

In vitro cytotoxic properties of *Ipomoea aquatica* leaf

Efforts are being made to develop anticancer agents from natural sources by many workers. In the past, plants have provided anticancer compounds like vincristine and taxol. Several members of the species *Ipomoea* (*Convolvulaceae*) are being used traditionally for the treatment of a large number of disease conditions. Among them *Ipomoea bahiensis* is reported to possess anticancer activity.^[1] *Ipomoea aquatica* leaf is being used as a green leafy vegetable and also in traditional medicine for nose bleed and high blood pressure with high antioxidant properties.^[2] No investigation has been carried out for its anticancer property till now. In view of these reports and ethnomedical uses of *Ipomoea aquatica* leaf, we studied the *in vitro* cytotoxic properties of its crude methanolic extract (CME), its column fraction (CF) and purified bioactive compound i.e., 7-O-β-D-glucopyranosyl-dihydroquercetin-3-O-α-D-glucopyranoside (DHQG) isolated from it.

The leaves of *Ipomoea aquatica* were collected from Mysore, Karnataka, India, authenticated by a taxonomist and a specimen preserved (specimen no: 9/1) at the Department of Botany, University of Mysore, Mysore. The leaves (628 g) were oven dried at 48°C for 36 h to prevent enzyme action,^[3] powdered and extracted with methanol (2.5 l) in a Soxhlet extractor for 8 h. The extract was concentrated under reduced pressure and controlled temperature to yield CME (146 g, 23.3%). This extract (60 g) was purified partially by using silica column chromatography to obtain the CF. This fraction was in turn purified by using XAD-4 resin column chromatography to obtain a pure compound (1.7 g). This 100% pure compound based on UV, NMR and GC-MS studies was identified as DHQG.^[2] Each test sample (CME, CF and DHQG) were separately dissolved in 1% dimethyl sulphoxide^[4] (DMSO) and the volume was made up to 10 ml with Dulbecco's modified Eagles medium (DMEM), pH 7.4 supplemented with 2% heat inactivated newborn calf serum (Maintenance medium, PAA Laboratories, Austria), to obtain a stock solution of 1 mg/ml concentration, sterilized by filtration and stored at -20°C till use.

The CME, CF and DHQG were investigated for cytotoxic properties against normal and cancer cell lines. Vero (normal African green monkey kidney) and Hep-2 (human larynx epithelial carcinoma) cell lines were obtained from Pasteur Institute of India, Coonor. A-549 (human small cell lung carcinoma) was obtained from National Center for Cell Sciences, Pune. The cultures were maintained in DMEM, pH 7.4 supplemented with 10% heat inactivated newborn calf serum,^[5] penicillin (100 IU/ml), streptomycin (100 µg/ml) and amphoterecin-B (5 µg/ml) and were grown in 25 cm² tissue culture flasks (Tarsons Products [P] Ltd., Kolkata, India) until confluent and used for cytotoxicity assays.

Cell lines in exponential phase were washed with serum free DMEM medium, trypsinised using 0.2% trypsin and re-

suspended in DMEM medium with 10% heat inactivated newborn calf serum.^[5] Cells were plated at 10,000 cells/well in 96 well microtiter plates (Tarsons Products [P] Ltd., Kolkata, India) and incubated for 24 h at 37°C, 5% CO₂ in a humidified atmosphere, during which period a partial monolayer was formed. The cells were then exposed to different concentrations (15.6, 31.2, 62.5, 125, 250, 500 and 1000 mg/ml, prepared by serial two-fold dilutions using maintenance medium from the stock solution) of test sample in quadruplicate. Control wells received only maintenance media. The cells were incubated at 37°C in a humidified incubator with 5% CO₂ for a period of 72 h according to method of Brown^[4] *et al.*, (2004). Morphological changes were examined using an inverted tissue phase-contrast microscope (Olympus Optical Co., Ltd., Japan, Model 1X70) at 24 h time intervals and compared with the control. At the end of 72 h, cellular viability was determined using standard 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT)^[6] and Sulphorhodamine B (SRB)^[7] assays and the CTC₅₀ value (concentration of the sample required to kill 50% of the cells) was calculated.

DHQG showed cytotoxicity towards cell cultures with CTC₅₀ values of 387 mg/ml against normal Vero cell line, whereas 156 and 394 mg/ml, against Hep-2 and A-549 cell lines respectively. The CME and CF gave CTC₅₀ values ranging from 41-332 mg/ml in Vero, 46 - 114 mg/ml in Hep-2 and 44 - 230 mg/ml in A-549 cell lines. [Table 1]

The CME was more potent than that of DHQG probably due to synergistic effects resulting from the combination of anthocyanins and other phenolic compounds.^[8] Presence of

Table 1

Cytotoxic activity of *Ipomoea aquatica* on different cell lines by MTT and SRB assays

Test samples	Cell line	*CTC ₅₀ (mg/ml) ^a		
		MTT	SRB	Mean
CME	Vero	44.3±4	37±1.4	41±2.7
	Hep-2	43±4.3	49±2.8	46±3.5
	A-549	47±5	41.3±0	44±2.5
CF	Vero	326.3±9.1	338±12.7	332±10.9
	Hep-2	116.6±4.9	112.3±4.9	114±4.9
	A-549	232.6±7	228±7	230±7
DHQG	Vero	390.6±5	385±2.8	387±3.9
	Hep-2	156.6±1.6	155.3±0	156±0.8
	A-549	393.6±7.7	395.3±3.5	394±5.6

^a Average of three independent determinations, 4 replicates, values are mean±SEM, *CTC₅₀= Concentration of the sample required to kill 50% of the cells. CME = Crude methanolic extract, CF = Column fraction, DHQG = 7-O-β-D-glucopyranosyl-dihydroquercetin-3-O-α-D-glucopyranoside. MTT assay = 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide, SRB assay = Sulphorhodamine B.

sugar moiety in DHQG may be attributed to its low activity, probably due to steric hindrance by addition of sugar moieties.^{19]} The results of CTC₅₀ values thus obtained indicate that DHQG showed cytotoxicity towards cancer cell lines tested. Hence further investigations are required to prove the toxicity of DHQG *in vivo*.

K.N. Prasad^a, G. Ashok^b, C. Raghu^b, G.R. Shivamurthy^a, P. Vijayan^b, S.M. Aradhya^c

^aDepartment of Studies in Botany,
Manasagangotri, Mysore-6,

^bJSS College of Pharmacy, Rocklands,
Ootacamund-643001 and

^cDepartment of Fruit and Vegetable Technology,
CFRTI, Mysore-570020.

E-mail: aradhysm@yahoo.co.in

References

1. Bieber LW, Filho AADS, Rosalia MO, Lima C, Chiappeta AD, Nascimento SC, *et al.* Anticancer and antimicrobial glycosides from *Ipomoea bahiensis*. *Phytochemistry* 1986;25:1077-81.
2. Prasad KN, Divakar S, Shivamurthy GR, Aradhya SM. Isolation of a free radical-scavenging antioxidant from water spinach (*Ipomoea aquatica* Forsk.). *J Sci Food Agric*. In press 2005.
3. Markham KR. *Techniques of flavonoid identification*. London: Academic Press; 1982.
4. Brown R, Boger-Brown U. *Methods in Molecular Medicine*. Vol 28: Cytotoxic Drug Resistance Mechanism. Totowa, NJ: Humana Press; 1999.
5. Freshney RI, *Culture of animal cells-A manual of basic technique*. New York: John Wiley & Sons, Inc Publications; 2000.
6. Francis D, Rita L. Rapid colorimetric assay for cell growth and survival modifications to the tetrazolium dye procedure giving improved sensitivity and reliability. *J Immunol Methods* 1986;89:271-7.
7. Skehan P, Storeng R, Scudiero D, Monks A, McMahon J, Vistica D, *et al.* New colorimetric cytotoxic assay for anti-cancer drug screening. *J Natl Cancer Inst* 1990;82:1107-12.
8. Seeram NP, Adams LS, Hardy ML, Heber D. Total cranberry extract versus its phytochemical constituents: Antiproliferative and synergistic effects against human tumor cell lines. *J Agric Food Chem* 2004;52:2512-7.
9. Koide T, Hashimoto Y, Kamei H, Kojima T, Hasgawa M, Terabe K. Antitumor effect of anthocyanin fractions extracted from red soybeans and red beans *in vitro* and *in vivo*. *Cancer Biother Radiopharm* 1997;12:277-80.

XXXVIII Annual Conference of the Indian Pharmacological Society



December 28 - 30, 2005
IMAGE Auditorium, Chennai

For further information please contact:

Dr. C. B. Tharani
Institute of Pharmacology,
Madras Medical College, Chennai.
E-mail: ips_38con@yahoo.co.in
Website: ips38chennai.com