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

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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 phytocompounds. 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/cm². Sample obtained through PCSL extraction was the most effective, with an IC₅₀ 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)
Background

Psoralens + UVA $\rightarrow$ PUVA (320-400 nm)

- Cutaneous T-cell lymphoma
- Vitiligo, psoriasis

Furanocoumarins

*Apiaceae*

*Fabaceae*

*Moraceae*

*Rutaceae*

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$^3$ and Francesco Menichini$^1$

$^1$Department of Pharmaceutical Sciences, University of Calabria, Italy; $^2$Operative Unit of Dermatology, A.O. of Cosenza, Italy

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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 in the range of 600-800 nm.

Photosensitizers (PS) react with oxygen to produce reactive oxygen species (ROS):

- $^1\text{O}_2$
- $\cdot\text{O}_2^-$
- $\text{H}_2\text{O}_2$
- $\cdot\text{OH}$

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

Applications of Natural Compounds in the Photodynamic Therapy of Skin Cancer

M. Marrelli$^1$, G. Menichini$^2$, E. Provenzano$^3$ and F. Conforti$^{1,*}$

Background

Natural Photosensitizers

Porfimer sodium

aminolevulinic acid, ALA

Hypericin

Polyacetylene structure

Thiophenes structure (α-terthienyl)

Curcumin

Pheophorbide a

Aloe emodina

β-Glucan

Previous works

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

Giallo Menichini, Carmine Alfano, Eugenio Provenzano, Mariangela Marrelli, Giancarlo A. Statti, Francesco Somma, Francesco Menichini and Filomena Conforti.

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

Mariangela Marrelli, Filomena Conforti, Chiara Toniolo, Marcello Nicoletti, Giancarlo Statti, and Francesco Menichini.

Cachrys pungens Jan

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.

<table>
<thead>
<tr>
<th>Sample</th>
<th>IC$_{50}$ µg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methanol</td>
<td>0.487 ± 0.037</td>
</tr>
<tr>
<td>Chloroform</td>
<td>0.286 ± 0.067</td>
</tr>
<tr>
<td>Coumarin fraction</td>
<td>0.209 ± 0.033</td>
</tr>
</tbody>
</table>

Data were expressed as mean ± S. E. M. (n=6).
**Cachrys libanotis L.**

**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

- 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.

**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.
Plant material and extraction procedure

<table>
<thead>
<tr>
<th>Extraction technique</th>
<th>Abbreviation</th>
<th>Yield (%)</th>
<th>Total phenolic content (mg/g)</th>
<th>Total flavonoid content (mg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maceration</td>
<td>TM</td>
<td>17.8</td>
<td>$25.0 \pm 0.2$</td>
<td>$1.29 \pm 0.04$</td>
</tr>
<tr>
<td>Naviglio®</td>
<td>PCSL</td>
<td>12.6</td>
<td>$12.8 \pm 0.1$</td>
<td>$0.09 \pm 0.01$</td>
</tr>
</tbody>
</table>

Photo from Saxifraga-Willem van Kruijsbergen
<table>
<thead>
<tr>
<th>Compound</th>
<th>Rt</th>
<th>Relative peak area percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>TM</td>
</tr>
<tr>
<td>Furanocoumarins</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Xanthotoxin</td>
<td>19.154</td>
<td>9.1</td>
</tr>
<tr>
<td>Bergapten</td>
<td>19.354</td>
<td>2.8</td>
</tr>
<tr>
<td>Isopimpinellin</td>
<td>20.571</td>
<td>3.4</td>
</tr>
<tr>
<td>Pyranocoumarins</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Seselin</td>
<td>19.462</td>
<td>0.6</td>
</tr>
<tr>
<td>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</td>
<td>24.423</td>
<td>-</td>
</tr>
<tr>
<td>Coumarins</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Osthol</td>
<td>19.822</td>
<td>2.8</td>
</tr>
<tr>
<td>Suberosin</td>
<td>20.388</td>
<td>2.7</td>
</tr>
<tr>
<td>Isogeijerin</td>
<td>21.154</td>
<td>1.2</td>
</tr>
<tr>
<td>Fatty acids</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Myristic acid</td>
<td>16.496</td>
<td>-</td>
</tr>
<tr>
<td>Palmitic acid</td>
<td>18.085</td>
<td>2.2</td>
</tr>
<tr>
<td>α-Linolenic acid</td>
<td>19.897</td>
<td>0.7</td>
</tr>
<tr>
<td>Terpenes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Estragole</td>
<td>11.141</td>
<td>0.1</td>
</tr>
</tbody>
</table>
**Quantitative analyses**

<table>
<thead>
<tr>
<th>Compound</th>
<th>TM (mg/mL ± SD)</th>
<th>PCSL (mg/mL ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Xanthotoxin</td>
<td>2.23 ± 0.14</td>
<td>4.98 ± 0.21</td>
</tr>
<tr>
<td>Bergapten</td>
<td>0.27 ± 0.02</td>
<td>0.59 ± 0.08</td>
</tr>
<tr>
<td>Isopimpinellin</td>
<td>0.46 ± 0.04</td>
<td>0.42 ± 0.03</td>
</tr>
</tbody>
</table>

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

**P < 0.01 (Students’ t test)**
## Antioxidant activity

<table>
<thead>
<tr>
<th>Sample</th>
<th>IC$_{50}$ (µg/mL)</th>
<th>DPPH</th>
<th>β-Carotene</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>30 min</td>
<td>60 min</td>
<td></td>
</tr>
<tr>
<td>TM</td>
<td>102.13 ± 0.79</td>
<td>19.22 ± 1.07</td>
<td>27.52 ± 1.73</td>
<td></td>
</tr>
<tr>
<td>PCSL</td>
<td>212.80 ± 6.91</td>
<td>81.20 ± 1.52</td>
<td>92.44 ± 1.08</td>
<td></td>
</tr>
<tr>
<td>Ascorbic acid*</td>
<td>2.00 ± 0.01</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Propyl gallate*</td>
<td>-</td>
<td>1.00 ± 0.02</td>
<td>1.00 ± 0.02</td>
<td></td>
</tr>
</tbody>
</table>

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.
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/cm²
- Cell viability 48h later: MTT test
- Unirradiated microtiter plates
- Positive control: Bergapten

**Photocytotoxic activity**

<table>
<thead>
<tr>
<th>Sample</th>
<th>IC$_{50}$ (µg/mL)</th>
<th></th>
<th>(\text{IC}_{50}) (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Irradiated</td>
<td>Unirradiated</td>
<td></td>
</tr>
<tr>
<td>TM</td>
<td>18.18 ± 1.33 (^b)</td>
<td>&gt; 100</td>
<td></td>
</tr>
<tr>
<td>PCSL</td>
<td>3.16 ± 0.21 (^a)</td>
<td>55.20 ± 1.65 (^c)</td>
<td></td>
</tr>
<tr>
<td>Bergapten*</td>
<td>0.191 ± 0.012 (^a)</td>
<td>n.d.</td>
<td></td>
</tr>
</tbody>
</table>

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

CONTROL

PCSL - 25 µg/mL + UV
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.
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.
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.

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.
Thanks for your attention