Flowers of *Woodfordia fruticosa* exhibit *in vitro* cytotoxic effect on HEP-2 and SK-N-MC cancer cells

Vikas Sharma

Division of Biochemistry and Plant Physiology, Sher-e- Kashmir University of Agricultural Sciences and Technology of Jammu, Chatha, Jammu, 180009, India; E-mail: vikas.skuast@gmail.com

**ABSTRACT**

The SRB assay was used for screening the extracts of *Woodfordia fruticosa* for *in vitro* cytotoxicity against six human cancer cell lines viz., lung cancer cells (A-549, NCI-H23), colon cancer cells (COLO-205, SW-620), liver cancer cells (HEP-2) and neuroblastoma cancer cells (SK-N-MC). Results demonstrated that the thanolic extract from the flowers of the plant showed *in vitro* cytotoxicity against two human cancer cell lines viz., HEP-2 and SK-N-MC. This extract did not exhibit any significant activity against other four human cancer cell lines. Surprisingly, the other two extracts (50% thanolic and hot water) were observed to be inactive against all the human cancer cell lines.

**Keywords:** *Woodfordia fruticosa*, *in vitro* cytotoxicity, cancer cells, thanolic extract

**INTRODUCTION**

The plant kingdom has always been the favourite source of medication in all healing traditions all over the world and the use of plants as medicine is as old as human civilization. It is estimated that about 70,000 plant species i.e., 28% of the total 2,50,000 species, from lichens to towering trees, have been used at one time or another for medicinal purposes. The herbs provide the starting material for the isolation or synthesis of conventional drugs [1]. The plants constitute one of the major raw materials for drugs for treating various ailments of human being and due to wide spread toxicity/harmful after effects associated with the long use of synthetic drugs and antibiotics, the Western Society prefer the drugs from natural sources and in USA and UK, the plant based drugs are being used substantially. India, in this regard, has a unique position in the world, where a number of traditional systems of medicine, viz., Ayurveda, Siddha, Unani etc. are utilized and practiced in the total health care system of the country and all of these are predominantly dependent upon medicinal plants [2].

Indian Materia Medica includes about 2000 drugs of natural origin, almost all of which are derived from different traditional system/folklore practices [3]. Many plants/plant extracts have been used as anticancer agents in folklore and traditional medicine. One such plant is *Woodfordia fruticosa* (Linn.) Kurz. (family: Lythraceae) commonly known as Fire-flame bush and found throughout India, but abundantly in north India up to 1600 m altitude. Extracts and metabolites of this plant, particularly those from flowers possess useful pharmacological/biological activities and chemical constituents [4]. The flowers of the plant are used in compound preparations and also in cosmetics of herbal origin [5]. The juice of its fresh flowers, when applied on the forehead, reduces the headache. To facilitate the dental eruption in children, the powder is massaged on the gums. A mixture of *W. fruticosa* powder, honey and rice water is extremely affected in diarrhoea, dysentery and piles. It can be safely used even in pregnancy. The decoction of flowers effectively quench the excessive thirst, especially in diabetic patients [6]. The flowers of this plant have various medicinal
uses, possess high content of tannins and have been used as an astringent tonic in disorders of mucous membrane [7,8]. The powder of dried flowers is sprinkled over ulcers and wounds to diminish discharge and promote granulation [9].

The flowers also showed hepatoprotective activity [10]. The previously isolated constituents from W. fruticosa flowers are ellagitannin dimers with astringent and haemostatic properties that affect histamine release [11]. As regards anticancer activity, a new compound, Woodfordin C, which is isolated from the methanolic extract of the leaves of W. fruticosa, showed remarkable activity against PC-1 and moderate activity against MKN-45 and KB human cancer cells. Besides, it demonstrated in vivo growth inhibitory activity on COLON-38 [12,13]. Thus, Woodfordin C, have a macro ring structure, was found to exhibit a significant antitumor activity [14,15]. Other constituents isolated were Woodfordin I that showed mitochondrial dysfunction in human leukemia K562 cells [16]. Keeping in view the above facts and due to enormous medicinal properties, the present study aims at in vitro cytotoxic activity of the extracts of W. fruticosa flowers against human cancer cell lines from four different origins.

MATERIALS AND METHODS

Plant material (flowers of *Woodfordia fruticosa*) were collected in the month of August from Nagrota region of Jammu district, Jammu and Kashmir, India. The dried flowers were homogenized to fine powder and further subjected to extraction. For the ethanolic extract, dried ground plant material (100 g) was percolated with 95% ethanol and then concentrated to dryness under reduced pressure. 50% ethanolic extract was prepared by percolating another lot of dried ground plant material (100 g) with 50% ethanol and then concentrating it to dryness under reduced pressure. The hot water extract was obtained by boiling dried ground plant material (100 g) for 30 min in distilled water (300 ml). Stock solutions of 20 mg/ml were obtained by dissolving ethanolic extract in Dimethyl sulfoxide (DMSO), the aqueous ethanolic extract in 50% DMSO and the hot water extract in sterile water. The microbial contamination was controlled by the addition of 1% gentamycin in complete growth medium i.e., used for dilution of stock solutions to prepare working test solutions of 200 µg/ml.

Positive controls, viz., Adriamycin, 5-Flourouracil and Mitomycin-C were prepared in distilled water and Taxol in dimethyl sulfoxide (DMSO) and then diluted in gentamycin medium to obtain desired concentrations of 2 x 10⁻⁴ M and 2 x 10⁻⁵ M. The human cancer cell lines were obtained from National Center for Cell Science, Pune, India and cultured in Rosewell Park Memorial Institute (RPMI)-1640 medium (pH 7.4), supplemented with Fetal Calf Serum (FCS) 10%, penicillin 100 units/ml, streptomycin 100 µg/ml and glutamine 2 mM.

Test material was subjected to in vitro anticancer activity against various human cancer cell lines [17]. For the assay (in brief), the cells were grown in tissue culture flasks in growth medium at 37°C in an atmosphere of 5% carbon dioxide and 90% relative humidity in a CO₂ incubator. The cells at subconfluent stage were harvested from the flask by treatment with trypsin (0.05% trypsin in phosphate buffer saline containing 0.02% ethylene diamine tetra acetic acid) and suspended in growth medium. Cells with more than 97% viability (trypan blue exclusion) were used for determination of cytotoxicity. An aliquot of 100 µl of cells (10⁴ cells/ml) was transferred to a well of 96 wells tissue culture plate. The cells were allowed to grow for 24 h. Test material was then added to the wells and cells were further allowed to grow for another 48 h.

The antiproliferative Sulphorhodamine B (SRB) assay was performed to assess growth inhibition which estimates cell number indirectly by obtaining total cellular protein with the dye SRB [18]. In brief, the cell growth was stopped by gently layering 50 µl of 50% (ice cold) trichloroacetic acid on the top of growth medium in all the wells. The plates were incubated at 4°C for an hour to fix the cells attached to the bottom of the wells. Liquid of all the wells were then gently
pipetted out and discarded. The plates were washed five times with distilled water and air dried. SRB 100 µl (0.4% in 1% acetic acid) was added to each well and the plates were incubated at room temperature for 30 min. Excess dye was removed by washing with 1% acetic acid and the bound dye was dissolved in tris buffer (100 µl, 0.01 M, pH 10.4). Plates were gently stirred on a mechanical shaker for 5 min and the optical density was recorded on ELISA reader at 540 nm.

Suitable blanks and positive controls were also included. Each test was done in triplicate and the values reported herein are mean values of three experiments. The cell growth was determined by subtracting average absorbance value of respective blank from the average absorbance value of experimental set. Percent growth in the presence of test material was calculated as Growth in the presence of test material / Growth in the absence of test material × 100. The percent growth inhibition in the presence of test material was calculated as 100 - percent growth in the presence of test material and the growth inhibition of 70% or above was considered active.

RESULTS AND DISCUSSION

Since medieval time, plants have been the source of medicine for the treatment of diseases. Regarding the availability of a wealth of synthetic drugs, plants remain (even in the 21st century) an integral part of the health care in different countries, especially the developing ones. Developing countries like India have a rich flora of medicinal plants that can be explored for the potential sources of new drugs and new biologically active substances. In this context, we have evaluated the in vitro cytotoxic potential of W. fruticosa (a traditionally used medicinal plant) via ethanolic, 50% ethanolic and hot water extract at 100 µg/ml, with appropriate positive controls against six human cancer cell lines namely of lung (A-549, NCI-H23), colon (COLO-205, SW-620), neuroblastoma (SK-N-MC) and liver (HEP-2) of four different tissues. Our results demonstrate that the 95% ethanolic extract of the above mentioned plant was found active as it inhibited the growth of two human cancer cell lines from two different tissues, viz., HEP-2 from liver origin and SK-N-MC from neuroblastoma origin. The other two extracts (50% ethanolic and hot water) did not exhibit cytotoxic effect on any of the human cancer cell line (Table 1).

Table 1. Growth inhibitory effect of W. fruticosa extracts along with positive controls against human cancer cell lines.

<table>
<thead>
<tr>
<th>Plant Name</th>
<th>Parts Used</th>
<th>Extract</th>
<th>Human Cancer Cell lines and Growth Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>A-549</td>
</tr>
<tr>
<td>Woodfordia</td>
<td>Flowers</td>
<td>Ethanol</td>
<td>0</td>
</tr>
<tr>
<td>fruticosa</td>
<td></td>
<td>50% Ethanol</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Hot water</td>
<td>12</td>
</tr>
<tr>
<td>Positive controls (Conc., M)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adriamycin</td>
<td>1 x 10^{-3}</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Mitomycin-C</td>
<td>1 x 10^{-3}</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5 - Flurouracil</td>
<td>1 x 10^{-4}</td>
<td>-</td>
<td>75</td>
</tr>
<tr>
<td>Taxol</td>
<td>1 x 10^{-5}</td>
<td>71</td>
<td>-</td>
</tr>
</tbody>
</table>

The concentration of extracts employed in each case was 100 µg/ml; Growth inhibition of 70% or above has been indicated in bold numbers. The mark (-) means that the particular human cancer cell line was not treated with that particular positive control.

The results obtained from our investigation confirmed the therapeutic potency of W. fruticosa. In addition, these results form a good basis for selection of the plant for further phytochemical and pharmacological investigation. The results of the present study support the folkloric usage of the studied plant and suggest that the plant extract possesses certain constituents with cytotoxic
properties that can be used for developing anticancer agents for cancer therapy. The most active extract can be subjected to isolation of the compounds and carry out further pharmacological evaluation as the promising extract of the plant will surely be the source for bioactive constituents having anticancer property. Thus, 95% ethanolic extract was most active as compared to other two extracts indicating that active constituents are polar in nature.

Cancer is one of the most deadly diseases facing the humanity today. It has emerged as an important health problem in the developed and developing countries. Worldwide, between 100 to 350 of each 100,000 people die of cancer every year. It has been recognized as the important cause of morbidity, mortality and disability in India also. The devastation caused by cancer is staggering to contemplate and it is considered that the management of cancer is still not up to the mark. Diverse biological disciplines such as cytogenetics, virology, cell biology, molecular genetics and biochemistry together with the clinical sciences have close links in their research of how cancer develops and to find remedies to stop the abnormal growth that is characteristics of cancerous cells. Despite of recent advances in cancer research and significant development in the field of synthetic drug chemistry, the global trend currently calls for discovery of new molecules of natural origin which are less toxic, endowed with fewer side effects and more potent in their mechanism of action.

Plants have played a significant role in maintaining human health and improving the quality of human life for thousands of years. Plants, being a rich source of therapeutic agents, have contributed to the drug industry for a long time and also they have a long history of use in the treatment of cancer. Plants played an important role as a source of effective anticancer agents and it is significant that over 60% of currently used anticancer agents are derived in one way or another from natural resources including plants, marine organisms and micro-organisms [19,20]. Natural products from a number of medicinal plants offer new sources of drugs, but there are still a large number of medicinal plants in which all the active constituents have not yet been fully investigated. Thus, Indian medicinal plants have assumed real significance and there is need to screen more Indian medicinal plants for their anticancer activity. Therefore, in the present research work an attempt was made to elucidate the in vitro anticancer potential of \( W. \) fruticosa. The promising extract of the plant i.e., 95% ethanolic extract will surely be the source for bioactive constituents having anticancer activity.

The striking results were shown by the 95% ethanolic extract from the flowers of \( W. \) fruticosa against neuroblastoma (SK-N-MC) and liver (HEP-2) cancer cells as 71-72% growth inhibition was observed in this case. Thus, we can say that the active ingredient from the plant can serve as a lead molecule in the development of anticancer drugs for neuroblastoma and colon carcinomas to provide a great promise and service to cancer patients.

Acknowledgments: The author is thankful to Indian Institute of Integrative Medicine (CSIR), Jammu for providing technical support and Dr. Madhulika Bhagat, Assistant Professor, School of Biotechnology, University of Jammu, Jammu for the collection of plant material.

REFERENCES