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NOTE

Cytotoxicity Effect of Methanolic Extract of Aerial Parts of *Viburnum punctatum* Buch-Ham. Ex D. Don

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The methanolic extract of aerial parts of *Viburnum punctatum* Buch (AX-M)-Ham. Ex D. Don has been studied for cytotoxicity against human liver cancer cells (HepG2) and human laryngeal epithelial carcinoma (Hep2) and assessed by the tetrazolium (MTT) and sulphorhodamine B (SRB) methods. Methanolic extract of viburnum punctatum (AX-M) showed potent cytotoxicity against HepG2 and Hep2 cells.

Key Words: Viburunum punctatum, Cytotoxicity, Tetrazolium assay, Sulphorhodamine B assay.

Viburnum punctatum Buch-Ham. Ex D. Don is a medicinal plant belonging to the family Caprifoliaceae. It is a medium sized tree or shrub, growing at an altitude not less than 1500 m, with other plants in Himalaya, Nilagiri and Coimbatore. The leaves were traditionally used for the treatment of fever, stomach disorder and mentioned to possess antiperiodic effect[1]. The preliminary phytochemical investigations shows presences of flavanoids, alkaloids, glycosides, phenolic compounds, tannins, proteins, amino acids, phytosterols and saponins[2-13]. The present work reports the cytotoxicity of methanolic extract of aerial parts of *Viburnum punctatum* Buch-Ham. Ex D. Don against human liver cancer cells (HepG2) and human laryngeal epithelial carcinoma (Hep2) by tetrazolium (MTT) and sulphorhodamine B (SRB) methods.

Viburnum punctatum was collected from Nilagiri hills, Tamil Nadu, India and authentificated by Dr. Chellathurai, Ex. Prof., Botany, Government Sidda Medical College, Thirunelveli, Tamil Nadu, India. This dried plant is extracted with methanol by Soxhlet extraction method[14]. The methanolic extract is concentrated under vaccum and then dried extract is subjected for preliminary phytochemical analysis and cytotoxicity effect.

Preparation of the plant extract: The collected plant material was dried at room temperature, pulverized by a mechanical grinder, sieved through 40 mesh. The powdered material was extracted with methanol by using Soxhlet extraction apparatus. The methanolic extract are concentrated and dried under reduced pressure. The free semi solid mass obtained was used for the experiment. Finally methanol extracted marc was then extracted with water. It is macerated for 48 h by occasional shaking[15]. After extraction, the material were filtered and concentrated by vacuum. The dried extract was dissolved in dimethyl sulfoxide and used for cytotoxicity studies.

Preparation of suspensions: The methanolic extract of *Viburnum punctatum* was dissolved in DMSO and the volume was made up to 10 mL with DMEM/MEM to obtain a stock solution of 1 mg/mL concentration and stored at -20 ºC prior to use. Further dilutions were made to obtain different concentrations ranging from 10.0-62.5 µg/mL with respective media and used for *in vitro* investigations.

Cell lines and growth media: Hep2 (Human laryngeal epithelial carcinoma) cells and HepG2 (human liver cancer) cells was cultured in MEM (minimum essential medium) and DMEM (Dulbecco's modified eagles medium) medium, respectively. The medium also contains 10 % fetal calf serum, penicillin (100 U) and streptomycin (100 µg).

***In vitro* cytotoxicity screening:** The ability of the cells to survive a toxic insult has been the basis of most cytotoxicity assays[16]. The monolayer cell culture was trypsinized and the cell count was adjusted to 1.0×10^5 cells/mL using medium containing 10 % new born calf serum. To each well of the 96 well microtitre plate, 0.1 mL of the diluted cell suspension (*ca.* 10,000 cells) was added. After 24 h, when a partial mono-

layer was formed, the supernatant was flicked off, washed the monolayer once and 100 µL of different drug concentrations was added to the cells in microtitre plates[17]. The plates were then incubated at 37 ºC for 3 days in 5 % CO_2 atmosphere and microscopic examination was carried out and observations recorded every 24 h. After 72 h, the drand MTT and SRB assay was performed.

The methanolic extract of *Viburnum punctatum* exhibits significant cytotoxicity against human liver cancer cells (HepG2) with CTC_{50} (cytotoxicity 50 %) values of 205.8 ± 1.92 and 233 ± 3.73 by MTT and SRB methods, respectively and against human laryngeal epithelial carcinoma (Hep2) with CTC_{50} (cytotoxicity 50 %) values of 197.3 ± 2.89 and 208 ± 2.9673 by MTT and SRB methods, respectively. AX-M showed significant toxicity against two different human cancer cells from liver and laryngeal origin, respectively. Hence this extracts merits further investigations to screen its anticancer activity using *in vivo* animal models (Tables 1 and 2).

TABLE-1
CTC_{50} BY USING MTT AND SRB ASSAY IN HepG2 (HUMAN LIVER CANCER) CELL CULTURES

Extract	Concentration (µg/mL)	CTC_{50} (µg/mL)	
		MTT	SRB
AX-M	250 125 62.5	205.8 ± 1.92	233 ± 3.73

Average of six determinations, values are mean ± SEM

TABLE-2
CTC_{50} BY USING MTT AND SRB ASSAY IN Hep2 (HUMAN LARYNGEAL EPITHELIAL CARCINOMA) CELL CULTURES

Extract	Concentration (µg/mL)	CTC_{50} (µg/mL)	
		MTT	SRB
AX-M	250 125 62.5	197.3 ± 2.89	208 ± 2.96

Average of six determinations, values are mean ± SEM

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