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Proceeding Paper

Cachrys ferulacea (L.) Calest. Extracts as Natural Photosensitizers: An In Vitro Photobiological Study †

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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 h 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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1. Introduction

PUVA-therapy, whose name is an acronym for psoralen (linear furanocoumarins) plus ultraviolet-A radiation, is a photochemotherapy commonly used in the treatment of psoriasis, vitiligo and other dermatologic diseases. Because of the ability of furanocoumarins to interact with DNA and disrupt its replication, there is a great interest in developing anti-cancer therapies based on the action of these compounds against malignant cells [1,2]. PUVA is one of the first line options for the treatment of mycosis fungoides, the most common type of cutaneous T-cell lymphoma, which is a heterogenous group of non-Hodgkin lymphomas arising in the skin [3,4].

Furanocoumarins are coumarins derivatives whose structure is based on a furan ring attached to the coumarin backbone. These compounds are divided into two groups according to the attachment place of the furan ring: linear (C6/C7) and angular (C7/C8) types. The first type is the most common, frequently found in plants belonging to Apiaceae and Rutaceae families, while the occurrence of the C7/C8 derivatives is limited to Apiaceae and Fabaceae [5,6].

The *Cachrys* genus (Apiaceae) is widely distributed in the Mediterranean basin. We previously investigated the photobiological properties of different *Cachrys* species, such as *C. pungens* Jan [7], *C. libanotis* L. and *C. sicula* L. [8].

Based on the promising previously obtained results, we aimed to investigate another species belonging to the same interesting genus: *C. ferulacea* (L.) Calest. The aerial parts were extracted using three different techniques: traditional maceration, pressurized cyclic solid-liquid extraction using Naviglio extractor® and supercritical CO_2. The phytochemical composition was verified with gas chromatography-mass spectrometry (GC-MS) and the photobiological properties were assessed in vitro on the melanoma C32 cell line.

2. Materials and Methods

C. ferulacea aerial parts were collected in Calabria (Southern Italy) and extracted with methanol (plant to-solvent ratio 1:10 g/mL) through traditional maceration (TM) and pressurized cyclic solid-liquid (PCSL) extraction with a Naviglio extractor® (Atlas Filtri SRL, Limena, PD, Italy). A further extract was obtained through supercritical CO_2 (S-CO_2)

The phytochemical profile was investigated with gas chromatography-mass spectrometry (GC-MS) [8]. Total phenolics were determined spectrophotometrically using the Folin-Ciocalteu method and flavonoids content was assessed by the formation of a complex with aluminum chloride after acid hydrolysis [9]. The DPPH and the β-carotene bleaching assays were used to verify the antioxidant potential of the extracts [10].

The photobiological properties of *C. ferulacea* samples were assessed in vitro on the C32 cell line (human melanoma cancer cells, ATCC no. CRL-1585). Cells were cultured in RPMI-1640 medium supplemented with 1% penicillin/streptomycin, 1% L-glutamine and 10% fetal bovine serum. Cells were then trypsinized, placed in 96-well plates (3.8×10^4 cells/well) and incubated to allow for cell attachment. Twenty-four hours later, the medium was removed and samples at different concentrations in Hanks' Balanced Salt Solution (100 μL) were added. After 30 min, microtiter plates were irradiated at 365 nm for 1 h at a dose of 1.08 J/cm^2 [8]. After the irradiation, sample solutions were replaced by fresh medium and the cytotoxicity was evaluated 48 h later using the 3-[4,5-dimethyl-2-yl]-2,5-diphenyl tetrazolium bromide (MTT) assay [11]. Experiments were run in quadruplicate and the well-known photoactive compound bergapten was used as positive control.

Immunoblotting analysis was also carried out in order to assess the apoptotic responses. After each treatment, C32 cells were lysed for total protein extraction, and proteins were then resolved on 10% SDS-polyacrylamide gel, transferred to a nitrocellulose membrane and probed with Cyclin D1, p21, p53, PARP and GAPDH antibodies (Santa Cruz Biotechnology). Membranes were then incubated with peroxidase-coupled goat anti-mouse or goat anti-rabbit antibodies and the antigen-antibody complex was shown using the ECL System (Amersham Pharmacia) [12].

Data were analyzed using GraphPad Prism Software (San Diego, CA, USA). Statistical analyses were performed using one-way analysis of variance (ANOVA) with Tukey's or Dunnett's post hoc test.

3. Results and Discussion

Traditional maceration allowed to obtain a significantly higher yield (14.7%) compared to the other two utilized techniques PCSL and S-CO_2 extraction (3.6% and 2.4%, respectively).

The phytochemical composition was assessed with GC-MS. Overall, the four linear furanocoumarins psoralen, xanthotoxin, bergapten, isopimpinellin were detected, together with their precursor marmesin, with the S-CO_2 extract showing the highest number of these compounds. The same sample contained the highest number of simple coumarins, being the coumarin derivative osthole the major one. Moreover, different fatty acids, such as myristic, palmitic, linoleic, behenic and lignoceric acids, and four terpenes were identified in *C. ferulacea* extracts.

The TM sample showed the highest phenolic content (17.99 ± 0.50 mg/g of plant material). Lower amounts were detected in the other two samples (4.14 ± 0.24 and 2.32 ± 0.09 mg/g for PCSL and S-CO_2, respectively). The same trend was observed for total flavonoids, with the TM extract showing the highest amount (0.63 ± 0.06 mg/g).

Consistently with the phenolic content, the sample obtained through traditional maceration showed the best radical scavenging activity (IC_{50} value = 77.37 ± 1.58 μg/mL) and the better results in the β-carotene bleaching test (IC_{50} = 19.57 ± 0.67 and 27.94 ± 0.48 μg/mL after 30 and 60 min of incubation, respectively).

The photobiological properties of *C. ferulacea* were investigated on C32 cell cultures irradiated with UVA light for 1 h at a dose of 1.08 J/cm^2. All the three *C. ferulacea* extracts induced a concentration-dependent photocytotoxic activity (Figure 1). IC_{50} values equal to 27.95 ± 0.67 and 25.90 ± 1.23 μg/mL were obtained for the TM and PCSL samples, respectively. The extract obtained through supercritical CO_2 extraction was the most active one, with an IC_{50} of 4.91 ± 0.15 μg/mL. Interestingly, no extract affected cell viability in the dark.

The proliferative arrest was evidenced by the down-regulation of Cyclin D1 in C32 UV-treated cells. However, a consistent reduction of the same protein, together with an up-regulation of the Cyclin-dependent kinase inhibitor p21, was given by the S-CO_2 extraction method in melanoma UV-treated cells. The latter protein, as previously reported, is a modulator of apoptotic responses through different pathways [13]. This is in line with the sustained increase of the proteolytic form of PARP, a marker of the DNA damage, observed in the melanoma cells underwent to UVA light.

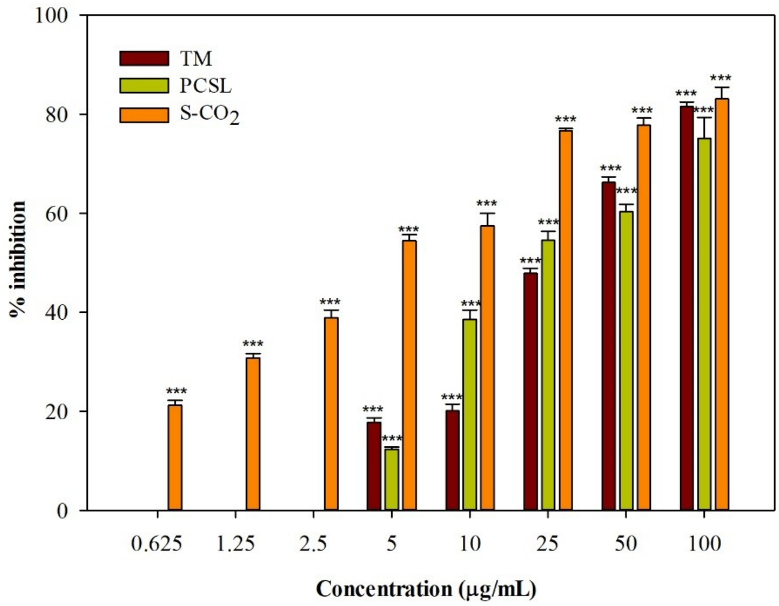

Figure 1. Photocytotoxic activity of *C. ferulacea* (L.) Calest. extracts. TM, traditional maceration; PCSL, pressurized cyclic sold-liquid extraction; S-CO_2, supercritical CO_2 extraction. Data are expressed as mean ± S.E.M. (n = 4). *** $p < 0.001$ compared to control (Dunnett's multiple comparisons test).

4. Conclusions

The present study demonstrated that *C. ferulacea* extracts, mainly the S-CO_2 sample, contain important photoactive constituents responsible for their photocytotoxic activity. Investigated samples induced promising cytotoxic effects on malignant melanoma cells upon irradiation with UVA light, without affecting cell viability in the dark. Future studies could be useful to further optimize the extraction method and to continue investigating the interesting photobiological properties of this species.

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