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In-vitro anti-proliferative activity of *Sambucus wightiana*

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Abstract
Sambucus wightiana is an important traditional medicinal plant that is used to relieve various health related issues. The demand for natural anti-cancer agents has gained a lot of momentum from last few years. Therefore, current research work was carried out to evaluate *In-vitro* anti-proliferative activity against breast cancer cell line (MCF-7) using MTT based assay. Nevertheless, results have showed that at tested concentrations, the cells did not show any significant anti-proliferative activity (IC50> 1000 µg/ml).

Keywords: anticancer, MCF-7, *Sambucus wightiana*, MTT assay

Introduction
Sambucus wightiana Wall. ex Wight & Arn. (Adoxaceae) is a perennial woody shrub with various ethno-medicinal properties e.g. to initiate vomiting for expelling poisonous substances, treat stomach disorders [1], as laxative, to treat skin diseases, as diuretic, anti-inflammatory, expectorant, diaphoretic, hypertensive and also as dye for coloring yarn [2]. The ethno-medicinal plants finds their application towards agricultural, cosmeceutical, food industries and also for addressing different diseases including life-threatening diseases like AIDS, Cancer, Diabetes [3].
Medicinal and aromatic plant species possesses various bioactive molecules that have potential to prevent and treat different diseases including cancers. The anti-cancer drugs are mostly natural in origin as 60% market based drugs are from natural sources [4, 5]. It is important to screen more and more number of natural sources such as plants to discover lead small biomolecules against prominent cancers. Among all cancer types, breast cancer is considered as a major cause of death among women of developed and developing countries [6]. Therefore, current investigation was carried out to evaluate *In-vitro* anti-proliferative activity of leaf portion of *Sambucus wightiana* against deadly breast cancer cell line.

Materials and Methods
Biological materials and culture medium
Sambucus wightiana leaf samples were collected from Ahribal region of Kashmir Himalayan region and voucher specimen was deposited at Centre for Biodiversity and Taxonomy, University of Kashmir herbarium (KASH-1732). The shade dried sample was proceeded for powder formation and phytochemical extraction was done using methanol [7]. Dried crude methanol extract was dissolved in distilled DMSO and stock solution of 1 mg/ml concentration was made. The serial dilutions were made to prepare different concentrations of the extract (62.5, 125, 250, 500 & 1000 µg/ml) to carry out cytotoxic studies.

Determination of *In-vitro* cytotoxicity activity
The cytotoxicity study (MTT assay) was carried out as described previously [8].

Results and Discussion
The results of *in-vitro* cytotoxicity and anticancer activity of methanolic leaf extract of *Sambucus wightiana* are presented in Table 1 and Fig.1. The morphological changes in two selected cell lines (L6- normal rat muscle and MCF7-human breast carcinoma) were observed by microscopic examination which revealed no marked cytotoxicity in the given extract concentration range against these two cell lines. The IC_{50} values against both the cell lines were found to be >1000µg/ml. The capacity of cells to resist toxic shock has remained the foundation of most cytotoxicity assays and MTT assay is based on the principle that dead cells or their products do not reduce tetrazolium. The assay depends both on the number of cells

present in the medium and the mitochondrial enzyme succinate dehydrogenase activity per cell. This enzyme brings cleavage of tetrazolium salt 3-(4, 5 dimethyl thiazole-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) into a blue coloured product (Formazan). It is found that number of cells present is directly proportional to the extent of formazan produced by the cells [8].

The inhibition of cell proliferation depends on various factors such as the type of the extract, cell line being used, stability of extract components in different media, length of treatment time, differential uptake of phenolics [9]. The absence of cytotoxicity against MCF-7 cell line could be because the current study used only single cancerous cell line (MCF-7). It is possible that cytotoxicity activity could be shown against other cancerous cells owing to the presence of its large pool of phytocompounds (Phenolics and flavonoids). It has been found previously that *Sambucus/adoxaceae* berries from different species have showed variation in their cytotoxicity activities against different types of cancerous cells such as human colon (HT29), MCF-7, human oral (KB and CAL27), prostate (LNCaP), SF-268(CNS-glioblastoma), HepG2 (human hepatocarcinoma), CT26 (Human colon carcinoma) etc. The cytotoxicity activity of extracts against any specific cancerous cell depends upon the type of phytoconstituents present in those extracts such as phenolic acids (hydroxycinnamic and hydroxybenzoic acids), flavonoids (anthocyanins, flavanols, flavonols), condensed tannins (proanthocyanins), hydrolysable tannins (ellagitannins and gallotannins), stilbenoids, lignans, triterpenes and sterols[9]. Therefore, it is recommended for future studies to include more number of cell lines to verify the anti-cancerous activities of different extracts of *Sambucus wightania.*

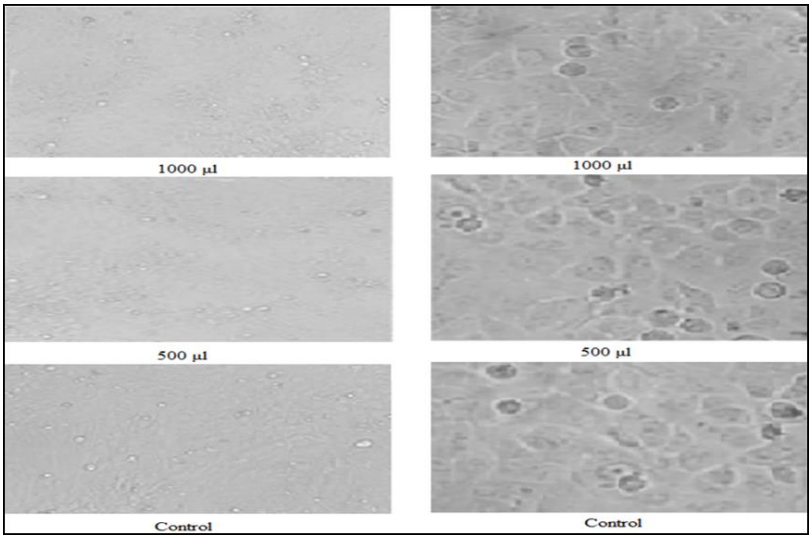

Fig 1: Microscopic examination of L6 (Normal rat muscle) and MCF-7 (Human breast carcinoma) cell lines after exposed to methanolic leaf extract of *Sambucus wightania.* No significant cytotoxicity was observed at tested concentrations. Values are mean ± SD, n=3, P<0.05

Table 1: *In-vitro* cytotoxicity effect of methanolic extract of *Sambucus wightiana* Wall. Against L6 (Normal rat muscle) and MCF-7 (Human breast carcinoma) cell lines.

S. No.	Name of Cell line	Test Conc. (µg/ml)	% Cytotoxicity	IC_{50} (µg/ml)
1.	L-6	1000	22.42±2.3	>1000
		500	17.89± 5.2	
		250	17.13±5.4	
		125	15.82±1.7	
		62.5	13.79±2.1	
2.	MCF-7	1000	22.23±0.8	>1000
		500	21.82±2.2	
		250	20.48±2.2	
		125	17.33±1.2	
		62.5	11.03±1.5	

References

1. Sharma R. Medicinal Plants of India, an encyclopedia. Daya Publishing house, Delhi, 2003, 221-222.
2. Vidyarthi OPS. Forest Flora of Kashmir. Working Plan Circle, Jammu and Kashmir Forest Department, 2010, 34.
3. Wiersum KF, Dold AP, Husselman M, Cocks M. Cultivation of medicinal plants as a tool for biodiversity conservation and poverty alleviation in the Amatola region, South Africa. Springer, 2006, 43-57.
4. Fernandez JA. Anticancer properties of saffron, *Crocus sativus* Linn. Adv. Phytomed. 2006, 313-330.
5. Rates SMK. Plants as source of drugs. Toxicon. 2001; 39:603-613.
6. Hany El-Shemy A. Drug discovery. In Tech, 2013, 515.
7. Tiwari P, Kumar B, Kaur M, Kaur G, Kaur H. Phytochemical screening and extraction. A Rev. IPS. 2011; 1:99-100.
8. Francis D, Rita L. Rapid colorometric assay for cell growth and survival modifications to the tetrazolium dye procedure giving improved sensitivity and reliability. J Immunol. 1986; 89:271-277.
9. Stoner GD, Wang LS, Casto BC. Laboratory and clinical studies of cancer chemoprevention by antioxidants in berries. Carcinogenesis. 2008; 29:1665-1674.