Supplementary material

Chemical composition and anticancer activity of the essential oil from *Vicia ochroleuca* Ten., Quite rare plant in Kabylia (Algeria)

Sara Boussaha[a,b], Massimo Bramucci [c*], Khellaf Rebbas [d], Luana Quassinti [c], Ratiba Mekkiou[a] and Filippo Maggi [c]

[a]*Unité de Recherche: Valorisation des Ressources Naturelles, Molécules Bioactives et Analyses Physicochimiques et Biologiques, Université Frères Mentouri, Constantine 1. Route d'Aïn El Bey, 25017, Constantine, Algérie.*

[b]*Ecole Nationale Supérieure de Biotechnologie Taoufik Khaznadar, Ville Universitaire Ali Mendjli, BP E66, 25100 Constantine, Algérie.*

[c] *School of Pharmacy, University of Camerino, Camerino, Italy, I-62032, Camerino, Italy*

[d] *University Mohamed Boudiaf of M'Sila, 28 000, Algeria. Agro-Biotechnology and Nutrition Laboratory in Arid and Semi-Arid Zones. Ibn Khaldoun University, Tiaret, Algeria*

*Corresponding author: Massimo Bramucci, School of Pharmacy, University of Camerino, Via Gentile III da Varano n.1, I-62032 Camerino (MC), Italy, Tel. +39-0737403262; fax: +39-0737403290; E-mail address: massimo.bramucci@unicam.it (M. Bramucci).

ORCID ID: https://orcid.org/0000-0003-4113-8199

Abstract

The search for new bioactive substances with anticancer activity and the understanding of their mechanisms of action are high priorities in the research effort toward more effective treatments for cancer. In this paper, we analyzed, for the first time, the chemical composition of the essential oil (EO) hydrodistilled from the aerial parts of *Vicia ochroleuca* Ten. (Leguminosae) by GC-MS. A total of sixteen compounds representing 82.2% of the total composition were identified. The major compounds were phytone (20.11%), hexadecanoic acid (10.23%), 1-octen-3-ol (9.84%), and 10-*epi*-α-cadinol (7.13%). Additionally, using the MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) method, the EO was tested *in vitro* against a panel of human cancer cells, including breast (MDA-MB 231), colon (HCT116), melanoma (A375), and glioblastoma (*T*98G), with corresponding IC_{50} values of 23.07, 47.05, 51.64, and 64.07 μg/mL, respectively. The results demonstrate cytotoxic activity and suggest that *V. ochroleuca* EO could be regarded as a natural bioactive source.

Keywords: *Vicia ochroleuca,* Essential oil, GC-MS analysis, anticancer activity

1. Experimental

1.1. Plant material

The studied sample was collected in the Akfadou region on 05 May 2017 and identified by Prof. Rebbas Khellaf. A voucher specimen (VOF-0517-Akf-ALG-53) has been deposited at the Herbarium of the Unit Research Valorization of Natural Resources, Bioactive Molecules and Physico-Chemical and Biological Analyzes, University of Constantine 1.

1.2. Essential oil isolation

The aerial parts (120 g) of *V. ochroleuca* were distilled in a Kaiser Lang apparatus for 3 hours. The obtained essential oil (EO) was collected and dried over anhydrous sodium sulfate and kept at 4°C until analysis. The yield of the oil was calculated in relation to the dry weight of the plant.

1.3. GC-MS analysis

Composition of the essential oils was determined by GC–MS analyses. They were achieved on an Agilent Technologies 6890 GC equipped with mass spectrometer 5973N detector using a HP-5MS (5% phenylmethylpolysiloxane, 30 m lenght× 0.25 mm internal diameter, 0.25 μm film thicknesses; J & W Scientific, Folsom, CA) capillary column. The carrier gas was helium at a flow rate of 1.0 mL/min. Initial column temperature was 60 °C and programmed to increase up to 280 °C at 4 °C/min. The split ratio was 40:1. The injector temperature was set at 300 °C. The acquisition range was 50–550 *m/z* in electron-impact (EI) mode using an ionization voltage of 70 eV. The EO was diluted 1:100 in *n*-hexane, then 0.1 μL were injected into the GC systems. The constituents were identified by comparison of their retention indices (Van Den Dool and Kratz 1963) and mass spectra with those recorded in the NIST 08 (NIST 08, 2008), ADAMS (Adams, 2007) and Willey MS libraries. The percentage composition (average of three independent analyses) was computed from the GC peak areas without using any correction factors.

1.4. Anticancer activity

The EO's antiproliferative potential was assessed using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) assay, which is based on the cleavage of the tetrazolium salt by mitochondrial dehydrogenases in viable cells, as previously reported by (Quassinti et al. 2013).

1.5. MTT assay

Briefly, the human glioblastoma multiforme cell line (T98G) was cultured in Eagle's minimum essential medium (EMEM) with 2 mM L-glutamine, 0.1 mM non-essential amino acids, 1mM sodium pyruvate, 100 IU/mL penicillin, 100 µg/mL streptomycin, and supplemented with 10% heat inactivated foetal bovine serum (HI-FBS) (Stein, 1979). The human malignant melanoma cell line (A375) and human breast adenocarcinoma cell line (MDA-MB231) were cultured in Dulbecco's modified Eagle's medium (DMEM) with 2 mM L-glutamine, 100 IU/mL penicillin, 100 µg/mL streptomycin, and supplemented with 10% HI-FBS (Cailleau et al. 1974). Human colon carcinoma cells (HCT116 cells) were cultured in RPMI1640 medium with 2 mM L-glutamine, 100 IU/mL penicillin, 100 µg/mL streptomycin and supplemented with 10% HI-FBS (Brattain et al. 1981).
Cells were cultured in a humidified atmosphere at 37°C in the presence of 5% CO_2.
Cells were seeded at a density of 2×10^4 cells/mL. After 24 h, EO was added at different concentrations (1.56–200 µg/mL). The anticancer drug cisplatin (0.05–50 µg/mL) was used as the positive control. Cells were incubated for 72h. At the end of incubation, each well received 10 µL of MTT solution (5 mg/mL in phosphate-buffered saline, PBS). After 4 h of incubation at 37°C, the MTT medium was removed and DMSO (100 µL) was added to dissolve the formazan crystals. The extent of MTT reduction was measured spectrophotometrically at 540 nm using a microplate spectrophotometer FLUOstar Omega (BMG Labtech). Experiments were conducted in triplicate. Cytotoxicity was expressed as the concentration of a compound inhibiting cell growth by 50% (IC_{50}). The IC_{50} values were determined with GraphPad Prism 5 computer program (GraphPad Software, S. Diego, CA, USA).

2. Statistical analysis

All assays were conducted at least three times with three different sample preparations. Analysis of variance was performed using InStat (GraphPad software, San Diego CA). A one-way ANOVA followed by Dunnett's multiple comparisons test was used for these analyses, and $P < 0.05$ was considered to be statistically significant.

References

Adams RP. 2007. Identification of essential oils by capillary gas chromatography/mass spectroscopy. Allured, Carol Stream, IL, USA.

Brattain MG, Fine WD, Khaled FM, Thompson J, Brattain DE. 1981. Heterogeneity of malignant cells from a human colonic carcinoma. Cancer Res 41(5):1751-1756.

Cailleau R, Young R, Olivé M, Reeves W J Jr. 1974. Breast tumor cell lines from pleural effusions. J Natl Cancer Inst 53(3):661-674.

NIST, 08, 2008. Mass Spectral Library (NIST/EPA/NIH). National Institute of Standards and Technology, Gaithersburg, USA

Quassinti L, Lupidi G, Maggi F, Sagratini G, Papa F, Vittori S, Bianco A, Bramucci M. 2013. Antioxidant and antiproliferative activity of *Hypericum hircinum* L. subsp. majus (Aiton) N. Robson essential oil. Nat Prod Res. 27(10):862-868.

Stein GH. 1979. T98G: an anchorage-independent human tumor cell line that exhibits stationary phase G1 arrest in vitro. J Cell Physiol 99(1):43-54.

Van Den Dool H, Kratz PD. 1963. A generalization of the retention index system including linear temperature programmed gas-liquid partition chromatography. J Chromatogr 11:463-471.