INTRODUCTION
Herbal drugs have been used since ancient times as medicines for the treatment of a range of diseases. Medicinal plants have played a key role in world health. An increasing number of research papers and reviews clearly indicate that medicinal plants exhibit a variety of therapeutic properties (Ahmad et al. 1998; Datta et al. 1998; Abou et al. 2000; Graf, 2000; Ankli et al. 2002; Neto et al. 2002) and provide health security to rural people in primary health care. In vitro cytotoxicity screening models provide important preliminary data to help select plant extracts with potential antineoplastic properties for future work (Cardellina et al. 1999). Bilal et al. 2003 showed the immunomodulatory effects of kengreek (Trigonella foenum graecum L) extract in mice. In Indian medicine both aqueous and ethanolic extracts are used for most of these species. Hence, two different extracts of each plant were made, one with hot water and other with ethanol, except in case Solanum nigrum where only alcoholic extract were used. Most of the negative results were neglected.

METHODS
Plant material:
The parts of each species, reported to be used against cancer in Indian folk medicines were collected from February to March 2004. The places of collection were from Chitrakoot region and Allahabad of Uttar Pradesh. Leaf of Calotropis procera, seed and inflorescence of Ocimum sanctum and leaf of Solanum nigrum, leaves and inflorescence of Cannabis sativa, seeds of Trigonella foenum graecum and leaves of Chenopodium rubrum were used. Authentication of plant materials were carried out at the herbarium of the Centre for Biotechnology, University of Allahabad, Allahabad. A duplicate set has been deposited in herbarium of the Centre.

Preparation of plant extracts:
Plant materials were dried at 37°C, powdered and extracted in different solvent. The aqueous SWW extract was obtained by boiling dried ground plant material (100 g) for 30 minutes in distilled water (300 ml). All extracts were fine-filtered and freeze-dried. For the ethanolic extracts, dried ground plant material (100 g) was percolated with 95% ethanol and concentrated to dryness under reduced pressure. The aqueous extracts were dissolved in sterile water and the ethanolic extracts in Dimethyl sulfoxide (DMSO) to form stock solutions 20mg/ml which were filter sterilized (0.2 μm) before testing on cell lines.

Human cell lines:
HT-15 and HT-29 human cancer cell lines of colon grown in RPMI medium were obtained from National Centre for Cell Sciences, Ganeshkhind, Pune, India. IMR-32 neuroblastoma cell line was grown in Minimal Essential Media (MEM) obtained from Centre for Cellular and Molecular Biology, Hyderabad, India and A-549 which was grown in RPMI was obtained from National Cancer Institute, DTCB, Frederick Cancer Research & Development Center, Madison, USA.

Preparation of test material:
Stock solution: Stock solution of 20 mg/ml were prepared. DMSO was used for 95% reconstitution of MeOH/EOH extract, and distilled water for hot aqueous extract. Stock solutions were prepared one day in advance. Multiple aliquots of each sample were stored for initial tests and retests, if necessary. Stock solutions were filtered sterilised and microbial contamination was controlled by addition of gentamycin to the complete growth medium.

Working test solution: On the day of assay, thaw an aliquot of frozen stock solution at room temperature. Prepared 100 μg/ml concentration of the extract by serial dilution of stock solution using the complete growth medium containing 50 mg/ml of gentamycin.

Positive controls
The positive controls used was Mitomycin-C.

In vitro Assay for cytotoxic activity
The anticaner activity is determined by the cytotoxic potential of the test material using human cancer cell lines which were allowed to grow on tissue culture plates in the presence of test material. The cell growth was measured on ELISA reader after staining with Sulforhodamine B dye (SRB) which binds to basic amino acid residues in the trichloroacetic acid (TCA) fixed cells.

Preparation of Cell suspension for assay:
The desired human cancer cell line were grown in multiple TCPS at 37°C in an atmosphere of 5% in CO2 and 90% relative humidity in complete growth medium to obtain enough number of cells as per requirement depending upon number of test samples. The flask with cells at sub-confluent stage were selected. Cells were harvested by treatment with Trypsin- EDTA and added to complete growth medium to stop the action of trypsin. Cells were separated to single cell suspension by gentle pipetting action and the viable cells were counted in a hemocytometer using trypan blue. Cell viability at this stage should be >97%. Viable cell density was adjusted to 5,000 - 40,000 cells/100μl depending upon the cell line (Monks et. al, 1991). Cell suspension is ready for addition to tissue culture plates. 100μl of cell suspension together with 100μl of complete growth medium was added into each well. The plates were incubated at 37°C for 24 hours in an atmosphere of 5% CO2 and 90% relative humidity in a CO2 incubator. After 24 hours, the test material, DMSO (vehicle control) and positive controls were added.
Addition of test materials

Added 100μl of working solutions of the test materials and positives controls along with equivalent complete growth medium into these wells in the tissue culture plate. It was prepared 24 hours in advance containing either cells or complete growth medium in a final volume of 100 μl. The plates were incubated at 37°C for 48 hours in an atmosphere of 5% CO₂ and 90% relative humidity. The cell growth was determined after 48 hours by SRB assay.

Sulforhodamine B (SRB) assay

Assay was carried out as described by Skehan et al (1990) using SRB dye. The microtiter plates were taken out after 48 hours incubation of cells with test materials and gently layered with 50μl of chilled 50% TCA on top of the medium in all the wells to produce a final concentration of 10%. Tissue culture plates were incubated at 4°C for one hour to fix the cells attached to the bottom of the wells.

All the contents of all the wells were pipetted out gently and the supernatant was discarded. The plates were washed five times with distilled water to remove TCA, growth medium, low molecular weight metabolites, serum proteins etc. For washing, the wells of tissue culture plates were filled with distilled water and then discarded the excess liquid in the wells by sharply flicking plate over a sink. Plates were air dried and stored until use. 100μl of SRB solution was added to each well of the plates and incubated at room temperature for 30 minutes. The unbound SRB was removed quickly (to avoid desorption of protein bound dye) by washing the wells five times with 1% acetic acid and then the plates were air dried.

100μl 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 and then the data was recorded.

Calculations

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

Percent growth in presence of test material

\[ \text{Percent growth in presence of test material} = \frac{\text{Growth in presence of test material}}{\text{Growth in absence of test material}} \times 100 \]

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

100- Percent growth in presence of test material

Criteria for Determination of Activity: The test sample showing growth inhibition of >70% at 100 μg/ml is considered to be active. Following table describes the results of in vitro cytotoxicity studies carried out against human cancer cell lines in the present investigations.

RESULTS AND DISCUSSION

The foregoing experiment shows various levels of in vitro cytotoxic activities of the alcoholic extracts, hot water extracts of different plants / their parts. The observations from the table 1, showed very few of them are active against cancer cell lines. This implies that the active extracts have specific cytotoxic activities against specific cell lines and that they are not generally cytotoxic. The activity was done using 100μg/ml.
The in-vitro cytotoxicity was performed on six Indian medicinal plants against four cell lines namely of lung (A-549), colon (HT-15, HT-29) and neuroblastoma (IMR-32).

The plant Ocimum sanctum, Calotropis procera, Cannabis sativa, Trigonella foenum graecum shows more than 70% of growth of inhibition and hence it has anticancer activity, Solanum nigrum, showed less than 70% of growth inhibition and therefore, these are showing no activity against any cell line. Chenopodium rubrum showed the activity against colon cell lines HT-29, HT-15 but had no activity against the cell lines, neuroblastoma IMR-32 and lung cancer cell line A-549.

Hence, the different extracts of plant in different solvents have cytotoxic activity showing a certain degree of selectivity against the different cell types. Alcoholic extract showed more degree of inhibition against the cell lines while aqueous extracts showed lesser degree of inhibition. It might be because the metabolites which are active against the cell line are best extracted in non polar solvents while some in polar solvents when it is hot extracted.

This piece of work shows that the different plant extracts used in our study respond very differently to the different cancer cell lines possibly since their specificity for these cell lines are different.

REFERENCES