

INVITRO ANTI-CANCER ACTIVITY OF AQUEOUS AND ACETONE EXTRACTS OF *TRIDAX PROCUMBENS* LEAF ON PC 3 CELL LINES

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ABSTRACT

Plants that possess therapeutic properties are exerting beneficial pharmacological effects on the animal body, generally designated as medicinal plants. One of the plant that can be used as herbal medicine is *Tridax procumbens* belonging to family Astraceae. It has found to possess significant medicinal properties. Therefore, present study designed to evaluate in-vitro anti-cancer activity of aqueous and acetone extracts of leaf of *Tridax procumbens* on PC 3 cell lines by MTT assay and tryphan blue exclusion assay. Tryphan blue assay is based on staining of cells, cells which exclude the stain are viable. MTT assay is based on the capacity of mitochondrial enzymes of viable cells to reduce the yellow soluble salt MTT [3-(4, 5-dimethyl -thiazole-2-yl)-2, 5-diphenyl tetrazolium bromide] to purple blue insoluble formazan precipitate which is then quantified spectrophotometrically at 570 nm. In both assays acetone extracts of *Tridax procumbens* produced potent anti-cancer activity *invitro*. This finding can be further exploited for the development of a potential therapeutic anti-cancer agent.

Keywords: Anti-cancer, *Tridax procumbens*, PC 3, MTT assay, Tryphan blue exclusion test.

INTRODUCTION

Nature always stands as a golden mark to exemplify the outstanding phenomenon of symbiosis. The plants are indispensable to man for his life. Nature has provided a complete store house of remedies to cure all ailments of mankind. The knowledge of drugs has accumulated over thousands of years as a result of man's inquisitive nature so that today we possess many effective means of ensuring health-care. Plant-based drugs have been used against various diseases since a long time. The nature has provided abundant plant wealth for all the living creatures, which possess medicinal virtues. The essential values of some plants have long been published, but a large number of them have remained unexplored to date. Therefore, there is a necessity to explore their uses and to conduct pharmacognostic and pharmacological studies to ascertain their therapeutic properties. Medicinal plants are of great importance to the health of individuals and communities.

Tridax procumbens is also one of the local weed commonly known as Coat buttons and tridax daisy in English¹. It has been found to possess significant medicinal properties against blood pressure, bronchial catarrh, malaria, dysentery, diarrhoea, stomach-ache, headache, wound healing; it also prevents falling of hairs and haemorrhage from cuts and bruises etc. Its flowers and leaves possess antiseptic, insecticidal and parasiticidal properties². A number of chemical constituents were also reported from the plant viz: alkaloids, flavonoids, carotenoids, β -Sitosterol, n-hexane, fumaric acid, luteolin, quercetin, oxoesters, lauric, myristic, palmitic, stearic, arachidic, benenic, palmitoleic, linoleic and tannin etc. Linolenic acid was also reported in the aerial parts except flower tops. Identification of some sterols by GC-MS and lipid constituents of *Tridax procumbens* were also been reported. A new flavonoid procumbenetin was also isolated from aerial parts of plant, has been characterized as 3, 6-dimethoxy-5, 7, 2', 3', 4'-pentahydroxyflavone 7-O- β -glucopyranoside on the basis of spectroscopic techniques and by chemical means^{3,4}. The plant also shows various pharmacological activities i.e. immunomodulatory⁵, anti-diabetic⁶, anti-hepatotoxic & anti-oxidant⁷, anti-inflammatory⁸, analgesic⁹ and marked depressant action on respiration. A reputed ayurvedic medicine "Bringraj" is also prepared by the same plant; which is used for various types of liver disorders¹⁰. Cancer is the abnormal growth of cells in body.

Cells usually invade and destroy normal cells. The major causes of cancer are smoking, dietary imbalances, hormones and chronic infections leading to chronic inflammation. Breast cancer is the most common form of cancer in women worldwide and prostate cancer is the most frequently diagnosed cancer among men. In fact, there are several medicinal plants all over the world, including India, which are being used traditionally for the prevention and treatment of cancer. However, only few medicinal plants have attracted the interest of researchers to investigate the remedy for neoplasm (tumor or cancer). Hence, an attempt has been made to investigate the anti-cancer activity of *Tridax procumbens*.

MATERIALS AND METHODS

Plant Material

The plant was identified based on the leaves, which are lobed with fine soft hairs; the flowers on the top are small creamy color with black colored seeds based on the features of the plant it was confirmed as *Tridax procumbens*. The identification was confirmed by plant taxonomist. The plant was collected from Indira Park and Public Gardens, Nampally, Hyderabad. The leaves were separated and shade dried. The separated leaves were powdered in a mixer and fine powder was collected by passing through sieve no: 40.

Preparation of extracts

The powdered dried leaves was extracted successively with aqueous and acetone by soxhlet apparatus. Both the extracts were dried under reduced pressure. A dose of crude material was prepared at a concentration of 50, 100, 200, 250 μ g/ml of aqueous and acetone extracts and tested on human prostate epithelial cancer cell line (PC 3) for anti-cancer activity.

Cell lines and culture conditions

The anti-cancer activity was studied on human prostate epithelial cancer cell line (PC 3). These cells were purchased from National Centre for Cell Science, Pune, India. Stock cells are routinely cultured in Dulbecco Modified Eagle's Medium containing 2 mM L-glutamine, 100 Units/ml penicillin and 100 μ g/ml streptomycin at 37°C under an air: CO₂ (9:1) atmosphere supplemented with 10% foetal calf serum and the medium was changed every 48 hrs. For the

experiments, cancer cells were seeded at a density of 1×10^5 cells/ml in multi well plate. After 24 hrs, medium was changed and the cancer cells were treated with respective plant extracts.

Anti-Cancer Studies

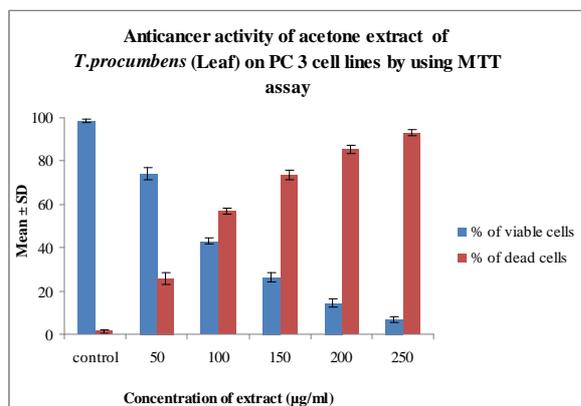
Cell viability by MTT assay

The cleavage of the soluble yellow tetrazolium salt MTT [3-(4, 5-dimethyl -thiazole-2-yl)-2, 5-diphenyl tetrazolium bromide] into a blue coloured formazan by the mitochondrial enzyme succinate dehydrogenase was used for assaying cell survival and proliferation. 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. For adherent cells all the media was removed completely from a multi well plate and 20 μ l of MTT (5 mg/ml) reagent was added. The wells were then incubated for 4hrs at 37 °C. Later 1 ml of DMSO was added to solubilize the formazan crystals. The absorbance was taken at 570 nm¹¹.

Percent specific cytotoxicity is calculated as follows:

Percentage of dead cells when extract at the concentration of 50,100, 200, 250 μ g/ ml is used

% Cell viability = $[(\text{O.D of control} - \text{O.D of test compound}) / (\text{O.D. of control})] \times 100$



Graph 1: Anticancer activity of acetone extract of *T. procumbens* leaf on PC 3 cell lines by using MTT assay

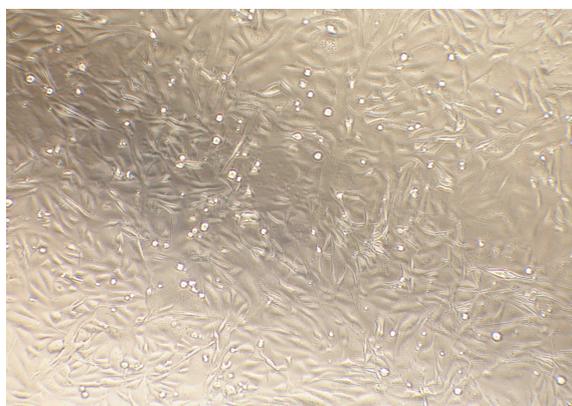


Fig. 1: Photograph showing PC 3 cell lines in control

(O.D=Optical density).

Tryphan blue exclusion assay

Tryphan blue is vital dye. The assay is based on 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. The cell suspension was diluted with 0.4% tryphan blue solution (1:1). Mixed thoroughly and was allowed to stand for 5 min at room temperature. Then hemocytometer was for cell counting. When observed under the microscope, non-viable cells were stained blue, viable cells remain unstained¹².

% Dead cell = $\text{No. of dead cells} / (\text{Sum of the live cells and dead cells}) \times 100$.

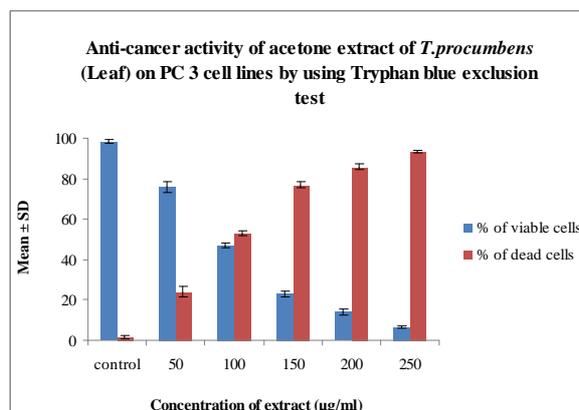
RESULTS

Effect of leaf extracts of *Tridax procumbens* on cell viability was estimated by MTT assay and Tryphan blue dye exclusion test. PC 3 cell lines were taken for investigation of anti-cancer activity of leaf extracts of *Tridax procumbens invitro*.

The aqueous extract of *Tridax procumbens* (leaf) has shown very little anti-cancer activity i.e., 6.6% cell death for 250 μ g/ml. The acetone extract of *Tridax procumbens* (leaf) has shown potent anti-cancer activity i.e., 93% cell death for 250 μ g/ml.

The results of both the MTT assay and Tryphan blue exclusion test of acetone extract were represented in graph 1 & 2 respectively.

Photographs showing effect on cancerous cells in control, aqueous leaf extract 250 μ g/ml and acetone leaf extract 250 μ g/ml by MTT assay were given in photograph 1, 2 and 3 respectively.



Graph 2: Anticancer activity of acetone extract of *T. procumbens* leaf on PC 3 cell lines by using Tryphan blue dye exclusion test.

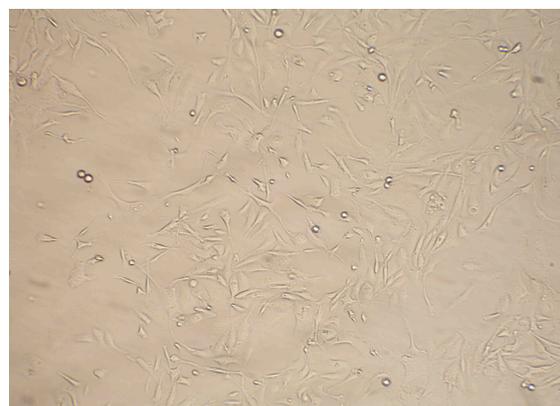


Fig. 2: Photograph showing the effect of aqueous extract of *T. procumbens* leaf on PC 3 cell lines at 250 μ g/ml

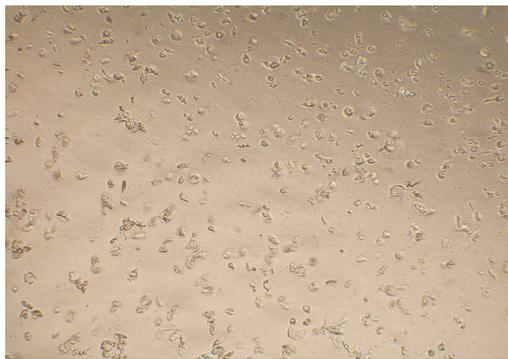


Fig. 3: Photograph showing the effect of acetone extract of *T. procumbens* leaf on PC 3 cell lines at 250 µg/ml

DISCUSSION

Harmful free radicals are generated in the body during normal metabolism and also upon exposure to environmental pollutants such as infectious agents, pollution, UV light, radiation and so on. When harmful free radicals are not neutralized by the body's primary and secondary defense mechanisms, an excess of harmful radicals exists. Clinical studies have also shown that supplemental levels of anti-oxidant vitamins (Vitamin E, Vitamin C and β -carotene) reduce an individual's risk for certain cancers¹³. Many of the medicinal plants have been found effective in experimental and clinical cases of cancers. Medicinal plants possess immunomodulatory and anti-oxidant properties, leading to anti-cancer activity. They are known to have versatile immunomodulatory activity by stimulating both non-specific and specific immunity¹⁴⁻¹⁶. Plants contain several phytochemicals, which possess strong anti-oxidant activities. The anti-oxidants may prevent and cure cancer and other diseases by protecting the cells from damage caused by free radicals – the highly reactive oxygen compounds. Thus consuming a diet rich in anti-oxidant plant foods (e.g. fruits and vegetables) will provide many phytochemicals from plants that possess health protective effects. Many naturally occurring substances present in the human diet have been identified as potential chemopreventive agents; and consuming relatively large amounts of vegetables and fruits can prevent the development of cancer^{13,17}. Phytochemicals such as vitamins (A, C, E, and K), carotenoids, terpenoids, flavonoids, polyphenols, alkaloids, tannins, saponins, pigments, enzymes and minerals have been found to elicit anti-oxidant activities¹⁸⁻²⁰. Ellagic acid and a whole range of flavonoids, carotenoids and terpenoids present in *Fragaria vesca* (strawberries) and *Rubus idaeus* (raspberries) have been reported to be responsible for anti-oxidant activity. These chemicals block various hormone actions and metabolic pathways that are associated with the development of cancer²¹⁻²².

Antioxidant activity of *Tridax procumbens* was evaluated by TLC. The powder of different parts of the plant were dissolved in methanol 100 % and spotted on silica gel sheets, developed in methanol:ethylacetate (2:1; v/v). The plates were air-dried and sprayed with 0.2 % solution of the stable DPPH (2, 2-diphenyl-1-picrylhydrazyl hydrate) radical and visualized for the presence of whitish spots, indicating anti-oxidant activity^{10,23}.

CONCLUSION

The medicinal plants with immunomodulatory and anti-oxidant properties leading to possess good anti-cancer activities. The anti-oxidant phytochemicals protect the cells from oxidative damage caused by free radicals. Thus, consuming a diet rich in anti-oxidant foods (e.g. fruits and vegetables) will provide health-protective effects. It is a significance to exploit novel anti-cancer drugs from the medicinal plants.

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