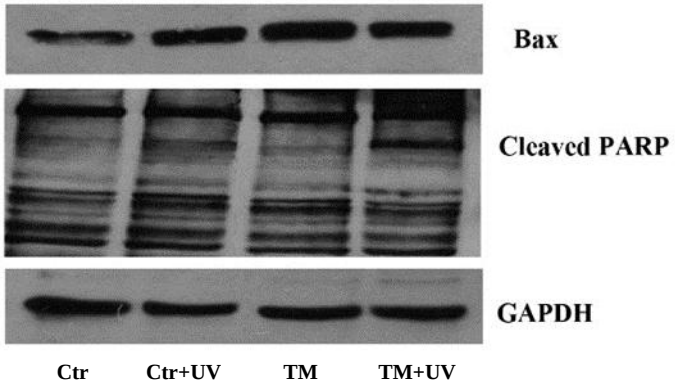


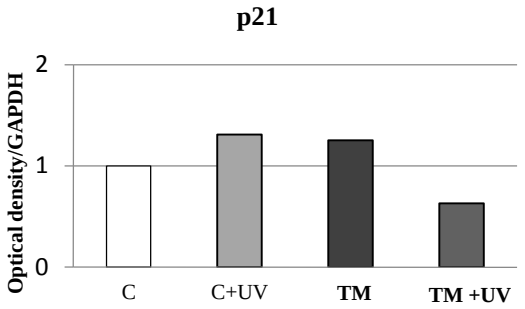
Immunoblots of p21, Bax and poly (ADP-ribose) polymerase (PARP) protein levels in C32 cells treated or not with PCSL extract for 24h. The histograms represent the mean ± SD of three separate experiments in which band intensities were evaluated as optical density (OD) and expressed as fold change vs. control samples.

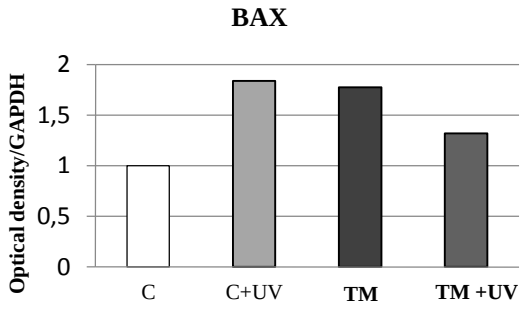


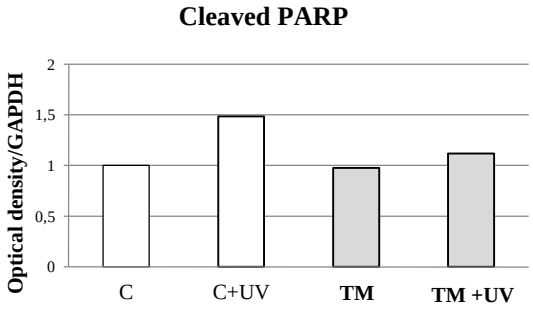
Immunoblotting Analysis: TM extract











Immunoblots of p21, Bax and poly (ADP-ribose) polymerase (PARP) protein levels in C32 cells treated or not with TM extracts for 24h. The histograms represent the mean ± SD of three separate experiments in which band intensities were evaluated as optical density (OD) and expressed as fold change vs. control samples.

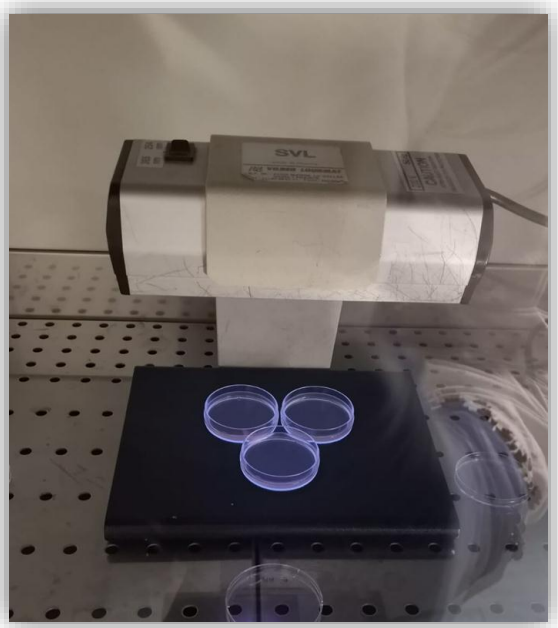
Conclusions

- Obtained results demonstrated the photocytotoxic activity of *C. libanotis* species.
- PCSL extraction allowed a better phytochemical composition for the anticancer activity compared to TM, inducing significant apoptotic effects on human melanoma cell line.

Future perspectives

- Investigated sample could be a promising candidate for further studies with the aim to find new potential drugs useful in the photochemotherapy of skin cancer.

Photo from Saxifraga-Willem van Kruijsbergen



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

IECPS 2020