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In vitro anticancer activity of small peptides from *Tridax procumbens*

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Abstract

Cancer is a hyper proliferative disorder and one of the most devastating diseases in both developing and developed countries. Chemotherapeutic drugs are enormously expensive and are associated with serious side effects. Still, the search continues for an ideal treatment that has minimal side effects and cost-effective. Over 50% of drugs in clinical trials for anti-cancer activity were isolated from natural plant sources. Plant species are enriched with potential anticancer proteins and peptides. *Tridax procumbens* phytochemicals having antiseptic, insecticidal, parasiticidal, anticancer properties, but the anticancer properties of peptides was not investigated. Thus the present investigation was aimed to evaluate anticancer activity of small peptides (SPs) from *T. procumbens*. The leaf, stem and flowers were shade dried and extracted with acetonitrile and formic acetic acid (50:1). The extracted SPs were purified by HPL Cand the purified SPs were tested for its cytotoxicity activity by MTT (-[3-(4, 5-dimethylthiazol-2yl)-2, 5-diphenyl tetrazolium bromide)] assay against human breast cancer metastatic cell line (MDA MB 231). Among all the tested SPs, leaf SPs showed lowest $IC_{50}$with 4µg/ml. In conclusion, the SPs showed potent cytotoxicity against breast cancer which can be further characterised.

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Introduction

Cancer is one of the most devastating diseases in both developing and developed countries. Several chemotherapeutic, cytotoxic and immunomodulation agents are available in Western medicine to treat cancer (Roa *et al.*, 2008). Due to a global increase in life expectancies, the incidents of cancer and related mortality rates are dramatically increasing. Treatment options are enormously expensive and associated with serious side effects and morbidity. Therefore the search continues for an ideal treatment option with minimal side effects and less costs (Newman *et al.*, 2003). Recently, a greater emphasis has been given towards the researches on complementary and alternative medicine that deals with cancer management. Over 50% of drugs in clinical trials for anti-cancer activity were isolated from natural plant sources (Shirisha *et al.*, 2014). The plants produce secondary metabolites which are having medicinal virtues. Besides these, plants also possess small peptides with biological properties such as anti-microbial, anti-viral, haemolytic, immuno suppressor and anticancer (Fu *et al.*, 20111). Plants species which are enriched with anticancer proteins and peptides are belonging to families such as Fabaceae, Brassicaceae, Solanaceae, Asteraceae and Cucurbitaceae (Damme *et al.*, 1998).

Tridax procumbens, belongs to Asteraceae and possesses antiseptic, insecticidal, parasiticidal, anti-cancer properties. The leaf of *T. procumbens* contains26% of crude proteins with wound healing, anti-diabetic, diarrhoea and immunomodulant properties (Nia *et al.*, 2003). In the importance view of *T. procumbens,* the present study was aimed to isolate and evaluate anticancer activity of small peptides from *T. procumbens*.

Materials and methods

Extraction and purification of SPs from T. procumbens

The plant material (leaf, stems and root) was collected from *Jawaharlal Nehru Technological University,* Hyderabad, Telangana, India. The collected samples were cleaned, air dried, and powdered. The powered samples were extracted with acetonitrile and formic acetic acid (50:1) at 1:1 (w/v) concentrations using percolation method.

The extract was filtered through What man No.1 filter paper and the filtrate was concentrated by lyophilizer and stored for further use. The extracted SPs were subjected to purified, first the SPs were captured on DEAE cellulose and polished with RPHPLC. The purified SPs were estimated according to standard procedure and subjected to size exclusion chromatography (SEC)- HPLC (Raymond and Weintraub, 1959;Sampson and Barlow, 1980; Mant, *et al.*, 2007).

Cytotoxicity assays

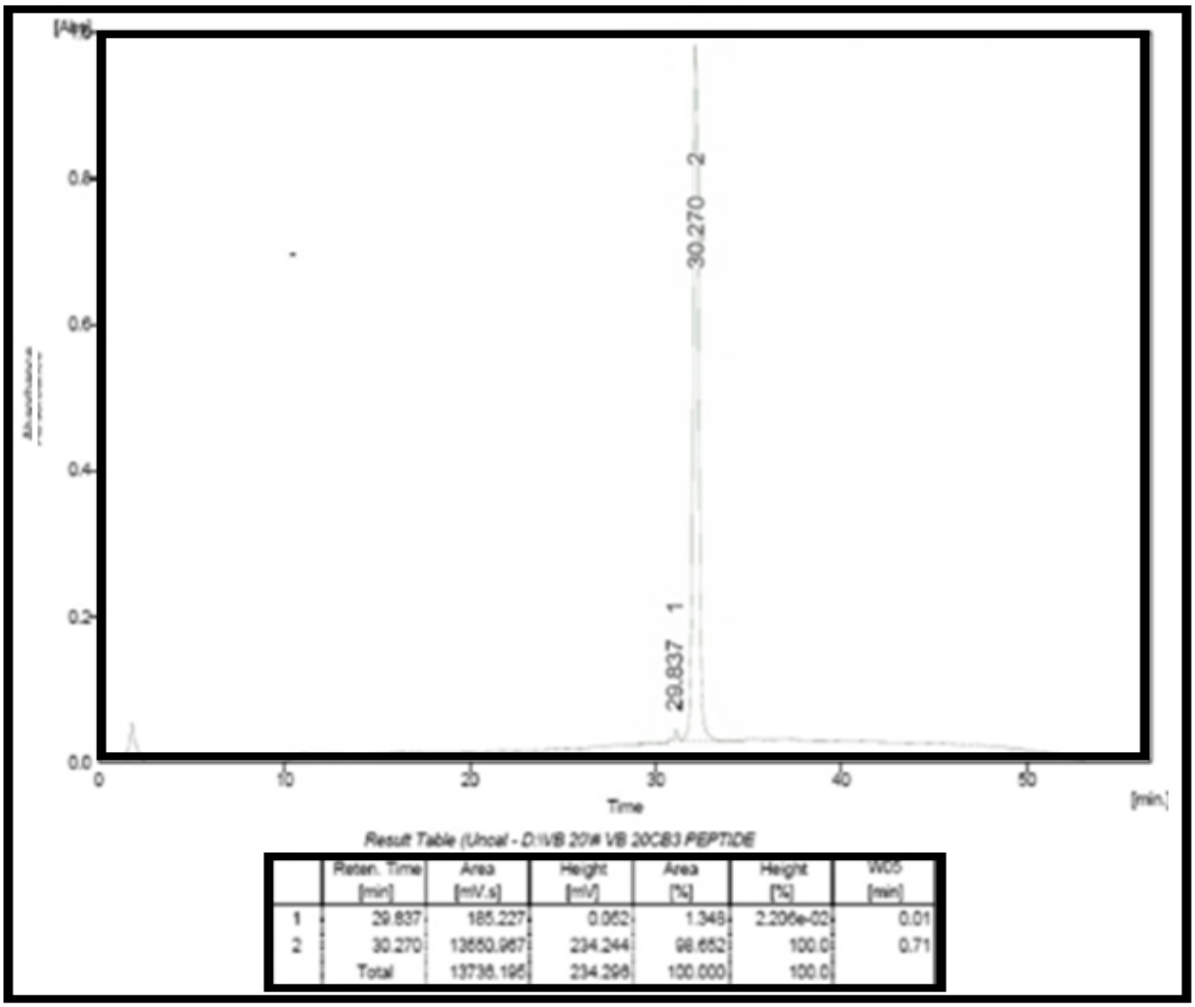
Cytotoxicity assay was performed by using MTT (-[3-(4, 5-dimethylthiazol-2yl)-2, 5-diphenyl tetrazolium bromide)] (Hansen *et al.*, 1989). Briefly, the exponentially growing human breast cancer metastatic cells (MDA MB 231)in log phase were harvested and centrifuged at 1000 rpm,10 minutes, 4°C. To the cell pellet 1ml of DMEM media was added and gently mixed. To a Petri plate, 10ml of sterile media with serum was added and to this, 1ml of cell suspension was added and swirled gently to mix the cells. 100µl of cell suspension was added to each well of a 96well plate (micro titre plate). The plate was incubated at 37°C in a CO_2 incubator for 24 hours. After incubation for 24 hours, different concentrations of SPs were added to the wells and incubated for 24 hours. A control and a blank were maintained simultaneously. After incubation for a specified time period, 20µl of 5mg/ml of MTT, prepared in phosphate buffer saline was added to each well and incubated in a dark chamber for 4 hours. After incubation, 100 µl of DMSO was added to each well to dissolve the for mazan crystals and the optical density (OD) was recorded in ELISA plate reader at 540nm. The percentage viability of the cells was calculated using the formula:

Percentage Inhibition = ODc-ODs×100

Where, ODs = Optical Density of the sample; ODc = Optical Density of the control.

Results and discussion

Extraction of SPs from leaf, stems and root was carried out, among all the samples leaf extraction showed highest yield of 2%. The solvents used for extraction 50% acetonitrile and 1% formic acid in the ratio of 1:1 showed maximum efficiency for extraction of SPs. The extracted SPs were subjected to purification, initially the SPs were captured on DEAE chromatography and polished on RPHPC. Among leaf, stem and flower extracts, leaf showed highest amount of purified SPs compared to stems and root. The concentration of SPs of leaf, stems and root were found to be 100, 50 and 30µg/ml. The purity of the SPs were determined by SEC-HPLC and the highest purity was found in leaf SPs (98.6%)(Fig. 1).



	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]
1	29.837	185.227	0.052	1.348	2.206e-02	0.01
2	30.270	13550.967	234.244	98.652	100.0	0.71
	Total	13736.196	234.296	100.000	100.0	

Fig. 1. SEC-HPLC analysis of leaf purified SPs.

The purified SPs from leaf, stem and flower extracts of *T. procumbens* were evaluated for their anticancer on breast cancer cells. The cells were exposed to different concentrations ranging from 1 to 25µg/ml and cell cytotoxicity was determined by the MTT assay (Fig.2).

The photomicrographs of the untreated cells, treated cells and the cells treated with highest concentration 25µg/ml showed changes in the cell morphology. The treated cells, when compared to the untreated, showed cell shrinkage, detachment from substratum and cell blabbing. In cells treated with 25µg/ml of leaf SPs, the presence of floating or detached dead cells was found (Fig.3).

Protein fraction isolated from *Trigonella foenum graecum* and *Lactuca sativa* seeds showed anticancer activities on HepG2 cell line with IC_{50} values of 6.1 and 7.7 µg/ml. Protein fractions isolated from

Raphanus sativus seeds and from *Linum usitatissimum* seeds showed IC_{50} values of 35.2 and 53.7 µg/ml, respectively (Sara *et al.*, 2014). In contrast to earlier research, the present study showed IC_{50} values of leaf, stems and root SPs were found to be 4, 4.9 and 4.33µg/ml respectively. The leaf SPs was found to have more cytotoxicity effect when compared rest of the two samples.

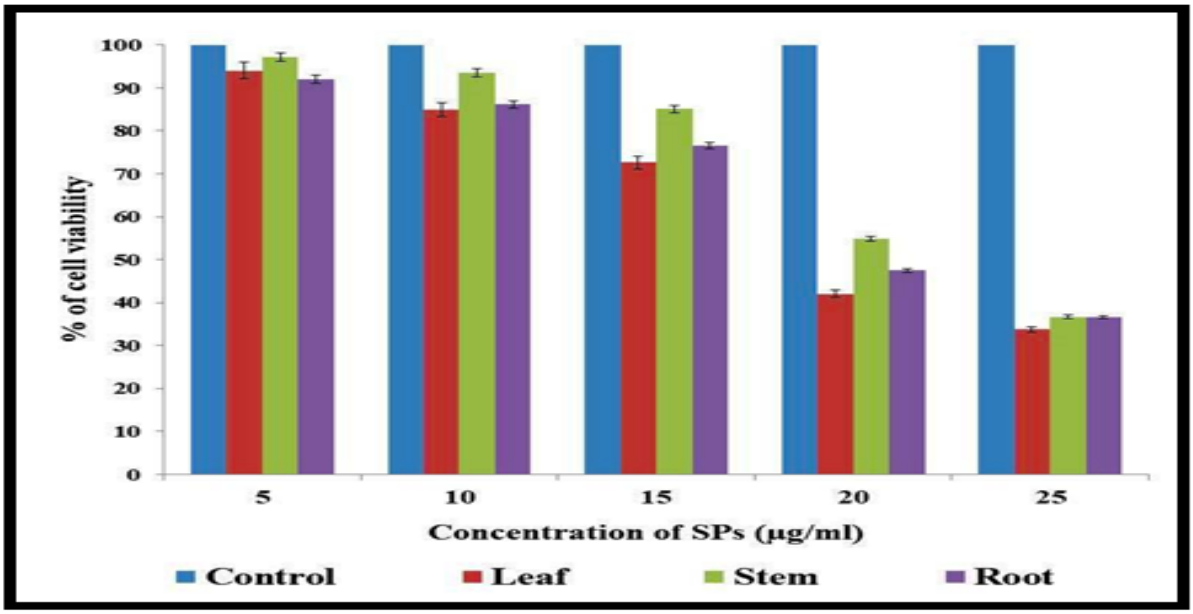


Fig. 2. Cytotoxic effect of purified leaf, stem, root SPs from *T. procumbens*: All results are mean±SE of three consecutive experiments.

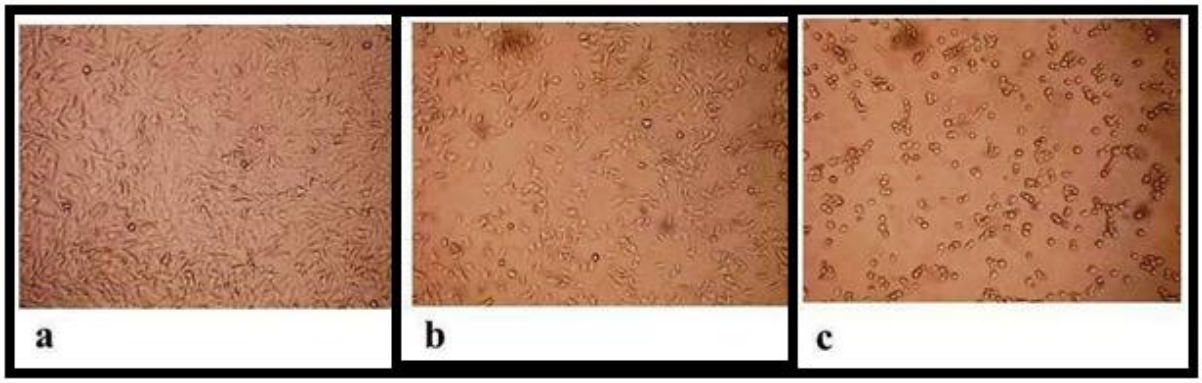


Fig. 3. Morphology of the MDA MB 231cells under the inverted microscope: (a) Untreated cells, (b) Cells treated with of 25µg/ml (c) Cells treated with IC50 value.

Conclusion

The present investigation was an attempt to evaluate the anticancer potential of SPs from *T. procumbens*. The study revealed the presence of potential anticancer peptides of *T. procumbens* and further explored characterisation of bioactive SPs.

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