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In vitro cytotoxic effect of chloroform soluble and n-Butanol soluble fractions of *Apium graveolens* on human cancer cell lines

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ABSTRACT

Ethanolic extract of seeds of *Apium graveolens* showed a remarkable *in vitro* cytotoxicity against four human cancer cell lines of colon, CNS, oral and cervical origin. Further, the fractions of the said extract were obtained and *in vitro* cytotoxic assay was performed against human cancer cell lines of different origins. Results revealed that the most significant cytotoxic effect was observed in case of chloroform soluble and n-butanol soluble fractions as both inhibited the growth of eight human cancer cell lines of lung, liver, colon, CNS and ovarian origin.

Key words : *Apium graveolens, in vitro cytotoxicity,* Human cancer cell lines, Ethanolic extract, Fractions.

Apium graveolens is an erect, annual/biennial herb. It is native to Europe, now naturalized and occurring wild in the foot hills of North – Western Himalayas (Punjab) or hills of Uttranchal (Bhattacharjee, 2001).The seeds of the plant have stimulant, cordial, tonic and carminative properties. They are used as antiseptic and useful in the bronchitis, asthma, spleen and liver problems (Gupta and Tandon, 2004). The methanolic extract of seeds has been reported to possess diuretic (Sharma *et al*.,1978)and hepatoprotective action (Singh and Handa,1995;Ahmad *et al*., 2002).The essential oil from its seeds and fruits showed antifungal activity (Kher and Chaurasia,1997; Mishra *et al*.,1993).In addition, there is evidence of 100% mortality on nematodes from bioactive compounds of the seed part of this plant (Rafikali and Muraleedharan,2001; Rafikali *et al*.,2000).

Hence, we felt it was worthwhile to evaluate the anticancer potential of the seeds of *Apium graveolens* in a well established *in vitro* system.

MATERIALS AND METHODS

Plant material:

The seeds of *Apium graveolens* were collected from Indian Institute of Integrative Medicine (IIIM), Jammu (Tawi), J&K-India in the month of May and were authenticated at site by Dr. Yashpal Sharma, Associate Professor, Department of Botany, University of Jammu, Jammu, (J&K), India. The freshly collected seeds were then chopped, shade dried, crushed/ground into powder. Powdered dried seed material was extracted with different solvents at room temperature to obtain extracts for bioevaluation.

Preparation of plant extracts:

For the ethanolic extract, dried ground plant material (100g) was percolated with 95% ethanol, then concentrated to dryness under reduced pressure. For aqueous ethanolic extract, another lot of dried ground plant material (100g) was percolated with 50% ethanol and concentrated to dryness under reduced pressure. The water extract was obtained by boiling dried ground plant material (100g) for 30 min in distilled water (300ml). The ethanolic extract was dissolved in DMSO, the aqueous ethanolic extract in 50% DMSO and the water extract in sterile water to form stock solutions 20mg/ml. Stock solutions were prepared atleast one day in advance and were not filtered/sterilized, but microbial contamination was controlled by addition of 1% gentamycin in complete growth medium, used for dilution of stock solutions to prepare working test solutions 200µg/ml. All extracts being freeze dried.

Preparation of plant fractions from ethanolic extract:

The ethanolic extract was fractionated into four fractions. For the hexane soluble fraction, dried ethanolic extract (10g) was taken in a stoppered conical flask (250ml) and vigorously shaken with hexane (100ml). After standing for 30 min, supernatant hexane was decanted. The procedure was repeated three times and the combined hexane soluble portion was concentrated to dryness under reduced pressure. For the chloroform soluble fraction, the residue left after removing the hexane soluble part was further macerated with chloroform (100ml) four times. Combined chloroform soluble portion was then concentrated to dryness under reduced pressure. For the