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In-vitro anticancer activity of *Achyranthes aspera* root extract against different human cancer cell lines

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ABSTRACT

Cancer is a public health problem all over the world. Developments in the treatment of cancer have lead to greatly improved survival and quality of life for the cancer patients by medicinal plants. The synthetic drugs are more toxic to animal body, whereas natural drugs play a dominant role in the pharmaceutical cure. Large number of plants and their isolated constituents has been shown to potential anticancer activity. 70% Ethanolic plant root extract of *Achyranthes aspera* L. showed in-vitro anticancer activity against different human cancer cell lines such as liver and colon. Sulforhodamine B dye (SRB) assay was done for in-vitro anticancer test assay. The in-vitro test was performed against two human cancer cell lines namely liver (Hep-2) & colon (HT-29). The activity was done using 100µg/ml of the root extract. It is confirmed that the plants root extract used for In-Vitro anti-cancer activity. *In-Vitro* experiments based on inhibition in the growth of the human cancer cells by the test sample. These observations clearly indicated that the *Achyranthes aspera* L. root extracts possess anticancer activity.

Key Words: Cancer, Sulforhodamine B, Ethanolic, Liver, In-Vitro.

INTRODUCTION

Cancer disease is characterized by the abnormal, uncontrolled cell growth and spread of abnormal cells. Cancer is a very complex disease involving numerous changes in cell physiology, which ultimately leads to malignant tumor (Anand, P.*et al*. 2008).

For thousands of year's natural products have played a very important role in health care and prevention of diseases. The ancient civilization of the Chinese, Indians and North Africans provide evidences for the use of natural sources for curing various disease.

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(Phillipson JD.2001). All herbal drugs constitute a major part in traditional systems of medicines. Herbal medicine is a achievement of popular therapeutic diversity. Plants and other products have been used for medicine from time immemorial because they have fitted the immediate personal need, are easily accessible and less expensive (P.K. Mukherjee, 2008). According to the WHO more than 82% of the world's population relies on traditional herbal medicine for their primary health care.(Vijayan Arun *et al*, 2007). Medicinal plants produce a number of compounds having some therapeutic properties (V. Duraipandiyan *et al*, 2012). Although conventional chemotherapeutic drugs induce cell death, they are limited by their toxicity to normal cells. Identification of natural agents in form of either plant extracts or a bioactive compound, which successfully exhibits apoptosis and cell cycle modulating properties and at the same time shows limited toxicity to normal cells and is therefore essential (M. Rebucci and C. Michiels, 2013).

A. aspera, belongs to Family Amaranthaceae, it is found throughout tropical Asia, Africa, Australia, America and it grows as wasteland herb everywhere. It is a shrub that grows up to 1-2 meter height;, obovate or greenish

spikes, elliptic, opposite; flowers- bracteates and bracteolate, leaves –simple, fruits- oblong urticle, seeds- single and inverse. It holds a reputed position as medicinal herb in different systems of medicine in India. According to Ayurveda, *A. aspera* plant is pungent, bitter, stomachic, laxative, carminative and little useful in treatment of vomiting, bronchitis, heart diseases, abdominal pains, piles, itching (Bhoomika, RG. *et al* 2007). A. aspera is also used in treatment of asthma (Bhoomika, RG. *et al* 2007) and as anti lipedimic (Latha, B.2010), analgesic (Geetha, K. *et al*, 2010) diuretic(Bafna, AR. & Mishra, SH. 2004), antibacterial (Aziz, M.D. *et al*, 2005), hepato protective (Jitendra, YN. 2009), anti-oxidant (Rama Mohan, RT. 2011), anti-fungal, anti-cancer (Vijaya, K. 2009), anti-inflammatory agent (Hullatti, KK. & Uma ,DM. 2010), immuno modulatory agent.(Jeetendra, YN. 2009).

The mechanism of action of antitumor medicinal plants could be anti-oxidative, anti-apoptotic effects, anti-proliferative, anti-differentiation, cell cycle arrest and inhibition of angiogenic and proliferation process. However, there is a continuing need for development of new, high potential novel anticancer drugs and chemotherapy strategies, by methodological and scientific exploration of natural products that give more efficiency, less toxicity and cost effective (Adel Abdel-Moneim and Alaa Magdy. 2016). In the development of cancer, spreading of apoptosis is one of the major factors resulting in overpopulation of cancer cells. Apoptosis is an active form of cell death guided by a set of prosurvival and anti-survival genes (Wong RSY. 2011).

Mechanisms leading to apoptosis and cell cycle arrest were also investigated by expression studies of caspase-3, caspase-9, Bcl-2, Bax, p21, p16 and p27 genes, followed by flow cytometric analysis for cell cycle distribution. Cytotoxicity screening of *A. aspera* extracts indicated greater cytotoxic activity of AAA extract against COLO-205 cells. There is a series of events marked by apoptosis which revealed the loss of cell viability,DNA fragmentation and chromatin condensation in AAA treated cells to a greater extent. The m-RNA expression levels of caspase-3, caspase-9, Bax p16, p21, and p27 were markedly increased in the AAA treated cells, along with decreased Bcl-2 expression. The cell cycle,S phase was arrest which was detected by flow cytometric analysis after treatment with AAA. (Arora, S. and Tandon, S. 2014).

The recent research work was undertaken to evaluate the anticancer activity from 70% Ethanolic root extract of *A. aspera* against Liver & Colon cancer cell lines.

MATERIALS AND METHODS

Plant material and Preparation of plant extract:

Whole plants were collected in the months of February to March -2012 from Botanical survey of India Dehradun, India. Plant roots are collected from the plant and was dried at room temperature in the laboratory and then powdered and extracted by Soxhlet apparatus using ethanol as a solvent.

Extract was fine-filtered, dried and freezed. Plant extract was re-dissolved in Dimethylsulphoxide (DMSO) to form stock solutions, which were filter and sterilized (0.2µm) before testing the cell lines.

Human cancer cell lines:

The selected human cancer cell lines namely liver (HEP-2) & colon (HT-29) were grown in RPMI-1640 media (sigma) with 2 mM L-glutamine medium of pH-7.2. Penicillin was dissolved in PBS and sterilized by filtering through 0.2µ filter paper in laminar air flow hood. The entire growth media was stored in refrigerator at low temperature (2-6^{0}C).The complete growth medium contain 10%FCS. The growth medium for cryopreservation contained 20% FCS and 10% DMSO. The cell lines were maintained at 37^{0}C in a 5% CO_2 atmosphere with 95% humidity (Verma S.K.,*et.al* , 2010).

In vitro study of cytotoxicity:

The anticancer activity was determined by evaluating the cytotoxicity potential of the plant extract using human cancer cell lines that were allowed to grown on tissue culture plates. The cell growth was measured using ELISA reader after staining with Sulforhodamine B dye (SRB) which binds to basic amino acid residues in the Trichloro- Acetic Acid (TCA) fixed cells.

Preparation of Cancer cell culture for assay:

Human cancer cell lines were grown in multiple tri-conical flasks (TCFs) at 37^{0}C in the atmosphere of 5% CO_2 and 90% relative humidity to obtain bulk number of cancerous cells. The TCF with cells at sub- confluent stage were selected and then harvested by treatment with Trypsin-EDTA solution. Cells were separated to single cell suspension by gentle pipetting and the viable cells were counted by Hemocytometer using trypan blue. The cell viability at this stage should be >97%. Viable cell density was adjusted between 5,000 - 40,000 cells/100µl depending upon the cancer cell lines (Monks, 1991). The cell suspension culture (100µl) together with 100µl of complete growth medium was added into each culture well. The plates were incubated at 37^{0}C for 24 hours in an atmosphere of 5% CO_2 and 90% relative humidity in a CO_2 incubator. After 24 hours of incubation, the test material, DMSO (vehicle control) and positive control were added in to the suspension culture medium.

Sulforhodamine B (SRB) assay:

Antiproliferative SRB assay was performed to assess the growth inhibition. It is a colorimetric assay which estimates the cell number indirectly by staining total cellular protein with SRB dye (Skehan, P.1990). The microtiter plates were taken out after 48 hours incubation and then gently layered with chilled 50% TCA in all the wells.The tissue culture plates were incubated at 4°C for one hour to fix the cells attached to the bottom of the wells. The supernatant was then

discarded. The plates were washed five times with distilled water to remove TCA, unused growth medium, light weight metabolites, serum proteins etc,then Plates were air dried and stored until further use. SRB solution was added to each well of the plates and incubated at room temperature for about 30-35 minutes. The unbound SRB was removed by washing the wells 3 to 6 times with 1% acetic acid and then air dried. 100µl of Tris buffer (0.01 M, pH 10.4) was added in each well and shaken gently for 3-5 minutes on a mechanical shaker. Optical density was recorded on ELISA reader at 515 nm.

Calculation:

Cell viability and growth in the presence of test material was calculated as:

Percent growth in presence of test material =(Growth in presence of test material/ Growth in absence of test material) X 100

Percent growth inhibition in presence of test material was calculated as:

= 100 ─ % growth in presence of test material

Criteria for Determination of Activity:

The test sample showing growth inhibition of >70% at 100 µg/ml was considered to be active. So keeping this in mind the results of *in vitro* cytotoxicity studies carried out against human cancer cell lines in the present investigations.

Statistical analysis:

The experiments were carried out and results are given as the mean ± S.D. The all data of the experiments were analyzed by Microsoft Excel 2007 the Student's *t*-test were showed significant result at $P < 0.05$.

RESULTS AND DISCUSSION

The roots of *Achyranthes aspera* stores different types of organic compounds. Chemically they could be phenolics, alkaloids, flavonoids, steroids, monoterpenes, sesquiterpenes, esters and fatty acids etc.

Experimentally, if the test samples showing growth inhibition more than 70% at 100 µg/ml then it is considered the significant result.

The whole experimental data were summarized in table form & graph was plotted as shown in figure-1.

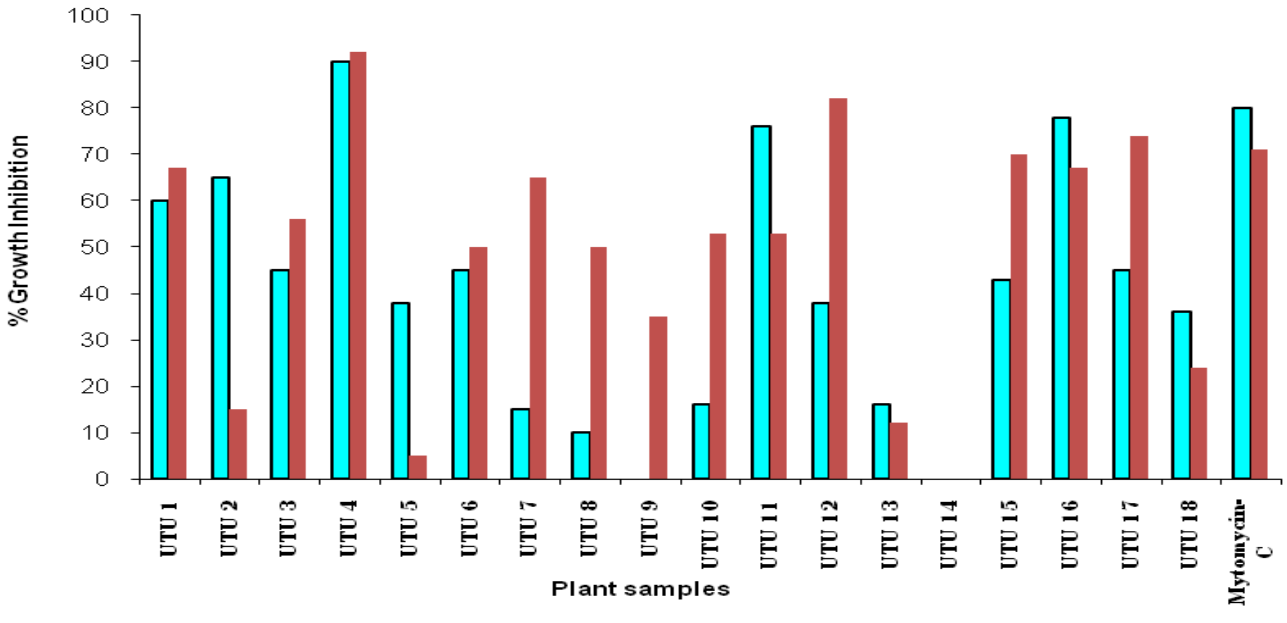
In-vitro cytotoxicity of plant sample was performed against liver cancer cell line (Hep-2) showed 90%,76% & 78% growth of inhibition of the sample number- UTU 4, UTU 11 & UTU 16 respectively while Mytomycin-C showed 80% growth of inhibition, where as in case of colon cancer cell line (HT-29), showed 92%, 82%, 70% & 74% growth of inhibition of the sample number-UTU 4,UTU 12,UTU 15 & UTU 17 respectively, while Mytomycin-C showed 71% growth of inhibition.

The statistical analysis (mean ± S.D) showed 81% growth of inhibition against Hep-2 while 79.5% growth of inhibition against HT-29 & the Student's *t*-test were showed significant result at $P < 0.05$.

The result showed that the Plants have rich source of bioactive compounds that possess unique medicinal properties. Therefore, plant roots containing these bioactive compounds may serve as a potential source in the treatment of colon & liver cancers.

So plant extracts have selective in-vitro cytotoxicity against some human cancer cell lines. The activity might be depended upon the physical morphology of cancer cell lines and mechanism of action of the plant extract. Many plant extract kill cancer cell lines through activating apoptosis and effecting growth regulators.

Figure-1. *In vitro* cytotoxicity of plant extracts on cell line Hep-2 & HT-29.



However, based on the published studies flavonoids, alkaloids phenolics, and terpenoids etc seem to be most likely bioactive compounds that eliciting in-vitro cytotoxicity effect. Its reported in-vitro cytotoxicity effects warrant further investigation for its use in the field of clinical anticancer activity.

CONCLUSION

The result of this study showed that the *A. aspera* root extract have some potential of anticancer activity against colon & liver cancer cell lines. The anticancer potential activities exhibited due to the presence of phytoconstituents, like alkaloid, phenolics, flavonoids, terpenoids etc; that have been demonstrated to act as cytotoxic agents. The experimental evidence obtained in the laboratory that provides a rationale for the traditional use of *A. aspera* plant. The research work are very interesting to know the chemical composition and better understanding the mechanism of action of the phytoconstituents of the root extract which exerting anticancer activities for developing it as a drug for therapeutic use in future. A possible herbal anticancer composition is proposed for make effective anticancer herbal formula that can be use alone or combine with chemical drugs to reduce toxicity as well as side effect.

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Conflict of Interests

Authors declare that there is no conflict of interests regarding the publication of this paper.

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