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Research Article

EVALUATION OF ANTICANCER ACTIVITY OF *TRIDAX PROCUMBENS* LEAF EXTRACTS ON A549 AND Hep G2 CELL LINES

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ABSTRACT

Objective: Evaluation of anticancer activity of various extracts of leaves of *Tridax procumbens* by 3-(4,5-dimethyl-thiazole-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay, and trypan blue dye exclusion assay against A549 (human lung cancer cell line), Hep G2 (human liver carcinoma cell line).

Methods: *In vitro* anticancer activity of ethanol, acetone, and aqueous leaf extracts of *T. procumbens* was evaluated on selected cancerous cells lines by MTT assay and trypan blue dye exclusion assay. MTT assay is based on the capacity of mitochondrial enzymes of viable cells to reduce the yellow soluble salt MTT to purple blue insoluble formazan precipitate which is then quantified spectrophotometrically at 570 nm. Trypan blue assay is based on staining of cells. Cells are then counted using hemocytometer under the microscope, non-viable cells were stained blue, viable cells remain unstained.

Results: The aqueous leaf extract of *T. procumbens* has not shown any anticancer activity. However, potent anticancer activity was shown by the acetone and ethanol leaf extracts of *T. procumbens* on A549 (human lung cancer cell line), Hep G2 (human liver carcinoma cell line).

Conclusion: The medicinal plant i.e., *T. procumbens* was studied by *in vitro* evaluation methods i.e., MTT assay and trypan blue exclusion assay. The acetone and ethanol leaf extract of *T. procumbens* have shown potent anticancer activity on selected cancerous cell lines. More efforts are needed to explore potent anticancer plants from the mother earth and save humans around the world from cancer.

Keywords: Anticancer activity, *Tridax procumbens*, 3-(4,5-dimethyl-thiazole-2-yl)-2,5-diphenyl tetrazolium bromide assay, Trypan blue exclusion assay, A549, Hep G2.

INTRODUCTION

Cell division in humans is mainly controlled by DNA of the cell. Main factors are responsible for the cause of cancer such as chemical carcinogens, viruses, chromosomal rearrangement or spontaneous transformation, and tumor suppressor genes. Cancer can be caused by any of the three ways improper diet, genetic factors, and environmental factors [1,2]. More than 35% of all cancers worldwide are caused by improper diet in the case of colon cancer; diet may account for more than 80% of the cases. Alcohol and cigarettes to their diet, the percentage cause of cancer may increase to 60%. Plants have been demonstrated clinical source for anticancer compounds. However, many of the plant products and their derivatives are approved for cancer control. Hence, the development of new drugs to play an important role in cancer control is greatly desired [3].

Tridax procumbens is an annual or short-lived perennial annual herb. Leaves are membranous, scaberulous, glabrata beneath, auricled at the base, irregularly toothed. Flower heads have long stalk, Yellow hard, rounded, 2.4-3.9 cm across, often 2-5 clustered together in the axils of leaves or terminal. Petals are about 2 cm long, tubular, yellow in color. Anther tails are fimbriate. Achenes are curved, compressed CA. 8 mm long, tip narrowed, with one rib on each face. In the Indian systems of medicine (Ayurveda, Siddha, and Unani) *T. procumbens* is used either as a single drug or in combination with other drugs. Traditionally, it is used for the treatment of bronchial catarrh, dysentery, malaria, stomachache, diarrhea, high blood pressure and to check hemorrhage from cuts, bruises, and wounds and to prevent falling of hair. The leaf extract has been extensively used in Indian traditional medicine as anticoagulant, anticancer, antifungal, and insect repellent [4]. The basic fraction from the leaves of *T. procumbens* has been identified as alkaloids, carotenoids, flavonoids (catechins and flavones), saponins, and tannins [5]. Dexamethasone luteolin, glucoluteolin, β-sitosterol quercetin, β-sitosterol-3-O-β-D-xylopyranoside, and flavonoid procumbenetin have been isolated from leaf [6]. Several workers have reported on the different biological activities of *T. procumbens* in various *in vitro* and *in vivo* test models. Different extracts of this plant have been found to exhibit antibacterial, anti-inflammatory, hepatoprotective, anti-ulcer and antioxidant, immunomodulatory [7-9].

The aim of the present study was to evaluate anticancer activity of various leaf extracts of *T. procumbens* on selected cancerous cell lines i.e., A549 and Hep G2 by *in vitro* evaluation by 3-(4,5-dimethyl-thiazole-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay and trypan blue dye exclusion assay.

METHODS

Plant material

The plant was identified and authenticated by plant taxonomist. The fresh leaves were separated from the plant and shade dried. The separated leaves were powdered in a mechanical grinder and fine powder was collected by passing through sieve no: 40.

Preparation of leaf extracts

For the preparation of leaves extracts 50 g of *T. procumbens* was separately extracted using ethanol, acetone, and aqueous solvents

using soxhlet extractor at a temperature 45°C. The extracts were concentrated and dried by using rotary evaporator and was stored in a refrigerator at 4°C.

Chemicals

The chemicals used in the present study, MTT, fetal bovine serum (FBS), phosphate buffered saline (PBS), and antibiotics from Sigma-Aldrich and Hi-media, Mumbai.

Cell lines and culture conditions

A549 and Hep G2 were procured from National Centre for Cell Science at Pune was maintained in RPMI-1640 supplemented with 10% FBS, antibiotic 2% (penicillin or streptomycin) in a humidified atmosphere of 5% CO_2 at 37°C until confluent. The stock cultures were grown in culture flask and the experiments were carried out in 96 well plate.

Anticancer studies

The anticancer activity of leaf crude extracted compound was studied. The anticancer activity was evaluated by MTT assay and trypan blue dye exclusion test.

MTT assay

The MTT assay is based on the cleavage of the soluble yellow tetrazolium salt MTT into a blue colored formazan by the mitochondrial enzyme succinate dehydrogenase. This assay is extensively used for measuring cell survival and proliferation. There is a direct proportionality between the formazan produced and the number of viable cells. However, it depends on the cell type, cellular metabolism, and incubation time with MTT. This method is based on the capacity of mitochondrial enzymes of viable cells to reduce the yellow soluble salt MTT to purple blue insoluble formazan precipitate which is quantified spectrophotometrically at 570 nm after dissolving in DMSO. Cells are plated on to 96 well plates at and allowed to grow in CO_2 incubator for 24 hrs (37°C, 5% CO_2). The medium is then removed and replaced by fresh medium containing different concentrations of leaf extract for 48 hrs. The cells are incubated for 24-48 hrs (37°C, 5% CO_2). Then, 20 µL MTT stock solution (5 mg/mL in PBS) is added to each well and incubated for 4 hrs. The medium is removed and 200 µL DMSO is added to each well to dissolve the MTT metabolic product. Then the plate is shaken at 150 rpm for 5 minutes, and the optical density is measured at 570 nm [10,11].

% Cell viability=([O.D of control–O.D of test compound]/[O.D. of control]) × 100
O.D: Optical density

Trypan blue exclusion assay[10,11]

This the most commonly utilized test for measuring cell viability. The assay is based on the fact that the chromophore is negatively charged and does not interact with the cell unless the membrane is damaged. Therefore, all the cells which exclude the dye are viable. In this assay, the cells are washed with Hank's buffered salt solution (HBSS) and centrifuged for 10-15 minutes at 10,000 rpm. The procedure is repeated thrice. The cells are suspended in a known quantity of HBSS, and the cell count is adjusted to 2×10^6 cells/ml. The cell suspension is distributed into Eppendorf tubes. The cells are exposed to various leaf extracts separately and incubated for 3 h at 37°C. After 3 hrs, dye exclusion test is performed. The cell suspension was diluted with 0.4% trypan blue dye solution (1:1). Mixed thoroughly and was allowed to stand for 5 minutes at room temperature. Cells are then counted using hemocytometer. When observed under the microscope, non-viable cells were stained blue, viable cells remain unstained [10,11].

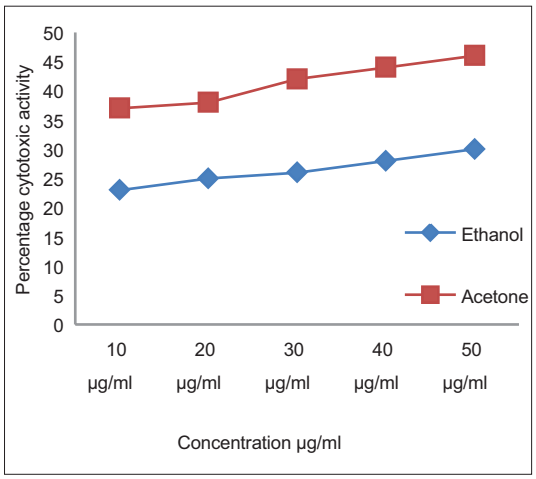
% Dead cell=No. of dead cells/(Sum of the live cells and dead cells) × 100

RESULTS

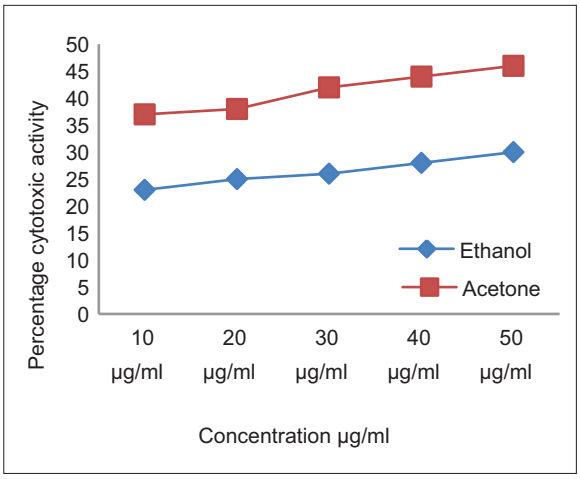
The anticancer activity of various leaf extracts of *T. procumbens* on selected cancerous cell lines, i.e., A549 and Hep G2 human cancerous cell lines was estimated by MTT assay and trypan blue dye exclusion test. The aqueous extract leaf of *T. procumbens* has not shown anticancer activity on selected cancer cell lines. The acetone and ethanol leaf extracts showed a potent anticancer activity on Hep G2 and A549 cancerous cell line by MTT assay and trypan blue dye exclusion assay.

The results of MTT assay of acetone and ethanol extracts of *T. procumbens* on Hep G2, A549 cancerous cell lines were represented in Graphs 1 and 2 respectively.

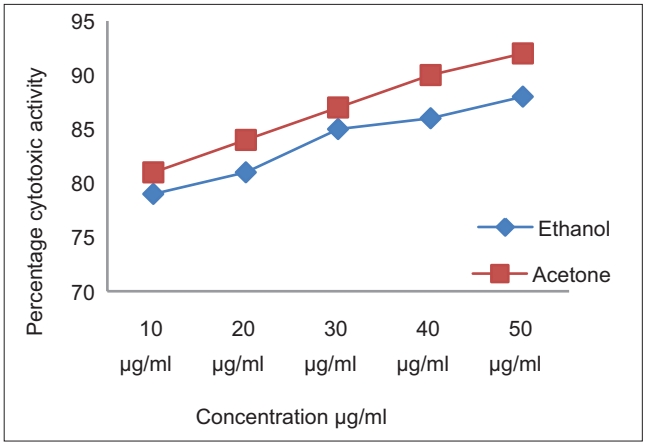
The results of trypan blue exclusion assay of acetone and ethanol extracts of *T. procumbens* on Hep G2 and A549 cancerous cell lines were represented in Graphs 3 and 4 respectively.



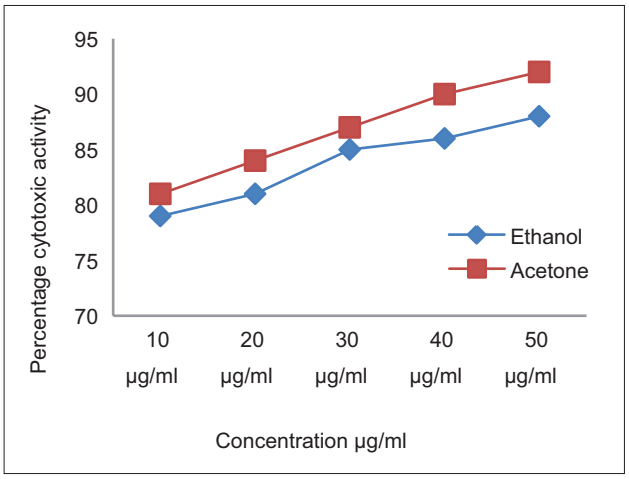
Graph 1: Anticancer activity of various leaf extracts of *Tridax procumbens* on Hep G2 cancer cell line by 3-(4,5-dimethyl-thiazole-2-yl)-2,5-diphenyl tetrazolium bromide assay



Graph 2: Anticancer activity of various leaf extracts of *Tridax procumbens* on A549 cancer cell line by 3-(4,5-dimethyl-thiazole-2-yl)-2,5-diphenyl tetrazolium bromide assay



Graph 3: Anticancer activity of *Tridax procumbens* on Hep G2 cancer cell line by trypan blue dye exclusion assay



Graph 4: Anticancer activity of *Tridax procumbens* on A549 cancer cell line by trypan blue dye exclusion assay

DISCUSSION

Natural products have received increasing attention over the past 30 years for their potential as a novel cancer preventive and therapeutic agents [12,13]. In parallel, there is increasing evidence for the potential of plant-derived compounds as inhibitors of various stages of tumorigenesis and associated inflammatory processes, underlining the importance of these products in cancer prevention and therapy. Approximately 60% of drugs currently used for cancer treatment have been isolated from natural products [14]. These include vinca alkaloids, Taxus diterpenes, camptotheca alkaloids, and podophyllum lignans. *Bidens pilosa* in cervix cancer [15,16]; *Citrullus colocynthis* in breast cancer [17,18]; *Crocus sativus* in cervical epithelioid carcinoma cancer [19,20]. At this time, more than 3000 plants worldwide have been reported to have anticancer properties. Globally, the incidence of plant-derived products for cancer treatment has increased. Hence, an attempt has made to study the cytotoxic activity of various extracts *Tridax procumbens* against various human cancerous cell lines.

CONCLUSION

The plant was initially selected and tested for anticancer activity based on their historical and other traditional uses. The leaf extracts of *T. procumbens* (acetone, ethanol, and aqueous) were prepared and tested for their potential as anticancer activity by *in-vitro* evaluation methods, i.e., MTT assay and trypan blue exclusion assay. This was done by closely monitoring the viability of cultured human cells exposed to the plant extracts. More efforts are needed to explore potent anticancer plants from the mother earth and save humans around the world from cancer.

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REFERENCES

1. World Health Organization, WHO Programmes and Projects: Cancer, (2009). Available from: http://www.who.int/cancer/en/. [Last accessed on 2013 July 10].
2. Mongelli E, Pampuro S, Coussio J, Salomon H, Ciccia G. Cytotoxic and DNA interaction activities of extracts from medicinal plants used in Argentina. J Ethnopharmacol 2000;71(1-2):145-51.
3. Younes RN, Varella AD, Sufredini IB. Selecao extracaoe identificac, aodedrogas novas anti-cancer de plantas brasileiras. Acta Oncol Bras 2000;20:15.
4. Sankaranarayanan S, Bama P, Ramachandran J, Kalaichelvan PT, Deccaraman M, Vijayalakshimi M, *et al*. Ethnobotanical study of medicinal plants used by traditional users in Villupuram district of Tamil Nadu, India. J Med Plants Res 2010;4(12):1089.
5. Ikewuchi JC, Ikewuchi CC, Ngozi IM. Chemical profile of *Tridax procumbens* Linn. Pakis J Nutri 2009;8(5):548-50.
6. Saxena VK, Albert S. β-Sitosterol-3-O-β-Dxylopyranoside from the flowers of *Tridax procumbens* Linn. J Chem Sci 2005:117(3):263-6.
7. Bama SS, Kingsley JS, Sankaranarayanan S, Bama P. Antibacterial activity of different phytochemical extracts from the leaves of *T. procumbens* Linn: Identification and mode of action of the terpenoid compound as antibacterial. Int J Pharm Pharm Sci 2012;4 Suppl 1:557-64.
8. Lee MG, Lee KT, Chi SG, Park JH. Costunolide induces apoptosis by ROS-mediated mitochondrial permeability transition and cytochrome C release. Biol Pharm Bull 2001;24(3):303-6.
9. Moeslinger T, Friedl R, Volf I, Brunner M, Koller E, Spieckermann PG. Inhibition of inducible nitric oxide synthesis by the herbal preparation Padma 28 in macrophage cell line. Can J Physiol Pharmacol 2000;78(11):861-6.
10. Priya VP, Radhika K, Rao SA. *In vitro* anti-cancer activity of aqueous and acetone extracts of *Tridax procumbens* leaf on PC 3 cell lines. Int J Pharm Pharm Sci 2011;3(4):1-4.
11. Ramamurthy SK, Pittu VP, Kotturi R, Devi P, Kumar S. *In vitro* cytotoxic activity of methanol and acetone extracts of Parthenium hysterophorus flower on A549 cell lines. Int J Pharm Sci Review and Research, 2011;10(2):95-9.
12. Gordaliza M. Natural products as leads to anticancer drugs. Clin Transl Oncol 2007;9(12):767-76.
13. Saklani A, Kutty SK. Plant-derived compounds in clinical trials. Drug Discov Today 2008;13(3-4):161-71.
14. Gerson-Cwilich R, Serrano-Olvera A, Villalobos-Prieto A. Complementary and alternative medicine (CAM) in Mexican patients with cancer. Clin Transl Oncol 2006;8(3):200-7.
15. Sundararajan P, Dey A, Smith A, Doss AG, Rajappan M, Natarajan S. Studies of anticancer and antipyretic activity of *Bidens pilosa* whole plant. Afr Health Sci 2006;6(1):27-30.
16. Abajo C, Boffill MA, del Campo J, Alexandra Méndez M, González Y, Mitjans M, *et al*. *In vitro* study of the antioxidant and immunomodulatory activity of aqueous infusion of *Bidens pilosa*. J Ethnopharmacol 2004;93(2-3):319-23.
17. Tannin-Spitz T, Grossman S, Dovrat S, Gottlieb HE, Bergman M. Growth inhibitory activity of cucurbitacin glucosides isolated from *Citrullus colocynthis* on human breast cancer cells. Biochem Pharmacol 2007;73(1):56-67.
18. Tannin-Spitz T, Bergman M, Grossman S. Cucurbitacin glucosides: Antioxidant and free-radical scavenging activities. Biochem Biophys Res Commun 2007;364(1):181-6.

19. Abdullaev FI. Cancer chemopreventive and tumoricidal properties of saffron (*Crocus sativus L*). Exp Biol Med (Maywood) 2002;227(1):20-5.

20. Escribano J, Alonso GL, Coca-Prados M, Fernandez JA. Crocin, safranal and picrocrocin from saffron (*Crocus sativus L*) inhibit the growth of human cancer cells *in vitro*. Cancer Lett 1996;100(1-2):23-30.