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Anticancer Potential of potent extracts of *Bergenia stracheyi*

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ABSTRACT
Medicinal plants have been a valuable source of natural products for maintaining human health, especially in the last decade, with more intensive studies for natural therapies. Many plants have been used because of their pharmacological traits, which are due to compounds synthesized in the secondary metabolism of the plant. In the present study, Bergenia stracheyi solvent extracts were screened for anticancer potential against liver (hep-2) cancer cell lines. it was observed that, liver (hep-2) cell line showed 65.78 % cell viability after treatment with *Bergenia stracheyi* hydro-alcoholic extract (20µg/ml) as determined by Trypan blue dye exclusion technique. The cytotoxicity assay of the *Bergenia stracheyi* hydro-alcoholic extract was determined against liver (hep-2) cell line, 50% cyto-toxicity was observed in 20 µg/ml with respect to hepatic cancer cell line studied. The standard positive drug viz. cyclophosphamide monohydrate showed 50% cyto-toxicity at 10 µg/ml against liver (hep-2) cell line. Thus, the IC50 value of the *Bergenia stracheyi* hydro-alcoholic extract was found to be 20µg/ml against hepatic cancer cell lines and IC50 values of cyclophosphamide monohydrate was found to be 10 µg/ml against liver (hep-2) cell line. The *Bergenia stracheyi* hydro-alcoholic extract (20µg/ml) was screened against cancer cell lines viz. liver (hep-2) cell line respectively via Sulphorodamine B assay and Microculture tetrazolium (MTT) assay. It was found that the extract was effective against hepatic cancer cell lines studied. It was found that the results of both the tests viz. Sulphorodamine B and MTT assay are correlated to each other. It was found that, the *Bergenia stracheyi* hydro-alcoholic extract (20µg/ml) causes 72.56 % inhibition of cancer cells in liver (hep-2) cancer cell line as observed by Sulphorodamine B assay.

Keywords: Anticancer potential, Bergenia stracheyi, solvent extracts, hepatic cancer cell lines

INTRODUCTION
Plants have served as a source of new pharmaceutical products and inexpensive starting materials for the synthesis of some known drugs. Components with medicinal properties from plants play an important role in conventional Western medicine [1]. At least 25% of Western medicine issued in the US and Canada were derived from or modelled after plant natural products and 119 secondary metabolites were used globally as drugs. The Himalayas have a great wealth of medicinal plants and traditional medicinal knowledge. The Central Himalayan Region covers the new state of Uttaranchal, which includes the major divisions of Kumaun and Garhwal. This region has played a significant role in the historical processes of Northern India and provides a mini model for understanding the Indian civilizational processes (Bist, 1994). Through the millennia different tribes and people - Protoaustroloids, Mundas, Kiratas, Mongoloids, Indo-Aryans, Khasas, Sakas and others - have been coming in and leaving their signatures and producing a mosaic of cultures [2]. Antioxidants help organisms deal with oxidative stress, caused by free radical damage. Free radicals are chemical species, which contains one or more unpaired electrons due to which they are highly unstable and cause damage to other molecules by extracting electrons from them to attain stability. In the present study, the plant extracts of *Bergenia stracheyi* were screened *in vitro* for human hepatic cancer cell lines liver (hep-2).

MATERIALS AND METHODS
The methanolic extract (SNME) of the plant extracts were further used to determine the anticancer activity. Different assays were done to determine the anticancer activity using two Human hepatic cancer lines (hep-2) [3-5].

Trypan blue dye exclusion technique
The cell suspension was prepared using fixed volume of cells (e.g. 1 ml). The sterility should be maintained throughout the procedure. Take 50 µL of cell suspension and mixed it with an equal volume of trypan blue. The solution was mixed well. Transferred to a hemocytometer and counted the live cells as clear form and dead cell as blue cells. After staining with trypan blue solution counting should commence less than 5 minutes. Using a pipette placed some of the cell suspension: trypan blue mixed into the hemocytometer and overlaid with a coverslip. The cell suspension was passed under the coverslip by capillary action unless there is an air bubble. Make sure that the wells are not overfilled, and that the coverslip is not moved once it is placed on the grid and the cell solution is added. Placed the hemocytometer on the stage of an inverted microscope. Adjusted focus and power until a single counting square fills the field.

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The numbers of viable cells were calculated (Masters, 2000) using the following formula:
Percent viability = [(live cell count/total cell count)]x 100

Determination of IC50 values/Cytotoxicity assay

The cytotoxicity property of SNME (crude extract) sample was determined against liver (hep-2) cell lines. The 2 mg of sample was dissolved in 200µl of DMSO (dimethyl sulfoxide) then 100µl of this solution was diluted to 10 ml with RPMI-1640 medium (containing 2 mM L-glutamine) the final concentration of this stock solution as 100µg/ml. Then,by serial dilution, varying concentrations were prepa red from the stock solution. The concentrations of SNME (crude extract) sample prepared were 100, 90, 80, 70, 60, 50, 540, 30, 20 and 10 µg/ml. Similar dilutions of the standard drug (positive control) viz. Cyclophosphamide monohydrate were also prepared. For negative control, 100 µl of DMSO was diluted to 10 ml with RPMI-1640 medium (containing 2 mM L-glutamine; pH 7.2).

The cells were incubated at a concentration of 1×10^6 cells/ml in culture medium for 3 h at 37°C and 5% CO2. Cells were seeded at a concentration of 5×10^4 cells/well in 100 µl culture medium and various amounts of compound (final concentration viz. 10-100 µg/ml) into micro-plates. Taken 50µL of cell suspension and mixed it with an equal volume of trypan blue. The solution was mixed well. Transferred to a hemocytometer and counted the live cells as clear form and dead cell as blue cells. Using a pipette placed some of the cell suspension: trypan blue mixed into the hemocytometer and overlaid with a coverslip. The cell suspension was passed under the coverslip by capillary action unless there is an air bubble. Care should be taken, that, the wells are not overfilled and that the coverslip is not moved once it is place on the grid and the cell solution is added. Placed the hemocytometer on the stage of an inverted microscope. Adjusted focus and power until a single counting square fills the field. The concentration at which 50% density of the population of the cancer cells gets inhibited in the cancer lines is known as IC50 values.

a) Sulphorodamine B assay

The anti-proliferative SRB assay was performed to assess growth inhibition. This is a colorimetric assay which estimates cell number indirectly by staining total cellular protein with the SRB dye (Skehan, 1990). The microtiter plates were taken out after 48 hours incubation of the both the cell lines with SNME (crude extract) sample and gently layered with chilled 50% TCA in all the wells to produce a final concentration of 10%. The 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, growth medium, low molecular weight metabolites, serum proteins etc. 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 30 minutes. The unbound SRB was removed quickly by washing the wells five times with 1 % acetic acid and then air dried. 100 µl of Tris buffer (0.01 M, pH 10.4) was added and shaken gently for 5 minutes on a mechanical shaker. Optical density was recorded on ELISA reader at 515 nm.

The percent inhibition of cancer cells was determined (Masters, 2000), using the following formula:
% inhibition of cancer cells = [(Total initial cell count- killed cell count)/Total initial cell count] x10

b) Micro culture tetrazolium (MTT) assay

The monolayer cell culture of each of the cell lines viz. liver (hep-2) and colon (502713 HT-29) were trypsinized and the cell count was adjusted to 3×10^5 cells/ ml using medium containing 10% newborn calf serum. To each well of 96 well microtiter plates, 0.1 ml of diluted cell suspension was added. After 24 hours, when the monolayer formed the supernatant was flicked off and 100 µl of extracts were added to the cells in microtitre plates and kept for incubation at 37ºC in 5 % CO2 incubator for 72 hour and cells were periodically checked for granularity, shrinkage, swelling. After 72 hour, the sample solution in wells was flicked off and 50µl of MTT dye was added to each well. The plates were gently shaken and incubated for 4 hours at 37ºC in 5% CO2 incubator. The supernatant was removed, 50 µl of propanol was added, and the plates were gently shaken to solubilize the formed formazan. The absorbance was measured using a microplate reader at a wavelength of 490 nm (Mosmann, 1983; Wilson, 2000; Masters, 2000).

The percent inhibition of cancer cells was determined (Masters, 2000), using the following formula:
% inhibition of cancer cells = [(Total initial cell count- Killed cell count)/Total initial cell count] x100

RESULTS AND CONCLUSION

The results of the study showed significant anticancer potential of solvent extracts of *Bergenia stracheyi* in liver cancer cell lines as determined in vitro. In the studies, the estimations were done as per absorbance values. The results of the study suggested that absorbance values which are lower as observed than the control cells indicate a reduction in the rate of cell proliferation. Conversely, a higher absorbance rate indicates an increase in cell proliferation. In-vitro viable cells of liver (hep-2) cell lines were determined. Percentage of viable cell was obtained by performing trypan blue dye exclusion

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technique. The cytotoxicity activity was carried out by using SRB assay and MTT assay. From the results as mentioned in **Table 1,** it was observed that, liver (hep-2) cell line showed 65.78 % cell viability after treatment with *Bergenia stracheyi* hydro-alcoholic extract (20µg/ml) as determined by Trypan blue dye exclusion technique. It was determined that the viable shows pink color after treating with the dye while non-viable cells showed blue color.

When the cytotoxicity assay of the *Bergenia stracheyi* hydro-alcoholic extract was determined against liver (hep-2) cell line, 50% cyto-toxicity was observed in 20 µg/ml with respect to hepatic cancer cell line studied. The standard positive drug viz. cyclophosphamide monohydrate showed 50% cyto-toxicity at 10 µg/ml against liver (hep-2) cell line. Thus, the IC50 value of the *Bergenia stracheyi* hydro-alcoholic extract was found to be 20µg/ml against hepatic cancer cell lines and IC50 values of cyclophosphamide monohydrate was found to be 10 µg/ml against liver (hep-2) cell line. DMSO did not show any cyto-toxic values. The results are shown in **Table 2.**

The numbers of cells (cells concentration) were increased in hepatic cancer cell lines before the anticancer assay. The *Bergenia stracheyi* hydro-alcoholic extract (20µg/ml) was screened against cancer cell lines viz. liver (hep-2) cell line respectively via Sulphorodamine B assay and Microculture tetrazolium (MTT) assay. It was found that the extract was effective against hepatic cancer cell lines studied. It was found that the results of both the tests viz. Sulphorodamine B and MTT assay are correlated to each other. It was found that, the *Bergenia stracheyi* hydro-alcoholic extract (20µg/ml) causes 72.56 % inhibition of cancer cells in liver (hep-2) cancer cell line as observed by Sulphorodamine B assay. The positive control, cyclophosphamide monohydrate (5 mg) showed 95% inhibition of cancer cells in liver (hep-2) cancer cell lines respectively as determined by Sulphorodamine B assay. The results are shown in **Table 3.**

The *Bergenia stracheyi* hydro-alcoholic extract (20µg/ml) causes 74.45 % reduction of cancer cells in liver (hep-2) cancer cell line as observed by MTT assay. The positive control, cyclophosphamide monohydrate (5 mg) showed 87% in liver (hep-2) cancer cell lines respectively as determined by MTT assay. The results are shown in **Table 4.** The results of the study revealed that, polar and non-polar extracts of whole plant, *Bergenia stracheyi* possessed anticancer activity as revealed in the study. The results of the study can act as a basis to isolate and identify such genes in order to develop the technology for fingerprinting multiple metabolites of *Bergenia stracheyi* to study different pharmacological properties [6-12].

Table 1: Percent cell viability and characterization of cell lines via Trypan blue assay (before anticancer assay)

Cell lines	**Percent viability**	**Live cell count**	**Total cell count**	**pH**
Liver (Hep-2)	65.78	$2.68x10^5$	$3x10^5$	7.2

Table 2: Cytotoxicity assay of *Bergenia stracheyi* extract against cancer cell lines (IC50 values determination)

Samples	**IC50 values (µg/ml)**
	Cancer cell lines used
	Liver (Hep-2)
Cyclophosphamide monohydrate	10.0
Bergenia stracheyi extract	20.0
DMSO	0.0

Table 3: Sulphorodamine assay in hepatic (hep-2) cell line

	Percent inhibition of cancer cells	
Sulphorodamine B assay In hepatic (hep-2) cell line	***Bergenia stracheyi* extract (20µg/ml)**	**Cyclophosphamide monohydrate (5 mg)**
	72.56	95.0

Table 4: MTT assay in hepatic (hep-2) cell line

	Percent inhibition of cancer cells	
MTT assay In hepatic (hep-2) cell line	***Bergenia stracheyi* extract (20µg/ml)**	**Cyclophosphamide monohydrate (5 mg)**
	74.45	87.0

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