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Original article

Viability-reducing activity of *Coryllus avellana* L. extracts against human cancer cell lines

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ABSTRACT

The increasing rate of cancer incidence has encouraged the search for novel natural sources of anticancer compounds. The presence of small quantities of taxol and taxanes in *Corylus avellana* L. has impelled new potential applications for this plant in the field of biomedicine. In the present work, the cell viability-reducing activity of stems and leaves from three different hazel trees was studiedagainst three human-derived cancer cell lines (HeLa, HepG2 and MCF-7). Both leaf and stem extracts significantly reduced viability of the three cell lines either after maceration with methanol or using taxane extraction methods. Since maceration reduced cell viability to a greater extent than taxane extraction methods, we scaled up the maceration extraction process using a method for solid/liquid extraction (Zippertex technology). Methanol leaf extracts promoted a higher reduction in viability of all cell lines assayed than stem extracts. Fractionation of methanol leaf extracts using silica gel chormatography led to the purification and identification of two compounds by HPLC–MS and NMR: (3R,5R)-3,5-dihydroxy-1,7-bis(4-hydroxy-phenyl) heptane 3-O-β-D-glucopyranoside and quercetin-3-O-rhamnoside. The isolated compounds decreased viability of HeLa and HepG2 cells to a greater extent than MCF-7 cells. Our results suggest a potential use of *C. avellana* extracts in the pharmacotherapy of cervical cancer and hepatocarcinoma and, to a lesser extent, breast cancer.

1. Introduction

Drug development has a long background of searching for active natural compounds in plants, many of which have been used in traditional medicine to treat a wide range of diseases and infections. Almost half the drugs approved since 1994 are based on natural products [1]. Concerning drugs used for cancer treatment, more than 60% have a natural origin and natural products are a major source for drug discovery [2].

The recent increase of cancer incidence has promoted a growing interest in finding new active compounds to be used to fight the disease. The discovery of taxanes in hazel plant extracts triggered an interest to study natural products from this plant and its secondary metabolites [3–6]. In *C. avellana*, over 100 compounds have been described and classified as organic acids, triacylglycerols, phytosterols, tocols, phenolic acids, diarylheptanoids, flavonoids, tannins, isoflavones, lignans, terpenes and taxanes.

Abbreviations: DCM, dichloromethane; DMEM, Dulbecco's Modified Eagle's medium; DMSO, dimethyl sulfoxide; DHHP, (3R,5R) 3,5-dihydroxy-1,7-bis(4-hydroxyphenyl) heptane 3-O-β-d-glucopyranoside; DW, dry weight; ESI, Electro-spray ionization; EtOH, ethanol; HeLa, human cervical cancer cells; HepG2, liver hepatocellular cells; HGF, normal human gingival fibroblast; HL-60, human promyelocytic leukemia cells; HPLC, high-performance liquid chromatography; HSC-2, oral squamous cell carcinoma; IC_{50}, half maximal inhibitory concentration; MCF-7, human breast adenocarcinoma cell line; MeOD, deuterated methanol; MeOH, methanol; MS, mass spectrometry; MTT, 3-(4 5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide); NMR, nuclear magnetic resonance; PPARs, peroxi-some proliferator-activated receptors; QR, quercetin-3-O-rhamnoside; SDS, sodium dodecyl sulfate; SK-Mes-1, human lung cancer cell line; THF-α, tumour necrosis factor alpha; NF-κβ, nuclear factor kappa-B.

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Previous studies reported a taxol-dependent antiproliferative activity in extracts of *C. avellana* cell cultures. Treatment of SK-Mes-1 cells (a human cell line derived from lung cancer) with extracts from *C. avellana* cell culture medium blocked cancer cells at the metaphase/anaphase transition more effectively than those treated with yew extract [3]. Recently, the effect of a *C. avellana* cell culture extract was evaluated in the MCF-7 cancer cell line (derived from breast cancer). The study confirmed the aforementioned activity, and suggested that the *C. avellana* cell culture extracts were more effective than pure taxol. It was therefore hypothesized that *C. avellana* extracts may present compounds that could enhance its effects [7]. Moreover, the antiproliferative activity of extracts of *C. avellana* trees has not been previously studied.

In the present study, bioassay-guided experiments were carried out to isolate and characterize compounds from *C. avellana* with potential anticancer activity. For this purpose, compounds from *C. avellana* leaves and stems were extracted using different methodologies, and their anticancer activity was evaluated using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay in three human cancer cell lines: HeLa (derived from cervical cancer cells), HepG2 (derived from liver hepatocarcinoma) and MCF-7.

2. Materials and methods

2.1. Mammalian cell lines

HeLa, HepG2 and MCF-7 cell lines were obtained from ATCC (ATCC nos.: CCL-2, HB-8065 and HTB-22, respectively). The cells were cultured in Dulbecco's Modified Eagle's (DMEM) medium without pyruvate, supplemented with 100 IU/mL penicillin, 100 μg/mL streptomycin and 10% (v/v) of heat inactivated fetal bovine serum. All the components were purchased from Gibco (Invitrogen, Barcelona, Spain). The cells were grown at 37 °C in 5% CO_2.

2.2. Plant material and extractions

Stems and leaves of *Corylus avellana* L. were collected in October 2014 from three mature trees in Catalonia: (I) wild type in Barcelona, (II) wild type in Terrassa, and (III) a cultivar in Tarragona. The plant materials were identified by Dr. Joan Vallès, Professor of Botany at the Faculty of Pharmacy at the University of Barcelona, and deposited in the herbarium of the Documentation Centre of Plant Biodiversity (CeDocBiV), University of Barcelona (voucher specimens: BCN 131396, BCN 131397 and BCN 131398, respectively). Plant material was air-dried overnight at 60 °C and powdered.

2.2.1. Screening of extraction procedures

Two different extraction procedures were tested in the screening assays. 1) E-extraction: Taxane extraction from 1 g freeze-dried leaves or stems using methanol:water (9:1,v/v) [8] and 2) M-extraction: Extraction by maceration from 1 g of freeze-dried leaves or stems in methanol (MeOH) 90%:H_2O 10% (40 mL, 24 h). All the extraction fractions were evaporated in a rotavapor (Buchi Labortechnik AG, Switzerland). Dry E-extracts (screening and Zippertex extractor) were resuspended in 0.5 mL of dimethyl sulfoxide (DMSO), and 1 mL of DMSO was necessary to resuspended the M-extracts. All samples were filtered through a DMSO-safe filter (0.22 μM; Tecknochroma).

2.2.2. Large-scale extractions

Following screening extraction assays, the tree whose leaf and stem extracts showed the highest antiproliferative activity was submitted to a scaled-up extract process using the accelerated solvent system, Zippertex extractor [9]. 25 g of stems and 40 g of leaves were extracted with dichloromethane (DCM) (2 × 100 mL), followed by MeOH (2 × 100 mL) at 100 bar. Afterwards, the solvents were evaporated and resuspended in DMSO at a concentration of 2 g dry weight (DW)/mL.

2.2.3. Plant extract fractionation

The MeOH extract from leaves was subjected to an additional fractionation procedure. Compounds of the extract were firstly separated by silica gel chromatography using a Combiflash-companion chromatograph (Serlabo) and ready-to-use RediSep column (40 g), obtaining 8 fractions which were resuspended in DMSO, at a concentration of 0.4 g DW/mL. The mobile phase consisted of DCM (A) and MeOH (B) with the following increasing polarity gradient (t (min), %B): (5, 0), (7–17, 5), (18–28, 10), (32–42, 15), (44–55, 20) in 60 min at 30 mL/min.

Flash chromatography was performed with two active fractions to purify the lead compounds. A Sunfire C_{18} III (10 × 250 mm) 5 μm column was used and the mobile phase consisted of water (A): acetonitrile (B), both added with 0.1% formic acid (v/v). A linear gradient of 100% A–100% B was performed in 20 min with a flow of 30 mL/min.

2.3. MTT assay

2.3.1. MTT cell assay conditions

Cell viability was determined by the MTT assay [10]. The cell growth medium was removed and 0.63 mM of MTT and 18.4 mM of sodium succinate (from Sigma-Aldrich, Madrid, Spain) were added to 1 mL of fresh culture medium and the cells were incubated for 3 h at 37 °C. Thereafter the medium was removed and formazan resuspended in DMSO supplemented with 0.57% CH_3COOH and 10% sodium dodecyl sulfate (SDS) (from Sigma-Aldrich, Madrid, Spain). Absorbance was measured at 570 nm in a UV2310 spectrophotometer (Dinko, Barcelona, Spain).

2.3.2. Vehicle validation

To determine the most suitable solvent and the appropriate volume to be used for the experiments DMSO and ethanol (EtOH) were analyzed at 0.01%, 0.02%, 0.1%, 0.2% and 1% (v/v) on HeLa cells. Cells were seeded at 3.4×10^4 cells/well in 12 well plates and after 24 h of growth, DMSO or EtOH was added to the medium and the cell viability assay was carried out for 48 h.

2.3.3. Plant extract assays

HeLa, HepG2 and MCF-7 cells were seeded at a density of 3.4×10^4 cells/well. Twenty-four hours later, different amounts of each plant extraction were added to the cell cultures: 15 mg DW/mL and 3.33 mg DW/mL obtained from E-extraction, 7.5 mg DW/mL and 1.66 mg DW/mL from M-extraction and 3.33 μg DW/mL from Zippertex extractor. For the different fractions obtained 3 mg DW/mL and 0.66 mg DW/mL were assayed. Cell viability was determined 48 h later.

2.3.4. Taxol toxicity

Taxol (ChromaDex, Irvine, CA, USA) was used as an antiproliferative control. The half maximal inhibitory concentration (IC_{50}) of taxol was determined in HeLa cell line. To this end, taxol was diluted in DMSO at the following concentrations: 0.5 nM, 5 nM, 50 nM, 500 nM and 1500 nM. 3.4×10^4 cells/well were seeded and grown for 24 h. Taxol solution was added to the medium to analyze cell viability 48 h later.

2.3.5. (3R,5R)-3,5-dihydroxy-1,7-bis(4-hydroxyphenyl) heptane 3-O-β-D-glucopyranoside and quercetin-3-O-rhamnoside assay

(3R,5R)-3,5-dihydroxy-1,7-bis(4-hydroxyphenyl) heptane 3-O-β-D-glucopyranoside (named as DHHP) and quercetin-3-O-