Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2017; 6(6): 1034-1037
Received: 08-09-2017
Accepted: 12-10-2017

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Antiproliferative activity of methanolic extracts of different *Iris* plant species against A549 and caco-2 cell lines

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Abstract
From long, plants were known to possess anticancer activities against different cancer cell lines. Our aim was to use the crude methanolic extracts of *Iris* plant species selected as per their ethno-pharmacological use and contain multiple molecules with anticancer potential that could be very effective in limiting the growth of cancer cell lines. The rhizome of plant material was collected from different ecogeographical regions of Kashmir valley, shade dried and extracted with methanol using cold extraction procedure. Anticancer activities were assayed with standard MTT colorimetric procedure against A549 and Caco-2 cell lines. The effect on cell proliferation was evaluated on successive population doubling times viz. 24hr and 48 hrs time points. The extracts showed a dose dependent inhibitory effect on proliferation of both A549 and Caco-2 cell lines. The effect against A549 cells for *Iris spuria* and *Iris kashmiriana* were lowest followed by *Iris germanica* and highest was observed in case of *Iris* ensata and *Iris* crocea respectively. However, this inhibitory effect became less pronounced against Caco2 cell lines treated with respective extracts, Our results revealed that metanolic extracts of different *Iris* plant species are promising anticancer reagents.

Keywords: *Iris* plant, methanolic extracts, MTT, A549, caco-2

Introduction
Iris plant, a member of the family Iridaceae is represented by several wild, cultivated and some endemic species. The genus *Iris* L. contains about 260 species which are distributed in temperate regions across the Northern Hemisphere, occurring mostly in Eurasia and North America. Some *Iris* species are found in wetland environments, however, most species occur in desert, semi-desert or dry, rocky habitats [1]. *Iris* species have been extensively used in traditional medicine for treatment of cancer, inflammation, bacterial and viral infections while its various constituents have been identified to possess different activities such as antiulcer, antibacterial, anti-inflammatory, piscicidal, antineoplastic, antioxidant, hypolipidemic, and antitubercolosis [2-9]. It is anticipated that plants can act as potential source of bioactive compounds that can be used in near future for the development of new 'leads' to combat against dread full diseases such as cancer [10, 11] and these fundamental advances continue to provide insights with potential applications in novel treatments.
Iris plants have immense medicinal importance and tremendous pharmacological potential and biological roles have been reported. Different *Iris* plant species are used in cancer, inflammation, bacterial and viral infections [12]. Many members of the genus *Iris* have also been used as traditional folk medicine for the treatment of various diseases. Its rhizomes are commonly used in Chinese folk medicine for clearing heat and detoxifying, eliminating phlegm, swelling, and pain in the throat [13]. The peeled and dried rhizomes of *Iris* collectively known as rhizome iridis enjoyed popularity due to their emetic, cathartic, stimulant, expectorant and errhine properties [14]. Rhizomes of this plant are used for fever and roots are used for kidney infections [15].
Globally the burden of new cancer cases in 2000 was estimated to be around 10 million with more than half of these cases originating from the developing world population. Although estimates vary it is estimated that by the year 2020 there will be almost 20 million new cases. Worryingly, it is not only in the number of new cases that will increase, the proportion of new cases from the developing world will also rise to around 70%. The magnitude of the problem of cancer in the Indian Sub-Continent in terms of sheer numbers is the most alarming. From the population census data for India in 1991, 609,000 new cancer cases were estimated to have been diagnosed in the country. This figure had increased to 806,000 by the turn of the century.

The estimated age standardized rates per 100,000 were 96.4 for males and 88.2 for females. The most common cancers found in males were cancers of the lung, pharynx, esophagus, tongue and stomach while among females cancers of the cervix, breast, ovary, esophagus and mouth were common [16]. The figures given here are only estimates since 70% of the Indian population resides in rural areas and accurate and widespread epidemiology data is not available. Cancer develops when one or more cells lose their ability to control cell division and begin to proliferate in an uncontrolled fashion. The origin of cancer lies in the genetic material of the cell and is a result of the accumulation of mutations that promote clonal selection of cells with an aggressive phenotype. This phenotype is underlined by a rapid proliferation rate and alterations in cell morphology. All oncogenic proteins participate in cellular functions that involve the transduction of signals from the extracellular environment through the membrane receptors into the cytoplasm and towards the nucleus, where transcription is initiated to generate proteins that will eventually contribute to the oncogenic phenotype [17]. Similar to genetic causes, external stimuli is equally important for the initiation and progression of cancers. If environmental factors such as radiation and exposure to carcinogens are excluded, a potential candidate for cancer causation is chronic inflammation [18]. Chronic inflammation accounts for 20% of all types of human cancers [19]. Population studies have demonstrated the increase susceptibility of cells to become cancerous when exposed to chronic inflammation.

Taking leads from the work that has already been done in relation to the medicinal properties of plant, anti-cancer in particular; the present study is thus aimed to investigate the Antiproliferative potential of methanolic extracts from selected indigenous species of *Iris* plant from Kashmir valley against A549 and Caco-2 Cell lines.

Material and Methods

Plant material

The plant material of *Iris* plant species were collected from different ecogeographical regions of Kashmir valley covering high altitude regions like Gulmarg, Sonamarg, Naranag (Table 1) etc and collected throughout the year. The collected plant material was properly identified and the specimen vouchers were deposited in the Indian Institute of Integrative Medicine, Sanat Nagar, Srinagar.

Table 1: Collection of samples of *Iris* plant species

Code	Samples	Sources	Collection date
IC-1	*Iris* croceae	Sonamarg, Kashmir, India	May, 2010
IC-2	*Iris* croceae	IIIM field, Srinagar, Kashmir, India	April, 2010
IC-3	*Iris* croceae	Gulmarg, Kashmir, India	June, 2010
IE-1	*Iris* ensata	Naranag, Kashmir, India	May, 2010
IE-2	*Iris* ensata	IIIM field, Srinagar, Kashmir, India	April, 2010
IG-1	*Iris* germanica	IIIM field, Srinagar, Kashmir, India	April, 2010
IG-2	*Iris* germanica	Gulmarg, Kashmir, India	June, 2010
IG-3	*Iris* germanica	Sonamarg, Kashmir, India	May, 2010
IK-1	*Iris* kashmiriana	Local graveyard, Srinagar, Kashmir India	April, 2010
IK-2	*Iris* kashmiriana	Naranag, Kashmir, India	May, 2010
IS-1	*Iris* spuria	Gulmarg, Kashmir, India	June, 2010
IS-2	*Iris* spuria	Sonamarg, Kashmir, India	May, 2010

Cell lines

Cell lines (A549, Caco2) were kindly provided by Hybridoma Lab., National Institute of Immunology, New Delhi. Cells were maintained in Dulbecco's minimal essential medium (DMEM) containing 10% heat inactivated fetal calf serum and supplemented with antibiotics penicillin-streptomycin (100U/ml) at 37°C in 5% CO2. The medium was changed every 2 days or until the cells became confluent and then the cells were used for the experimentation.

Buffers and Reagents

Cell culture materials like DMEM, FBS, Antibiotics (Gentamycin) were obtained from PAA, Germany. All other reagents were of molecular biology/ cell culture grade.

Extract preparations from plant rhizome

The shade dried underground part (rhizome) of different *Iris* plant species viz, *Iris kashmiriana, Iris croceae, Iris spuria, Iris ensata, Iris germanica* (2 Kg) was finely ground and soaked in methanol (10L x 4) at room temperature for 30 hrs. The resulting extract was concentrated to a gum. The extract percentage were calculated and recorded. The extracts were stored at 4deg until use.

Anti-proliferation assay using MTT method

This is a colorimetric assay that measures the reduction of yellow 3-(4, 5-dimethythiazol- 2-yl)-2,5-diphenyl tetrazolium bromide (MTT) by mitochondrial succinate dehydrogenase. The MTT enters the cells and passes into the mitochondria where it is reduced to an insoluble, coloured (dark purple) formazan product. The cells are then solubilised with an organic solvent (e.g. isopropanol) and the released solubilized formazan is measured spectro-photometrically. Since reduction of MTT can only occur in metabolically active cells the level of activity is a measure of the viability of the cells. A549 & Caco-2 cells were seeded in 24-well plates (1 × 104 cells/well). After 24 hrs, various concentrations of crude extracts were added to wells in fresh media. The cells were allowed to grow for 24hrs and 48 hrs after drug treatment. All experiments were performed at least in triplicate.

The Cell growth was evaluated by MTT assay by adding 80µl of MTT solution followed by incubation for 4h in 37^0C in dark. Media was then removed from wells and added with 200µl acidic isopropanol and mixed well. The plate was placed in dark on shaking rotor. 50µl of solution was harvested from 24-well plate and transferred to 96 well plate and read at 570nm (background wavelength is 630nm).

Results

Effect on Cell proliferation

The effect of crude extracts from *Iris* plant was evaluated using cell proliferation assays on model cancer cell lines viz. A549 which is a human type-II pulmonary epithelial cell line; and Caco-2 cell line, derived from human epithelial colorectal adenocarcinoma tissue.

Effect of crude extract of *Iris* plant rhizomes on the proliferation of A549 and Caco2 cell lines.

Methanolic crude extracts of *Iris* plant rhizomes from five species viz *Iris* ensata, *Iris spuria*, *Iris* croceae, *Iris germanica and Iris kashmiriana* were tested for their ability to modulate growth of A549 and Caco2 cell lines using MTT cell proliferation assay. The effect on cell proliferation was evaluated on successive population doubling times viz. 24hr and 48 hrs time points. The extracts showed a dose dependent inhibitory effect on proliferation of both A549 and Caco-2 cell lines. The IC50 values against A549 cells for *Iris spuria* (123.04 µg/ml) and *Iris kashmiriana* (128.7 µg/ml) were lowest followed by *Iris germanica* (134.72 µg/ml), *Iris* ensata

(137.98 µg/ml) and *Iris* crocea (149.80 µg/ml) respectively. However, this inhibitory effect became less pronounced against Caco2 cell lines (Table 2 - 6) treated with respective extracts viz. *Iris germanica* (230.82 µg/ml), *Iris kashmiriana* (237.76µg/ml), *Iris* spuria (302.94 µg/ml), *Iris* ensata (358.81 µg/ml), *Iris* crocea (368.88 µg/ml)

Table 2: Percentage inhibition of A549 and Caco-2 cells treated with methanolic extract of *Iris ensata* at various doses w.r.t control group.

DOSE (per ml)	A549						Caco-2					
	24 hour			48 hour			24 hour			48 hour		
Extract 50µg	32.02	±	0.93	59.70	±	0.53	13.07	±	1.77	17.06	±	1.22
Extract 100µg	45.87	±	0.98	60.24	±	0.98	27.05	±	1.78	32.08	±	1.13
Extract 200µg	56.50	±	0.98	62.96	±	1.17	43.09	±	1.07	48.07	±	1.88
Extract 400µg	65.09	±	1.19	76.95	±	1.35	49.03	±	1.98	56.09	±	1.28

N=3. Data is presented as Mean± SEM percentage inhibition w.r.t control group (DMSO treated). Data is analyzed using one-way ANOVA with multiple comparisons using Dunnetts test vs. control group. Values were considered as significant for $p < 0.05$. (Cell conc.: $2x10^3$ seeded in 96well plate, PDT: 24hrs)

Table 3: Percentage inhibition of A549 and Caco-2 cells treated with methanolic extract of *Iris spuria* at various doses w.r.t control group.

DOSE (per ml)	A549						Caco-2					
	24 hour			48 hour			24 hour			48 hour		
Extract 50µg	27.05	±	1.35	50.99	±	1.93	7.067	±	1.23	14.06	±	1.33
Extract 100µg	37.09	±	1.03	51.34	±	1.98	17.05	±	1.13	22.08	±	1.43
Extract 200µg	47.02	±	2.17	61.42	±	0.98	33.09	±	1.89	42.07	±	1.54
Extract 400µg	58.05	±	0.98	76.33	±	1.19	45.03	±	1.29	49.09	±	1.37

N=3. Data is presented as Mean± SEM percentage inhibition w.r.t control group (DMSO treated). Data is analyzed using one-way ANOVA with multiple comparisons using Dunnetts test vs. control group. Values were considered as significant for $p < 0.05$. (Cell conc: $2x10^3$ seeded in 96well plate, PDT: 24hrs)

Table 4: Percentage inhibition of A549 and Caco-2 cells treated with methanolic extract of *Iris croceae* at various doses w.r.t control group.

DOSE (per ml)	A549						Caco-2					
	24 hour			48 hour			24 hour			48 hour		
Extract 50µg	44.70	±	1.93	51.00	±	1.28	7.067	±	1.57	14.34	±	1.22
Extract 100µg	47.65	±	1.87	51.34	±	0.93	12.05	±	1.68	27.03	±	1.13
Extract 200µg	52.08	±	1.18	69.94	±	0.98	23.09	±	1.24	36.03	±	1.88
Extract 400µg	63.02	±	1.55	77.88	±	0.98	32.03	±	1.98	49.39	±	1.28

N=3. Data is presented as Mean± SEM percentage inhibition w.r.t control group (DMSO treated). Data is analyzed using one-way ANOVA with multiple comparisons using Dunnetts test vs. control group. Values were considered as significant for $p < 0.05$. (Cell conc: $2x10^3$ seeded in 96well plate, PDT: 24hrs)

Table 5: Percentage inhibition of A549 and Caco-2 cells treated with methanolic extract of *Iris germanica* at various doses w.r.t control group.

DOSE (per ml)	A549						Caco-2					
	24 hour			48 hour			24 hour			48 hour		
Extract 50µg	37.10	±	1.94	51.00	±	1.93	17.07	±	1.34	24.05	±	2.23
Extract 100µg	47.02	±	1.87	51.34	±	1.87	32.05	±	2.22	37.07	±	1.99
Extract 200µg	53.08	±	1.19	61.42	±	1.18	43.09	±	1.86	56.09	±	1.96
Extract 400µg	63.09	±	1.56	76.34	±	1.28	52.03	±	1.22	69.09	±	2.85

N=3. Data is presented as Mean± SEM percentage inhibition w.r.t control group (DMSO treated). Data is analyzed using one-way ANOVA with multiple comparisons using Dunnetts test vs. control group. Values were considered as significant for $p < 0.05$. (Cell conc: $2x10^3$ seeded in 96well plate, PDT: 24hrs)

Table 6: Percentage inhibition of A549 and Caco-2 cells treated with methanolic extract of *Iris kashmiriana* at various doses w.r.t control group.

DOSE (per ml)	A549						Caco-2					
	24 hour			48 hour			24 hour			48 hour		
Extract 50µg	27.84	±	1.99	34.56	±	1.82	13.99	±	2.13	27.89	±	2.03
Extract 100µg	47.14	±	1.29	49.08	±	1.77	23.22	±	2.56	36.07	±	1.83
Extract 200µg	50.97	±	1.73	59.71	±	1.62	44.83	±	1.81	53.17	±	1.97
Extract 400µg	57.85	±	1.94	80.16	±	1.61	48.20	±	1.67	56.56	±	1.29

N=3. Data is presented as Mean± SEM percentage inhibition w.r.t control group (DMSO treated). Data is analyzed using one-way ANOVA with multiple comparisons using Dunnetts test vs. control group. Values were considered as significant for $p < 0.05$. (Cell conc: $2x10^3$ seeded in 96well plate, PDT: 24hrs)

Discussion

There are several plant extracts or "phytomedicines" in clinical trials for the treatment of various diseases including cancers. With the availability of new hyphenated analytical methods drug discovery from plants has been accelerated to a large extent paving way to establish new approaches for future medicines. Iris species are considered as one of the most important medicinal plants owing to their huge reserves of secondary metabolites viz. flavanoids, isoflavanoids, quinones, triterpenoids, flavones and xanthones. The most essential class among these secondary metabolites are those of flavanoids and isoflavanoids-the entities known to have varied medicinal properties ranging from anti-bacterial or anti-inflammatory to anti-cancer activities. This differential

activity of the molecules points towards their differential targets that influence cellular signaling and hence cancer cell survival. Cancer cell survival not only depends on the tumor cell phenotype but is majorly defined by communication between the tumor cells and the surrounding cells—the microenvironment. The tumor microenvironment consists of tumor cells, fibroblasts, leukocytes, bone marrow-derived cells, blood and lymphatic vascular endothelial cells. The interplay between these cell types determines the fate of tumor cell survival and progression. Recent evidence indicates that the microenvironment provides essential cues to the maintenance of cancer initiating cells and to promote the seeding of cancer cells at metastatic sites. Furthermore, inflammatory cells and immunomodulatory mediators present in the tumor microenvironment polarize host immune response toward specific phenotypes impacting tumor progression. A growing number of studies demonstrate a positive correlation between angiogenesis, carcinoma-associated fibroblasts, and inflammatory infiltrating cells and poor outcome, thereby emphasizing the clinical relevance of the tumor microenvironment to aggressive tumor progression. Thus, the dynamic and reciprocal interactions between tumor cells and cells of the tumor microenvironment plan events critical to tumor evolution toward metastasis, and many cellular and molecular elements of the microenvironment are emerging as attractive targets for therapeutic strategies [20]. Aggressive inflammation is associated with the process of tumorigenesis whereas number of reports suggests that progression of tumor is mainly due to immune-suppressive microenvironment around the tumor cells, indicating that immune-editing might be one of the ways to restrict cancer growth. Further research is required for precise evaluation of specific targets for tested extracts.

Acknowledgement
This study was supported jointly by Department of Biotechnology, University of Kashmir, Srinagar and Indian Institute of Integrative Medicine (CSIR), Srinagar. We are also thankful to Dr. Ayub Qadri from National Institute of Immunology, New Delhi for providing the Caco-2 cell line.

References

1. Wilsom CA. Subgeneric classifi cation in Iris re-examined using chloroplast sequence data. Taxon. 2001; 60:27-35.
2. Rahman AU, Nasim S, Biag I, Jalil S, Orhan I, Sener B, *et al*. Anti-inflammatory isoflavonoids from the rhizomes of Iris germanica. J Ethnopharmacol. 2003; 86:177-180.
3. Benoit-Vical F, Imbert C, Bonfils JP, Sauvaire Y. Anti-plasmodial and antifungal activities of iridal, a plant triterpenoid. Phytochemistry. 2003; 62:747-751.
4. Bonfils JP, Pinguet F, Culine S, Sauvaire Y. Cytotoxicity of iridals, triterpenoids from *Iris*, on human tumor cell lines A2780 and K562. Planta Med. 2001; 67:79-81.
5. Choudhary MI, Naheed S, Jalil S, Alam JM, Rahman AU. Effect of ethanolic extract of Iris germanica on lipid profile of rats fed on a high-fat diet. J Ethnopharmacol 2005; 98:217-220.
6. Han J. Traditional Chinese medicine and the search for new antineoplastic drugs. J Ethnopharmacol. 1988; 24:1-17.
7. Hideyuki I, Yoko M, Takashi Y. New piscicidal triterpenes from Iris germanica. Chem. Pharm. Bull. 1995; 43:1260-1262.
8. Orhan I, Nasim S, Sener B, Ayanoglu F, Ozguven M, Choudhary M. Two isoflavones and bioactivity spectrum of the crude extracts of Iris germanica rhizomes. Phytother. Res. 2003; 17:575-577.
9. Wollenweber E, Stevens JF, Klimo K, Knauft J, Frank N, Gerhäuser C. Cancer chemopreventive in vitro activities of isofl avones isolated from Iris germanica. Planta Med. 2003; 69:15-20.
10. Orhan I, Nasim S, Sener B, Ayanoglu F, Ozguven M, Choudhary M. Two isoflavones and bioactivity specturm of the crude extracts of Iris germanica rhizomes. Phytotherapy Research. 2003; 17:575-577.
11. Rigano D, Formisano C, Grassia A, Grassia G, Perrone A, Piacente S. Antioxidant flayonoids and isoflavonoids from rhizomes of Iris pseudopumila. Planta Medica. 2007; 73:93-96.
12. Nadkarni KM. In Indian Materia Medica; Popular Prakashan: Bombay. 1976; 1:541.
13. Zhao GP, Dai S, Chen RS, Zhongyao Da Cidian. (The Dictionary of Chinese Crude Drugs), Edited by Nanjing University of Chinese Medicine (Shanghai Science and Technology Press, 2006.
14. Steinegger E, Hansel R. Lehrbuch der pharmakognosie and phytopharmazie, 4. Ed Springer Veralag, 1988.
15. Bhattacharjee SK. Handbook of Medicinal Plants. Pointer Publisher, Jaipur, India. 1998, 192-193.
16. Rao DN, Ganesh B. Estimate of cancer incidence in India in 1991. Indian J Cancer. 1998; 35:10-18.
17. Hanahan D, Weinberg RA. The hallmarks of cancer. Cell. 2000; 100:57-70.
18. Balkwill F, Coussens LM. An Inflammatory Link. Nature. 2004; 23:405.
19. Coussens LM, Werb. Inflammation and cancer. Nature 2002; 420:860-867.
20. Girieca, Lorusso, Curzio, Ruegg. The tumor microenvironment and its contribution to tumor evolution toward metastasis. Histochem Cell Biol. 2008; 130:1091-1103.