Antiproliferative Activity on Human Cancer Cell Lines after Treatment with Polyphenolic Compounds Isolated from *Iris pseudopumila* Flowers and Rhizomes

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The present study describes the antiproliferative properties of *Iris pseudopumila* flowers and rhizomes extracts and fourteen constituents isolated from them. The *in vitro* cytotoxic activity assay against two human cancer cell lines, large lung carcinoma (CORL-23) and amelanotic melanoma (C32), showed that the most antiproliferative extract was the MeOH extract from flowers with a percentage of inhibition of 50.9 at 100 μg/ml against amelanotic melanoma cells. The most antiproliferative compounds against amelanotic melanoma cells were kaempferol-3-*O*-β-D-glucopyranoside and irisolidone with a percentage of inhibition of 100 and 96.6, respectively, and against large lung carcinoma cells with a percentage of inhibition of 82.1 and 84.6, respectively. Significant activity on the amelanotic melanoma cell line was also showed by irigenin-7-*O*-β-D-glucopyranoside, with a percentage of inhibition of 89.3. The compounds isovitexin and isoorientin-6-*O*''-β-D-glucopyranoside showed a selective activity against amelanotic melanoma cells with a percentage of inhibition of 83.2 and 79.8, respectively.

Key words: Iris pseudopumila, Antiproliferative Activity, Phenolic Compounds

Introduction

A high number of new drugs derived from plant secondary metabolites have been throughout applied in the treatment and/or prevention of various diseases (Balunas and Kinghorn, 2005). Investigations on natural products have recently regained prominence because of increasing understanding of their biological significance and increasing recognition of the origin and function of their structural diversity. Since 1990, there has been a 22% increase in cancer incidence and mortality, with over 10 million new cases and over 6 million deaths worldwide in 2000 (excluding non-melanoma skin) (Parkin, 2001). Important progress has been made in cancer chemotherapy, a considerable portion of which can be attributed to plant-derived drugs (Balunas and Kinghorn, 2005). The search for more effective and safer antiproliferative compounds has continued to be an important area of active research and, according the recommendations made by WHO, investigation on antitumour compounds from medicinal plants has become an important aspect of this project.

Iris is the largest genus in the Iridaceae family and comprises about 210 species occurring in Eurasia, North Africa, and North America (Mabberley, 1997). Peeled and dried rhizomes of various *Iris* species, collectively known as *Rhizoma iridis*, enjoyed popularity in traditional medicine due to their emetic, cathartic, diuretic, stimulant, antispasmodic and expectorant properties (Steinegger and Hansel, 1988). In some countries, *Iris* species are used in the treatment of cancer, inflammation, bacterial and viral infections (Han, 1988). Literature reports that various *Iris* sp. possess different activities such as antiulcer, antibacterial, anti-inflammatory, piscicidal, antineoplastic, antioxidant, hypolipidemic and antituberculosis (Orhan *et al.*, 2003; Wang *et al.*, 2003; Choudhary *et al.*, 2005).

Iris pseudopumila Tineo flowers and rhizomes (Iridaceae) is a dwarf, bearded species endemic of Southern Italy, where it grows as an ornamental plant. It can be yellow or violet, and it grows in shallow, stony soils (Agrawal, 1989). Some of eth-

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nomedical and reported biological activities of *Iris* sp. may be due to their antioxidant nature; for this reason, we have recently assayed the methanolic extract and its constituents from *I. pseudopumila* rhizomes using luminol-dependent chemiluminescence, and found a significant antioxidant activity (Rigano *et al.*, 2007). Furthermore, the same extract showed good antimicrobial activity against different Gram-positive and Gram-negative bacteria (Rigano *et al.*, 2006). We also demonstrated that methanolic extracts from *I. pseudopumila* rhizomes and flowers showed free radical scavenging and antioxidant activities while a chloroform fraction from the rhizomes showed high cytotoxic activity on the amelanotic melanoma cell line (C32) (Rigano *et al.*, 2009).

The aim of the present study was to evaluate the cytotoxic activity of extracts and their constituents from *I. pseudopumila* against two human cancer cell lines, CORL-23 and C32, using the MTT assay.

Material and Methods

Plant materials

The flowering aerial parts and rhizomes of *Iris pseudopumila* Tineo were collected in May 2006 in the "Parco Nazionale del Cilento" (Salerno, Southern Italy). A voucher specimen (NAP # 68) is deposited at the Herbarium Neapolitanum (NAP), Dipartimento di Biologia Vegetale, Università degli Studi di Napoli "Federico II", Naples, Italy.

Preparation of the methanolic extracts and isolation of compounds

The obtainment of methanolic extracts from flowers and rhizomes of *I. pseudopumila* and the isolation of phenolic compounds from them were described previously (Rigano *et al.*, 2007, 2009).

Cell line and cell culture

Two cancer cell lines, large lung cell carcinoma CORL-23 (ECACC No. 92031919) and amelanotic melanoma C32 (ATCC No. CRL-1585) (Sigma-Aldrich, Milan, Italy), were used in this experiment. The cells were cultured in RPMI 1640 medium supplemented with 10% foetal bovine serum, 1% L-glutamine, 1% penicillin/streptomycin.

Cell counts and viability were performed using a standard trypan blue cell counting technique. The cell concentration was adjusted to $2 \cdot 10^4$ cells/ml. 100 μl of this cell concentration were cultured in a 96-well plate for 1 d to become nearly confluent. Concentrations ranging from 5–200 μg/ml of the samples were prepared from stock solutions by serial dilution in the medium to give a volume of 100 μl in each well of a microtiter plate (96-well). Then cells were cultured with vehicle, extracts, and their constituents for 48 h.

Cytotoxic activity assay

Cytotoxicity was determined using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, Sigma) assay reported by Tubaro *et al.* (1996) with some modification. The assay for each concentration of samples was performed in triplicates, and the culture plates were kept at 37 °C and 5% (v/v) CO_2 for 1 d. After incubation, 100 μl of medium were removed from each well. Subsequently, 100 μl of 0.5% w/v MTT (Sigma, Italy), dissolved in phosphate buffered saline, were added to each well and allowed to incubate for further 4 h. Then, 100 μl of DMSO were added to each well to dissolve the formazan crystals. Absorbances at 550 nm were measured with a microplate reader (GDV DV 990 B/V, Roma, Italy). Cytotoxicity was expressed as IC_{50} which is the concentration to reduce the absorbance of treated cells by 50% with reference to the control (untreated cells).

Statistical analysis

Data were expressed as means ± SD. Statistical analysis was performed by using Student's *t* test. Differences were considered significant at $P \leq 0.05$. The 50% inhibitory concentration (IC_{50}) was calculated from the Prism dose response curve (Prism Graphpad, Prism version 4.0 for Windows, GraphPad Software, San Diego, CA, USA) obtained by plotting the percentage of inhibition versus the concentrations.

Results and Discussion

Compounds isolated from I. pseudopumila MeOH extracts

The major constituents (Fig. 1) in the MeOH extracts of flowers and rhizomes of *I. pseudopumila* were isolated by RP-18 silica gel high

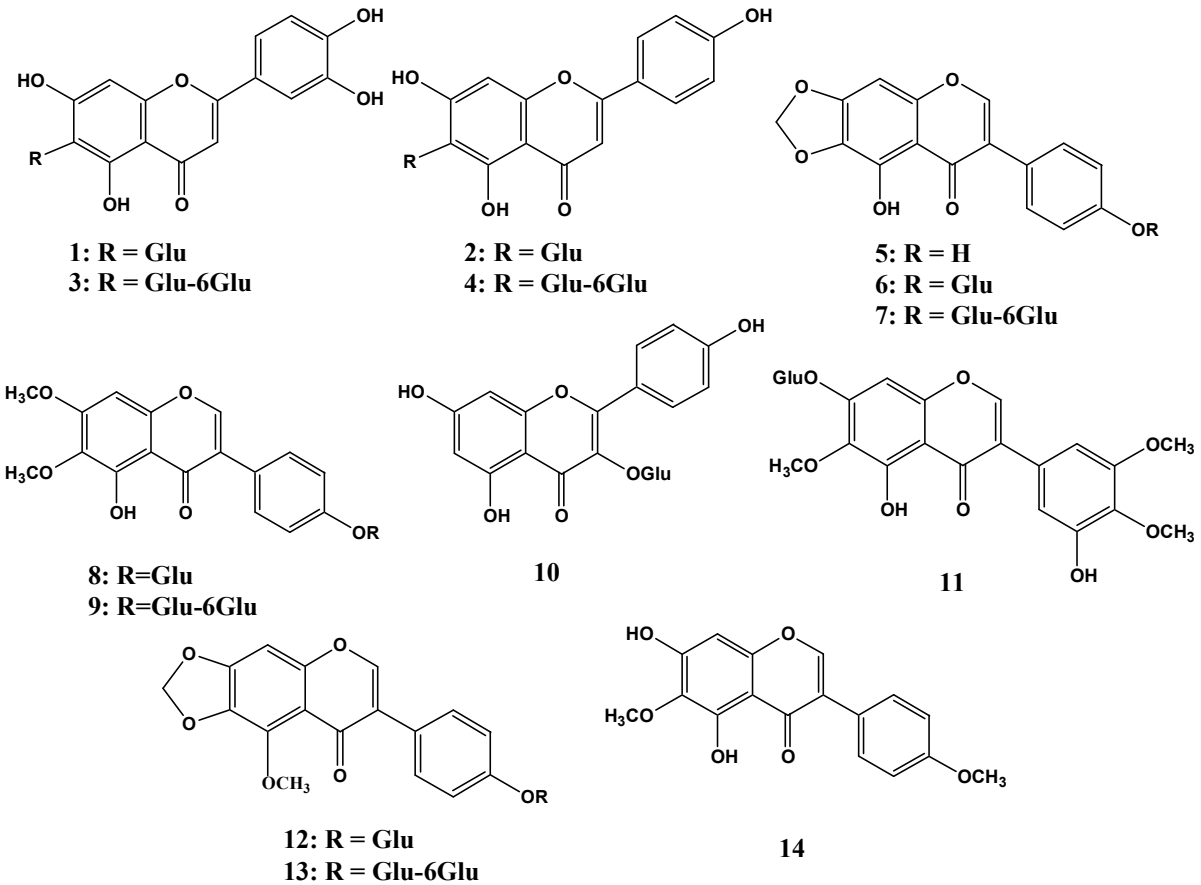

Fig. 1. Chemical structures of the tested compounds from *I. pseudopumila.*

performance liquid column chromatography using several eluations. Four compounds, isoorientin (**1**), isovitexin (**2**), isoorientin-6-*O*''-*β*-D-glucopyranoside (**3**), and isovitexin-6-*O*''-*β*-D-glucopyranoside (**4**), were isolated from the flowers (Rigano *et al.*, 2009) and ten compounds, irilone (**5**), irilone-4'-*O*-*β*-D-glucopyranoside (**6**), irilone-4'-*O*-[*β*-D-glucopyranosyl-(1→6)-*β*-D-glucopyranoside] (**7**), 7-methyl-tectorigenin-4'-*O*-*β*-D-glucopyranoside (**8**), 7-methyl-tectorigenin-4'-*O*-[*β*-D-glucopyranosyl-(1→6)-*β*-D-glucopyranoside] (**9**), kaempferol-3-*O*-*β*-D-glucopyranoside (**10**), irigenin-7-*O*-*β*-D-glucopyranoside (**11**), irisolone-4'-*O*-*β*-D-glucopyranoside (**12**), irisolone-4'-*O*-[*β*-D-glucopyranosyl-(1→6)-*β*-D-glucopyranoside] (**13**), and irisolidone (**14**), were isolated from the rhizomes (Rigano *et al.*, 2007).

Antiproliferative activity of I. pseudopumila extracts and their constituents on CORL-23 and C32 cells

The *Iris pseudopumila* flowers and rhizomes extracts and their constituents were evaluated for their *in vitro* antiproliferative properties against two human cancer cell lines: large lung carcinoma CORL-23 and amelanotic melanoma C32. The two human tumour cell lines were capable of attachment to form a homogeneous monolayer on the plastic substratum of the culture wells, what is ideal for the MTT assay. The MTT test is a simple bioassay used for primary screening of crude plant extracts and isolated compounds. For each cell line, there was a linear relationship between cell number and absorbance, measured at 550 nm in both control and drug-treated wells. After 48 h

Table I. Antiproliferative activity of methanolic extracts and compounds isolated from *I. pseudopumila* flowers and rhizomes against human tumour cell lines.

Extract (100 μg/ml) or compound (100 μM)	% Inhibition	
	CORL-23[a]	C32[a]
MeOH extract of rhizomes	31.5 ± 2.6*	48.7 ± 2.6*
MeOH extract of flowers	25.4 ± 2.6*	50.9 ± 2.6*
1	35.3 ± 0.63**	69.6 ± 0.87**
2	34.4 ± 0.72**	83.2 ± 0.72**
3	27.2 ± 0.56**	79.8 ± 0.63**
4	49.8 ± 0.71**	78.3 ± 0.71**
5	39.4 ± 0.83**	69.1 ± 0.83**
6	40.0 ± 0.85**	78.6 ± 0.85**
7	38.6 ± 0.89**	73.7 ± 0.89**
8	41.5 ± 0.89**	78.2 ± 0.65**
9	31.3 ± 0.68**	77.6 ± 0.68**
10	82.1 ± 0.21**	100 ± 0.21**
11	60.1 ± 0.43**	89.3 ± 0.43**
12	52.9 ± 0.43**	86.5 ± 0.64**
13	59.0 ± 0.61**	86.1 ± 0.61**
14	84.6 ± 0.23**	96.6 ± 0.23**

Exposure time was 48 h. Data are expressed as means ± SD (n = 3). Vinblastine (2 μg/ml) was used as positive control.
[a] CORL-23, large lung carcinoma; C32, amelanotic melanoma.
Multicomparison Dunnett's test: ** $p < 0.01$; * $p < 0.05$.

of treatment, the antiproliferative activity was determined. The results on the growth of the human tumour cell lines are given in Table I. The most antiproliferative extract was the MeOH extract from flowers with a percentage of inhibition of 50.9 at 100 μg/ml against amelanotic melanoma cells. The MeOH extracts from rhizomes showed also good activity against amelanotic melanoma cells with a percentage of inhibition of 48.7 at 100 μg/ml.

All compounds showed a significant antiproliferative activity against amelanotic melanoma cells with growth inhibition higher than 50%. The most antiproliferative compounds against amelanotic melanoma cells were kaempferol-3-*O*-*β*-D-glucopyranoside (**10**) and irisolidone (**14**) with a percentage of inhibition of 100 and 96.6, respectively, and against large lung carcinoma cells with a percentage of inhibition of 82.1 and 84.6, respectively. Significant activity against the amelanotic melanoma cell line was also showed by irigenin-7-*O*-*β*-D-glucopyranoside (**11**), with a percentage of inhibition of 89.3.

The compounds isovitexin (**2**) and isoorientin-6-*O*''-*β*-D-glucopyranoside (**3**) showed a selective activity against amelanotic melanoma cells with a percentage of inhibition of 83.2 and 79.8, respectively, while showed weak activity against large lung carcinoma cells with a percentage of inhibition of 34.4 and 27.2, respectively.

8 and **14** are both isoflavonoids that differ from each other only through the presence of an extra methoxy group and a glucose unit in **8**. However, the activity of **14** is around 100% higher than that of **8** against large lung carcinoma cells. Therefore, the 7-methoxy group at the A ring and glycosylation in position 4' of ring B is important to reduce the antiproliferative activity in this series. **2** and **1** are both flavones that differ from each other only through the presence of an extra hydroxy group in **1**. However, the activity of **2** is higher than that of **1** against amelanotic melanoma cells. Therefore, the 3'-hydroxy group at ring B is important to reduce the antiproliferative activity in this series. **10**, which is a flavanol, showed the highest activity among these compounds.

Some isoflavones isolated from *I. germanica* were previously shown to have cancer chemopreventive activity against mouse Hepa cells (Wollenweber *et al.*, 2003), while kaempferol-3-

O-*β*-D-glucopyranoside suppressed the growth of leukaemia cells (Lee *et al.*, 1981).

Cell type cytotoxic specificity is observed in some plant extracts. This specificity of plant extracts is likely due to the presence of different classes of compounds in the extract, as it has been documented in the case of known classes of compounds (Cragg *et al.*, 1994). Previous pharmacological studies showed that flavonoids are generally responsible for the pharmacological activity of *Iris* species (Fang *et al.*, 2008).

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