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RESEARCH ARTICLE

Exploring *in vitro* anti-proliferative efficacy of Jammu botanicals

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SUMMARY

Cancer is becoming a big load on families and economies. Cancer cases related deaths on rise in J&K during past four years with the total of 11,815 cancer cases and 5,198 mortality cases have been reported in the state during the current year. Cancer research has, therefore, become a major area of scientific research supporting the foundations of modern biology to a great extent. Chemotherapy is a major treatment modality for cancer, but most of the drugs used in cancer chemotherapy exhibit cell toxicity and can induce genotoxic, carcinogenic and teratogenic effects in non tumor cells. Therefore, the research for alternative drugs of natural origin, which are less toxic, endowed with fewer side effects and more potent in their mechanism of action, is an important research line. In the present investigation, methanolic and aqueous extracts from two medicinal plants (*Allium sativum and Holarrhena antidysenterica*) selected from Jammu region were evaluated against eight human cancer cell lines from six different origins, *viz.*, A-549 (lung), NCI-H322 (lung), HCT-116 (colon), COLO-205 (colon), MCF-7 (breast), PC-3 (prostate), THP-1 (leukemia) and U-87-MG (glioblastoma) at the concentration of 100 µg/ml using sulphorhodamine blue (SRB) assay. Results revealed that methanolic extract from the stem-leaves of *H. antidysenterica* displayed *in vitro* cytotoxic effect against leukemia and colon cancer cells.

Key Words : Cancer cells, *Allium sativum, Holarrhena antidysenterica,* SRB assay

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Medicinal plants have played a key role in world health. Population rise, inadequate supply of drugs, prohibitive cost of treatment, side effects of several allopathic drugs and development of resistance to currently used drugs for infectious diseases, have led to increased emphasis on the use of plant material as a source of medicine for a wide variety of ailments. Out of total 250,000 plant species existing on earth approximately one thousand have anticancer potential. A large number of plant species have been screened through bioassays for the search of novel plant based anticancer drugs. The State of J&K has great botanical diversity and widespread use of traditional medicine practice known as ayurvedic medicine, however, only a relatively small number of these plants have been subjected to accepted scientific evaluation for their potential anticancer effects. Garlic is a relative of the onion and leek, and other related species containing the aromatic sulphur-based compounds,

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which contribute to the characteristic odour and taste, as well as garlic's beneficial healing effects (Linnaeus, 1957). Garlic has historically been used to treat leprosy, deafness, diarrhea, constipation, parasitic infections and relieve ear and stomach aches (Hahn, 1996). The use of garlic in the treatment of tumors dates back to 1550 BC when ancient Egyptians administered it orally and topically, but modern era begins in 1950s when its thiosulfinate extracts inhibited the growth of malignant cells and prevented growth of sarcoma 180 ascites tumor *in vitro* and *in vivo* (Weisberger and Pensky, 1958). *A. sativum* is widely used as herbal vegetable and has been suggested as an anticancer agent for several decades in epidemological studies (Thomson and Ali, 2003). Fresh garlic aqueous extract possesses antiproliferative potential against neoplastic transitional cell carcinoma cells (Talebi *et al*., 2006). The plant displayed anti-tumor activity in sarcoma, mammary carcinoma, hepatoma, colon cancer, squamous cell carcinoma of skin and esophagus (Lamm and Riggs, 2001).Two main water soluble constituents of garlic, S-allylcysteine (SAC) and S-allylmercaptocysteine (SAMC), were able to suppress prostate cancer cell proliferation and proved their novel anticancer effect (Chu *et al*., 2006). *A. sativum* >24 kg/ year had 60 per cent reduced risk of gastric/stomach cancer compared to those with low consumption <11.5 kg/year (You *et al*., 1989). *In vivo* and *in vitro* studies have shown that S-allylcysteine (SAC) and S-allylmercaptocysteine (SAMC) are not only able to suppress the skin, esophageal, stomach, colon, liver, lung and breast cancer growth in animal models (Thomson and Ali, 2003; Herman-Atosiewiez and Singh, 2004), but also directly inhibit proliferation of a variety of cancer cell lines derived from colon, lung, leukaemia, skin, breast and prostate cancer *in vitro* (Herman-Atosiewiez and Singh, 2004). The stem bark crude aqueous and alcoholic extracts of *H. antidysenterica* exhibit anti-bacterial activity against the known enteric pathogens (Ballal *et al*., 2001). Efficacy of aqueous extract of seed for the management of diabetes was also carried out in experimental model in rat (Ali *et al*., 2009; Wahab and Yousuf, 2004). This plant also showed *in-vitro* antioxidant potential according to ferric thiocyanate assay method (Zahin *et al*., 2009). Bitter oleander (*H. antidysenterica*) has been considered as a popular remedy for the treatment of dysentery, diarrhea and intestinal worms (Kavitha *et al*., 2004) and derivative of 2, 6-diisopropylphenol (propofol) was prepared by coupling with 9-hydroxy-11-Z-octadecenoic acid (isolated from seed oil of *H. antidysenterica*) with the C_1-α-hydroxy function of 2, 6-diisopropylphenol and the analog was found cytotoxic against HeLa, MCF-7 and HL-60 cancer cells (Mohammad *et al*., 2010). In the present study, *in vitro* anticancer potential of garlic and bitter oleander has been investigated against eight human cancer cell lines from six different tissues.

MATERIAL AND METHODS

Preparation of extracts :

Bulb of garlic and stem-leaves of bitter oleander were collected in the month of July-August from Baisht village of Udhampur, Jammu and Herbal Garden of SKUAST-Jammu, J&K, India respectively. Extraction of the plant material was carried out as per Jabar and Al-Mossawi, 2007 and Kandil *et al*., 1994.

Cell lines and positive controls :

The human cancer cells were obtained from National Centre for Cell Science, Pune, India and were further grown and maintained in RPMI-1640 medium. Positive controls like adriamycin and 5-fluorouracil were prepared in distilled water, while paclitaxel was prepared in DMSO. These were further diluted in gentamycin medium to obtain desired concentrations.

In vitro assay for cytotoxic activity :

Extracts were subjected to *in vitro* anticancer activity against various human cancer cell lines (A-498, A-549, HCT-116, MCF-7, MDA-MB-435, OVCAR-5, PC-3, SF-295, T-47D) (Monks *et al.*, 1991). In brief, the cells were grown in tissue culture flasks in growth medium at 37°C in an atmosphere of 5 per cent CO_2 and 90 per cent relative humidity in a CO_2 incubator (Hera Cell, Heraeus; Asheville, NCI, USA). The cells at sub-confluent stage were harvested from the flask by treatment with trypsin (0.05% trypsin in PBS containing 0.02% EDTA) and suspended in growth medium. Cells with more than 97 per cent viability (trypan blue exclusion) were used for determination of cytotoxicity. An aliquot of 100 µl of cells (10^5 cells/ml) was transferred to a well of 96-well tissue culture plate. The cells were allowed to grow for 24 h. Extracts (100 µl/ well) were then added to the wells and cells were further allowed to grow for another 48 h.

The anti-proliferative SRB assay which estimates cell number indirectly by staining total cellular protein with the dye SRB was performed to assess growth

inhibition. The SRB staining method is simpler, faster and provides better linearity with cell number. It is less sensitive to environmental fluctuations and does not require a time sensitive measurement of initial reaction velocity (Skehan *et al.*, 1990). The cell growth was stopped by gently layering 50 µl of 50 per cent (ice cold) trichloro acetic acid on the top of growth medium in all the wells. The plates were incubated at 4°C for 1 h to fix the cells attached to the bottom of the wells. Liquid of all the wells was 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.

The unbound SRB was quickly removed by washing the cells five-times with 1 per cent acetic acid. Plates were air-dried, tris buffer (100 µl, 0.01 M, pH 10.5) was added to all the wells to solubilize the dye and then plates were gently stirred for 5 min on a mechanical stirrer. The optical density (OD) was recorded on ELISA reader at 540 nm. Suitable blanks (growth medium and DMSO) and positive controls (prepared in DMSO and distilled water) were also included. Each test was done in triplicate and the values reported were 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. Per cent growth in presence of test material was calculated as under:

–OD change in presence of control = Mean OD of control – Mean OD of blank

–OD change in presence of test sample = Mean OD of test sample – Mean OD of blank

–Per cent growth in presence of control = 100/OD change in presence of control

–Per cent growth in presence of test sample = Per cent growth in presence of control × OD change in presence of test sample

–Per cent inhibition by test sample = 100 – Per cent growth in presence of test sample.

The growth inhibition of 70 per cent or above was considered active while testing extracts, but in testing of active ingredients at different molar concentrations, the growth inhibition of 50 per cent or above was the criteria of activity.

RESULTS AND DISCUSSION

The methanolic extract from the bulb part of *Allium sativum* did not exhibit significant cytotoxic effect (growth inhibition of 70% or above) against any of the human cancer cell line. The extract is considered inactive

Table 1 : Growth inhibitory effect of *Allium sativum* along with positive controls against human cancer cell lines

Plant part used	Extract	Conc.(µg/ml)	Human cancer cell lines from six different tissues							
			Lung A-549	Colon COLO-205	Colon HCT-116	Breast MCF-7	Lung NCI-H322	Prostate PC-3	Leukemia THP-1	Glioblastoma U-87-MG
			Growth inhibition (%)							
Bulb	Methanolic	100	08	42	37	56	41	00	27	04
	Aqueous	100	00	00	51	26	19	19	05	15
Positive controls		Conc.(Molar)								
5-Flurouracil		2×10^{-5}M	-	51	68	-	-	-	73	60
Paclitaxel		1×10^{-6}M	79	-	-	-	52	-	-	-
Adriamycin		1×10^{-6}M	-	-	-	60	-	59	-	-

The mark (-) indicates that particular human cancer cell line was not treated with that particular positive control

Table 2 : Growth inhibitory effect of *Holarrhena antidysenterica* along with positive controls against human cancer cell lines

Plant part used	Extract	Conc.(µg/ml)	Human cancer cell lines from six different tissues							
			Lung A-549	Colon COLO-205	Colon HCT-116	Breast MCF-7	Lung NCI-H322	Prostate PC-3	Leukemia THP-1	Glioblastoma U-87-MG
			Growth inhibition (%)							
Stem-Leaf	Methanolic	100	59	78	19	58	50	0	82	22
	Aqueous	100	63	64	60	0	27	0	19	8
Positive controls		Conc.(Molar)								
5-Flurouracil		2×10^{-5}M	-	51	68	-	-	-	73	60
Paclitaxel		1×10^{-6}M	79	-	-	-	52	-	-	-
Adriamycin		1×10^{-6}M	-	-	-	60	-	59	-	-

Growth inhibition of 70% or above has been indicated in bold numbers

The mark (-) indicates that particular human cancer cell line was not treated with that particular positive control

as the growth inhibition by this extract was observed in the range of 0-56 per cent. Similarly, aqueous extract from the bulb part of the same plant was found inactive as the growth inhibition by this extract was observed in the range of 0-51 per cent which is not considered active (Table 1). The methanolic stem-leaf extract from *H. antidysenterica* showed *in vitro* anticancer potential against two human cancer cell lines as 82 per cent growth inhibition was observed against THP-1 (a human cancer cell line from leukemia origin) and 78 per cent growth inhibition was observed against COLO-205 (a human cancer cell line from colon origin). However, the aqueous extract from the stem-leaf part of the same plant did not exhibit *in vitro* cytotoxicity against any of the human cancer cell line. The growth inhibition by this aqueous extract was observed in the range of 0-64 per cent, which is not considered significant (Table 2). Cancer is a deadly disease facing the humanity today and has emerged as an important health problem in the developed / developing countries and recognized as the important cause of morbidity, mortality, disability in India also. Cancer originates within a single cell and can be classified by the type of cell in which it originates or by the location of the cell. Presently, more than hundred types of cancer are known, the most commonly occurring ones are breast, colon, cervical, liver, lung, oral, ovary and prostate cancer. In recent years, cancer research has become a major area of scientific research supporting the foundations of modern biology to a great extent. Diverse biological disciplines such as cytogenetics, virology, cell biology, molecular genetics, epidemiology 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 characteristic of cancerous cells. Despite the recent advances in surgery, endocrine therapy, radiotherapy and chemotherapy, it is considered that the management of cancer is still not upto the mark and we are in emergent need of drugs for the treatment of cancer having no side effects. Natural products or related substances or extracts of folk medicine accounted for 30 per cent of the top 35 worldwide natural product-based drugs sold and it has been estimated that > 50 per cent of all patients diagnosed with cancer explore complementary and alternative medicine – especially herbal medicine. Current evidence suggests that garlic, green tea, tomatoes and soy intake as part of the diet may be useful in preventing various cancers. A number of exciting researches suggest that vegetables, fruits, whole grains, herbs, nuts and seeds contain an abundance of polyphenolic compounds, terpenoids, sulphur compounds, pigments and other natural antioxidants, that have been associated with protection from or treatment of conditions such as cancer. Therefore, we can say that natural products have been a prime source of highly effective conventional drugs for the treatment of many forms of cancer. In the search of potential anticancer agents from natural products, the present research work was carried out to examine the *in vitro* cytotoxic potential of Jammu botanicals against human cancer cell lines originated from different tissues.

To conclude, the present study demonstrated significant *in vitro* antitumor potential of *H. antidysenterica* and active ingredients from the promising methanolic extract of the plant can act as lead molecules for the development of anticancer drugs to provide a great service and promise to cancer patients.

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REFERENCES

Ali, K.M., Chatterjee, K., De, D., Bera, T. K. and Gosh, D. (2009). Efficacy of aqueous extract of seed of *Holarrhena antidysenterica* for management of diabetes in experimental model rat: A correlative study with antihyperlipidemic activity. *Internat. J. Appl. Res. Nat. Prod.,* **2**: 13-21.

Ballal, M., Surjan, D., Bhat, K.K., Shirwaikar, A. and Shivananda, P.G. (2001). Antibacterial activity of *Holarrhena antidysenterica* [Kurchi] against the enteric pathogens. *J. Pharmacol.,* **33**: 392-393.

Chu, Q., Ling, M. T., Feng, H., Cheung, H.W., Tsao, S.W., Wang, X. and Wong, Y.C. (2006). A novel anticancer effect of garlic derivatives: inhibition of cancer cell invasion through restoration of *E- cadherin* expression. *J. Carcinog.,* **27**: 2180-2189.

Hahn, G. (1996). Garlic: The science and therapeutic application of *Allium sativum* L. and related species. In: Koch, H.P., Lawson, L.D. (Eds.). pp. 1-24. Baltimore Williams and Wilkins.

Herman-Antosiewicz, A. and Singh, S.V. (2004). Signal transduction pathways leading to cell cycle arrest and apoptosis induction in cancer cells by *Allium* vegetable-derived organosulfur compounds: a review. *Mut. Res.*, **555**: 121–131.

Jabar, M.A. and Al-Mossawi, A. (2007). Susceptibility of some multiple resistant bacteria to garlic extract. *Afr. J. Biotechnol.*, **6**: 771-776.

Kandil, O., Radwan, N. M., Hassan, A. B., Amer, A.A.M., El-banna, H.A. and Amer, W.M.M. (1994). Extracts and fractions of *Thymus capitatus* exhibit antimicrobial activities. *J. Ethnopharmacol.*, **45**: 97-111.

Kavitha, D., Shilpa, P.N. and Devraj, S.N. (2004). Antibacterial and antidiarrhoeal effects of alkaloids of *Holarrhena antidysenterica* Wall. *Indian. J. Exp. Biol.*, **42**: 589-594.

Lamm,D.L. and Riggs, D.R. (2001). Enhanced immunocompetence by garlic: role in bladder cancer and other malignancies. *J. Nutr.*, **131**: 1067–1070.

Linnaeus, C. (1957). *Species planatarum*: a facsimile of the Ist Ed. LONDON, U.S.A.

Mohammad, A., Faruqi, F.B. and Mustafa, J. (2010). Synthesis, characterization, anti-tumor and anti-microbial activity of fatty acid analog of 2,6-diisopropylphenol. *J. Adv. Sci. Res.*, **1**: 12-18.

Monks, A., Scudiero, D., Skehan, P., Shoemaker, R., Paull, K., Vistica, D., Hose, C., Langley, J., Cronise, P., Vaigro-Wolff, A., Gray-Goodrich, M., Campbell, H., Mayo, J. and Boyd, M. (1991). Feasibility of a high-flux anticancer drug screen using a diverse panel of cultured human tumor cell lines. *J. Natl. Cancer Inst.*, **83**: 57-66.

Skehan, P., Storeng, R., Scudiero, D., Monks, A., McMohan, J., Vistica, D., Warren, J. T., Bokesch, H., Kenney, S. and Boyd, M.R. (1990). New colorimetric cytotoxicity assay for anticancer-drug screening. *J. Natl. Cancer Inst.*, **82**: 1107-1112.

Talebi, S., Afshari, J.T., Rakhshandeh, H., Seifi, B. and Boskabadi, M. H. (2006). *In vitro* antiproliferative effect of fresh red garlic on human transitional cell carcinoma (TCC-5637 cell line). *Internat. J. Agric. Biol.*, **8**: 609-614.

Thomson, M. and Ali, M. (2003). Garlic [*Allium sativum*]: a review of its potential use as an anti-cancer agent. *Curr. Cancer Drug Tar.*, **3**: 67–81.

Wahab, M.A. and Yousaf, M. (2004). Ethano-medical uses of some medicinally active plants for treating diabetes in Chittagong Hill Tracts, Bangladesh. *Hamdard Medicus*, **47**: 36-38.

Weisberger, A.S. and Pensky, J. (1958). Tumor inhibition by sulfhydryl-blocking agent related to an active principle of garlic (*Allium sativum*). *Cancer Res.*, **18**: 1301-1308.

You, W. C., Blot, W. J., Chang, Y. S., Ershow, A., Yang, Z. T., An, Q., Henderson, B. E., Fraumeni, J. F. and Wang, T. G. (1989). *Allium* vegetables and reduced risk of stomach cancer. *J. Natl. Cancer Inst.* **81**: 162–164.

Zahin, M., Aqil, F. and Ahmad, I. (2009). *In vitro* antioxidant activity and total phenolic content of four Indian medicinal plants. *Internat. J. Pharm. Pharm. Sci.*, **1**: 88-95.