Short communication

**In Vitro** screening for anticancer activity of petroleum ether and ethyl acetate extracts of *Conyza canedensis* growing in Kashmir region.

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**Abstract**

Cancer is a major cause of death throughout the world. In the U.S., the number of deaths caused by cancer is second highest to that from the cardiovascular disease. This study was an attempt towards the *in vitro* screening of petroleum ether and ethyl acetate extracts of root parts of *Conyza canedensis* on various human cancer cell lines viz; Neuroblastoma (SF-295, IMR-32, SK-NSH), Prostate (PC-3), Lung (A549) and Breast (MCF-7). Cell viability was evaluated by MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay. Petroleum ether extract was found to be significantly effective against Neuroblastoma (IMR-32, SK-NSH) and Prostate (PC-3) cell lines at all the three concentrations, with a maximum inhibitory effect of 87% against Neuroblastoma (IMR-32) cell line at a concentration of 100 µg/ml, but exhibited no marked inhibition against Neuroblastoma (SF-295) and Lung (A549) cell lines at lower doses, except of 55% and 54%, respectively at 100 µg/ml concentration.

**Keywords:** *Conyza canedensis*, Neuroblastoma, Ethyl acetate extract, Anticancer activity.

**Introduction**

Medicinal plants have proved to be promising source of novel chemotherapeutic agents against various diseases including cancer and interestingly about 60% of anticancer drugs used nowadays are obtained from natural sources [1].

Interest in herbal drugs and natural medicine is undergoing a renaissance in the present age. Higher plant derivatives represents around 25% of the total number of clinically used drugs [2]. It has been reported that plants and other sources of natural products are superior sources of molecular diversity and novel molecular chemo-types, particularly in the areas where potential synthetic leads do not exist [3]. About 250000 living plant species contain a much greater diversity of bioactive compounds than any chemical library made by humans.

Cancer is a major cause of death through the out world. In the U.S., the number of deaths caused by cancer is second highest to that from the cardiovascular disease [4]. Cancer can develop in almost any organ or tissue, such as the lung, colon, breast, skin, bones or nerve tissue [5].

Further, the chemical modification of plant derived natural products has resulted in better anticancer activity well exemplified by topotecan and irinotecan, the synthetic derivatives of camptothecin [6]. Therefore, to achieve better therapeutic impact, i.e., lower sensitivity and higher efficacy, chemical modification of natural products apparently is an interesting proposition.

Genus *Conyza* (*Family* Astereaceae) consists of more than 70 species and is an annual or perennial plant. The plants are mainly distributed in tropical and sub-tropical regions.
The species *Conyza canadensis* Linn. is native to North America and is distributed in almost all parts of the world. In India, *Conyza canadensis* is widespread in northern areas. The whole plant is locally used for the treatment of edema, hematuria, hepatitis and cholecystitis [7].

Earlier, aerial parts of *Conyza canadensis* have been shown to possess sphingolipids [8-9], phenolic acids [9], steroids and triterpenoids [9-10], acetylenes [10] and phenyl esters [11].

**Experimental**

**Plant Material**

The root part of *Conyza canadensis* (5.4 Kg) were collected from Hazratbal, Srinagar (J&K, India) in June 2007. The specimen was identified by Akhtar H. Malik, Curator, Centre for Biodiversity & Taxonomy, University of Kashmir (Specimen deposited under accession No. 33214 and Collection No. 1202- Javid, Kash).

**Preparation of Extracts**

The air dried, finely powdered root material (1Kg) was extracted for 72 hours sequentially with petroleum ether (60-80°C) and ethyl acetate in a soxhlet apparatus to afford the respective extracts, which were concentrated under reduced pressure.

**Cell culture**

The cell lines used were human Neuroblastoma (SF-295, IMR-32, SK-NSH), Prostate (PC-3), Lung (A549) and Breast (MCF-7). These cell lines were maintained in cell culture as recommended by the ATCC (LGC Promochem GmbH, Wesel Germany) and were grown in DMEM, medium supplement with 10% heat inactivated fetal bovine serum, 1% of 2 mmol/l L-glutamine, 50 U/ml penicillin, and 50 µg/ml streptomycin. Cells were maintained in humidified atmosphere in 5% CO₂ at 37°C.

**In vitro anti-proliferative assay**

All the human cancer cell lines Neuroblastoma (SF-295, IMR-32, SK-NSH), Prostate (PC-3), Lung (A549) and Breast (MCF-7) were obtained from ATCC via Sigma/Aldrich St. Louis, Mo, USA. Cells used were grown in RPMI-1640 medium containing 10% foetal bovine serum (FBS), 100 unit penicillin/100 mg Streptomycin per ml medium. Cells were allowed to grow in carbon dioxide incubator (Thermo scientific USA) at 37°C with 98% humidity and 5% CO₂ gas environment in case of MTT assay. In the present study, all cell lines seeded in flat-bottomed 96-well plates were allowed to adhere overnight, and then media containing different samples (varying concentrations) were added. Cell viability of the extract treated cells was measured by using MTT assay. Briefly, cells (10⁴ cells/well) were cultured in 96 well tissue culture plates and treated with different concentrations of extracts ranging from 30 µg/mL, 50 µg/mL and 100µg/mL for 48 h. At the end of incubation, 20 µL of MTT (2.5mg/ml) was added to the wells and incubated for 4 h. After incubation the media was aspirated out and 150µl of DMSO was added in-order dissolve the formazan crystals. Absorbance was recorded at 570 nm using ELISA Plate Reader. Adriamycin (1×10⁻⁶µg/mL), Mitomycin (1×10⁻⁶µg/mL and 5-FU (2×10⁻⁵µg/mL) were used as positive controls.

**Results and Discussion**

Anticancer activity of petroleum ether and ethyl acetate extracts of root parts of *Conyza canadensis* was performed on various human cancer cell lines viz: Neuroblastoma (SF-295, IMR-32, SK-NSH), Prostate (PC-3), Lung (A549) and Breast (MCF-7) at different concentrations (30 µg/ml, 50 µg/ml and 60 µg/ml). The cells were treated with test compounds (dissolved in DMSO) at the above said concentrations and kept in serum media for 48h to observe anti-proliferative activity using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay. The results revealed significant sensitization of the cell lines by inhibiting their cell growth (Table 1).

Petroleum ether extract was found to be significantly effective against Neuroblastoma (IMR-32, SK-NSH) and Prostate (PC-3) cell lines at all the three concentrations, with a maximum inhibitory effect of 87% against Neuroblastoma (IMR-32) cell line at a concentration of 100 µg/ml, but exhibited no marked inhibition against Neuroblastoma (SF-295) and Lung (A549) cell lines at lower doses, except of 55% and 54%, respectively at 100 µg/ml concentration (Figure 1).

Both extracts CCP and CCE displayed negligible inhibitory effect against Breast (MCF-7) cell line at all concentrations, except an inhibition of 62%, exhibited by CCP at 100 µg/ml concentration.

CCE displayed marked inhibitory effect against Prostate (PC-3) cell line at all the three concentrations with a maximum inhibitory effect of 97% at 100 µg/ml concentration. It was found to be in effective against all other cell lines at 30 µg/ml concentration. At 50 µg/ml concentration, CCE showed a marked inhibitory effect of 50%, 62%, 65%, 53% against Neuroblastoma (IMR-32, SK-NSH), Prostate (PC-3) and Lung (A549) cell lines.
respectively. CCE displayed significant inhibition of 82%, 93%, 97% 97% and 62% against Neuroblastoma (SF-295, IMR-32, SK-NSH), Prostate (PC-3) and Breast (MCF-7) cell lines, respectively, at 100 µg/ml concentration, with a maximum inhibitory effect of 100% against Lung (A549) cell line (Figure 2).

Table 1: Cytotoxic activity at 30 µg/mL, 50 µg/mL and 100 µg/mL of petroleum ether (CCP) and ethyl acetate (CCE) extracts of Conyza canadensis against SF-295, IMR-32, SK-NSH, PC-3, A-549 and MCF-7 cell lines with MTT assay. Adriamycin (1×10⁻⁶ µg/mL), Mitomycin (1×10⁻⁶ µg/mL and 5-FU (2×10⁻⁵ µg/mL) were used as positive controls.

<table>
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<th>PC-3</th>
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<td>Prostate</td>
<td>Lung</td>
<td>Breast</td>
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<td>% Growth Inhibition</td>
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<td>76</td>
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<tr>
<td>5-FU</td>
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</tbody>
</table>

Figure-1: Cytotoxic activity of petroleum ether extract of Conyza canadensis against SF-295, IMR-32, SK-NSH, PC-3, A-549 and MCF-7 cell lines at three different concentrations.

Figure-2: Cytotoxic activity of ethyl acetate extract of Conyza canadensis against SF-295, IMR-32, SK-NSH, PC-3, A-549 and MCF-7 cell lines at three different concentrations.
Acknowledgments

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Conflict of Interest

The authors declare that there is no conflict of interest to reveal.

References