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In Vitro Anticancer Activity of Acetone and Chloroform Extracts of *Tridax procumbens* Leaf on Breast Cancer Cell Line MCF-7

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Abstract : The Phytochemical screening of acetone and chloroform leaf extracts *T. procumbens* showed the presence secondary metabolites such as Alkaloids, Terpenoids, Tannins, Saponins, Flavanoids and Steroids, Amino acids, Phenol, Proteins and Glycosides. The Hemostatic activity of the acetone extract of the leaves of *T. procumbens* reduces the clotting time uniformly in the blood samples of all the subjects, it can be suggested that the same possesses hemostatic activity, thus affecting hemostasis.
Keywords : Anticancer activity, Tridax procumbens, MCF7, Breast Cancer, Invitro

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, commonly known as coat buttons or tridax daisy, is a species of flowering plant in the daisy family. It is best known as a widespread weed and pest plant. It is native to the tropical Americas but it has been introduced to tropical, subtropical, and mild temperate regions worldwide. 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*1

Collection of Plant Materials and Identification

The leaves of *Tridax procumbens*(Fig. 1) were collected from in and around Erode district, Tamilnadu, India. The collected leaves were identified by Dr. Nagarajan Department of Botany kongunadu Arts and Science College Coimbatore, The collected plant leaves *Tridax procumbens*(*T. procumbens*)were washed twice with tap water and rinsed with distilled water to remove or dust particles attached with leaves and the plant leaves subjected to dry in shade. Followed by this step, the dried plant leaves were then subjected to cold percolation method to obtain *T.procumbens* leaves powder.

Fig.1. Study plant

Preparation of Plant Extract

About 10 g of air dried powder was taken in 100 mL of methanol. Plugged with cotton wool and then kept on a rotary shaker at 220 rpm for 24 h. Then the supernatant was collected and the solvent was evaporated to make the final volume one-fourth of the original volume and stored at 4 ^{o}C in air tight container.

Qualitative Analysis of Phytochemicals

The acetone extracts and chloroform extract *T. procumbens* was screened for the presence of secondary metabolites using the procedures of 2, 3.

Alkaloids (Meyer's test)

To 1mL of the extract 2 mL of Meyer's reagent was added. Appearance of dull white precipitate indicates the presence of alkaloids.

Flavonoids (Sodium Hydroxide NaOH Test)

A small amount of the extract was treated with aqueous NaOH and HCl later, the sample was observed for the formation of yellow orange colour.

Glycosides

A small amount of extract was dissolved in 1.0 mL of water and then an aqueous sodium hydroxide solution was added. Formation of a yellow colour indicates the presence of glycosides.

Steroids

To 1.0 mL of extract, 1.0 mL of conc. H2SO4 was added, followed by the addition of 2.0 mL of acetic anhydride solution. A greenish colour developed and turned blue indicates the presence of steroids.

Tannins (Ferric Chloride test)

To 1.0 mL of extract, few drops of 5 % aqueous FeCl3 solution was added. A bluish black colour, which disappears on the addition of a 1.0 mL of dilute H2SO4, was followed by the formation of a yellowish brown precipitate.

Terpenoids(Salkwoski test)

About 5.0 mL of leaf extract was mixed in 2.0 mL of chloroform and concentrated H2SO4 (3.0 mL) was carefully added to form a layer. A reddish brown coloration in the inter phase formed to show positive results for the presence of terpenoids

In vitro* anticancer activity of *T. procumbens

The human breast cancer cell line (MCF 7) was obtained from National Centre for Cell Science (NCCS), Pune and grown in Eagles Minimum Essential Medium containing 10% fetal bovine serum (FBS). The cells were maintained at 37^0C, 5% CO2, 95% air and 100% relative humidity. Maintenance cultures were passaged weekly, and the culture medium was changed twice a week.

Cell Treatment Procedure

The monolayer cells were detached with trypsin-ethylenediaminetetraacetic acid (EDTA) to make single cell suspensions and viable cells were counted using a hemocytometer and diluted with medium containing 5% FBS to give final density of $1x10^5$ cells/mL. One hundred microlitres per well of cell suspension were seeded into 96-well plates at plating density of 10,000 cells/well and incubated to allow for cell attachment at 37^0C, 5% CO_2, 95% air and 100% relative humidity. After 24 h the cells were treated with serial concentrations of the test samples. They were initially dissolved in neat dimethylsulfoxide (DMSO) and an aliquot of the sample solution was diluted to twice the desired final maximum test concentration with serum free medium. Additional four serial dilutions were made to provide a total of five sample concentrations. Aliquots of 100 µl of these different sample dilutions were added to the appropriate wells already containing 100 µl of medium, resulting in the required final sample concentrations. Following sample addition, the plates were incubated for an additional 48 h at 37^0C, 5% CO_2, 95 % air and 100 % relative humidity. The medium containing without samples were served as control and triplicate was maintained for all concentrations.

MTT Assay

3-[4,5-dimethylthiazol-2-yl] 2,5-diphenyltetrazolium bromide (MTT) is a yellow water soluble tetrazolium salt. A mitochondrial enzyme in living cells, succinate-dehydrogenase, cleaves the tetrazolium ring, converting the MTT to an insoluble purple formazan. Therefore,the amount of formazan produced is directly proportional to the number of viable cells. After 48 h of incubation, 15µl of MTT (5mg/mL) in phosphate buffered saline (PBS) was added to each well and incubated at 37^0C for 4h. The medium with MTT was then flicked off and the formed formazan crystals were solubilized in 100µl of DMSO and then measured the absorbance at 570 nm using micro plate reader. The percentage cell viability was then calculated with respect to control as follows,

$$\% \text{ Cell viability} = [A] \text{ Test} / [A]\text{control} \times 100$$

$$\% \text{ Cell inhibition} = 100 - [A] \text{ Test} / [A]\text{control} \times 100$$

Results

Medicinal plants possess immunomodulatory and anti-oxidant properties, leading to anticancer 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. 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 chemo preventive agents; and consuming relatively large amounts of vegetables and fruits can prevent the development of cancer13,17.

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 Fragariavesca (strawberries) and Rubusidaeus (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.

Cancer is an abnormal growth and proliferation of cells. It is a frightful disease because the patient suffers pain, disfigurement and loss of many physiological processes. Cancer may be uncontrollable and incurable, and may occur at any time at any age in any part of the body. It is caused by a complex, poorly understood interplay of genetic and environmental factors. It continues to represent the largest cause of mortality in the world and claims over 6 millions. Cancer kills annually about 3500 per million populations around the world. A large number of chemo preventive agents are used to cure various cancers, but they produce side effects that prevent their extensive usage. Although more than 1500 anticancer drugs are in active development with over 500 of the drugs under clinical trials, there is an urgent need to develop much effective and less toxic drugs 23

T. procumbens compounds were tested for cytotoxicity against human lung cancer by MTT assay. The compound of Rf value 0.66 showed 90% reduced cell viability.NMR, MS and IR spectra revealed the compound as Lupeol. The anticancer potential of the Lupeol against human lung cancer has been evaluated by colonogenic survival determination, cell cyclecontrol, Cell based assay for inhibition of COX-2activity and DNA fragmentation analysis, an amount of 320 µg/ml concentration of Lupeol compound exhibited potential anticancer property 24.

Effect of leaf extracts of *T. 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 *T. procumbens In vitro*. The aqueous extract of *T. procumbens*(leaf) has shown very little anti-cancer activity i.e., 6.6% cell death for 250 µg/mL. The acetone extract of *T. procumbens*(leaf) has shown potent anticancer activity i.e., 93% cell death for 250 µg/mL.(Vishnu Priya, 2011).The chloroform leaf extracts of *T. procumbens* were tested for their anticancer activity potential againstMCF-7, breast cancer cell line . The extract *T. procumbens* were found to be inhibits the growth of MCF-7, breast cancer cell line. The chloroform leaf extract of *T. procumbens* showed a remarkable inhibition in the maximum concentration of 150-300 µg/mL to an extent of 99.5% of cell growth. The lower concentration of the extract 18.75 µg/mL showed 18.14, 37.5 µg/mL 12.9, while 75 µg/mL inhibited 24.7% of the cell growth. The IC_{50} value leaf extract of *T. procumbens* 47.5µg/mL. The regression value is 0.974 µg/mL.

An effect leaf extracts of ***T. procumbens on cytotoxicity was estimated by mtt assay*** **and** yellow water soluble tetrazolium salt. MCf-7 cell line were taken for investigation of anticancer activity of leaf extracts of ***T. procumbens in vitro*** The chloroform leaf extract of *T. procumbens* were tested for their anticancer activity potential against MCF-7, breast cancer cell line (fig b). The extract *T. procumbens* were found to be inhibit the growth of MCF-7,breast cancer cell line The chloroform leaf extract of *T. procumbens* showed a remarkable inhibition in the maximum concentration of 150-300 µg/mL to an extent of 99.5% of cell growth. The lower concentration of the extract 18.75 µg/mL showed 8.14, 37.5 µg/mL 12.9, while 75 µg/mL inhibited 24.7% of the cell growth. The IC_{50} value leaf extract of *T. procumbens* are is 47.5 µg/mL the regression value is 0.974 µg/mL.

The acetone leaf extract of *T. procumbens* were tested for their anticancer activity potential againstMCF-7, breast cancer cell line. The extract T. *procumbens* were found to be inhibit the growth of MCF-7, breast cancer cell line(fig:d). The chloroform leaf extract of *T. procumbens* showed a remarkable inhibition in the maximum concentration of 150-300 µg/mL to an extent of 99.5% of cell growth. The lower concentration of the extract 18.75 µg/mL showed -1.46, 37.5 µg/mL 3.18, while 75 µg/mL inhibited 30.20 of the cell growth. The IC_{50} value leaf extract of *T. procumbens* is 256.6 µg/mL. The regression value is 0.9646 µg/mL. The results of both the MTT assay chloroform and acetone extract were represented in graph 1 & 2 respectively. Photographs showing effect on cancerous cells in control, aqueous leaf extract 300 µg/ml and acetone leaf extract 300 µg/ml by MTT assay were given in photograph 1, 2 and 3 respectively.

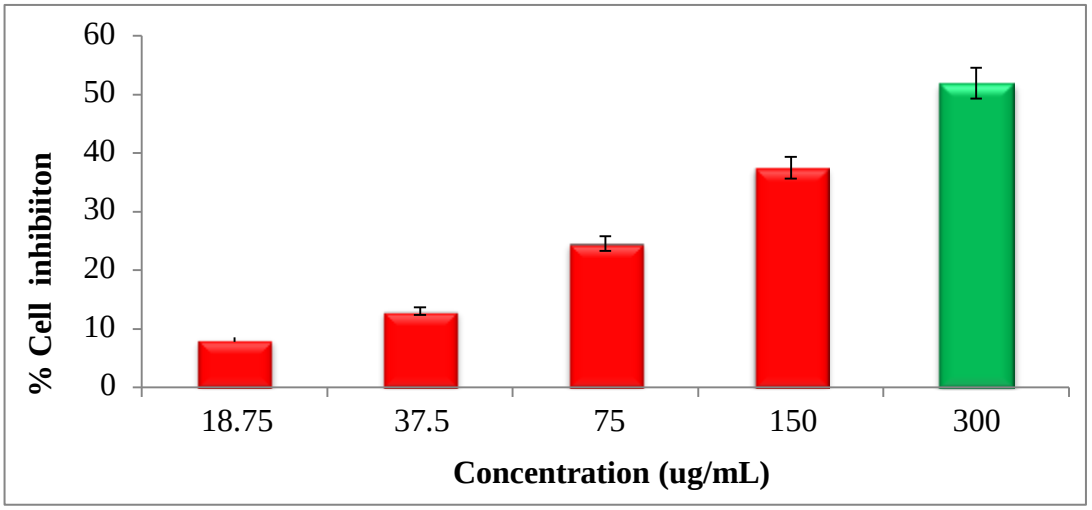

Fig. 1: Anticancer activity of chloroform extract of *T. procumbens* leaf on mcf-7 lines by using MTT assay

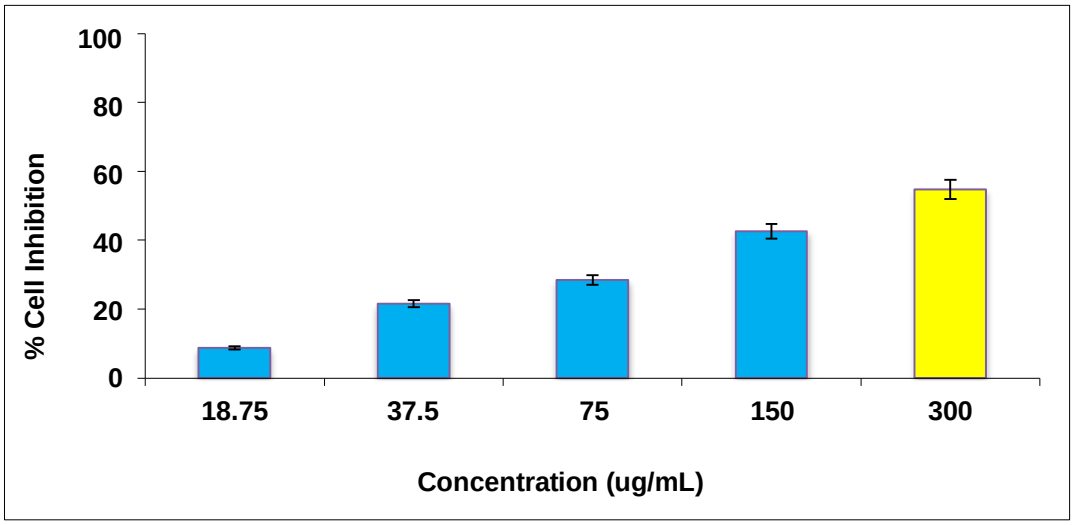

Fig. 2 anticancer activity of acetone extract of *T.procumbens* leaf on mcf-7 cell line by using

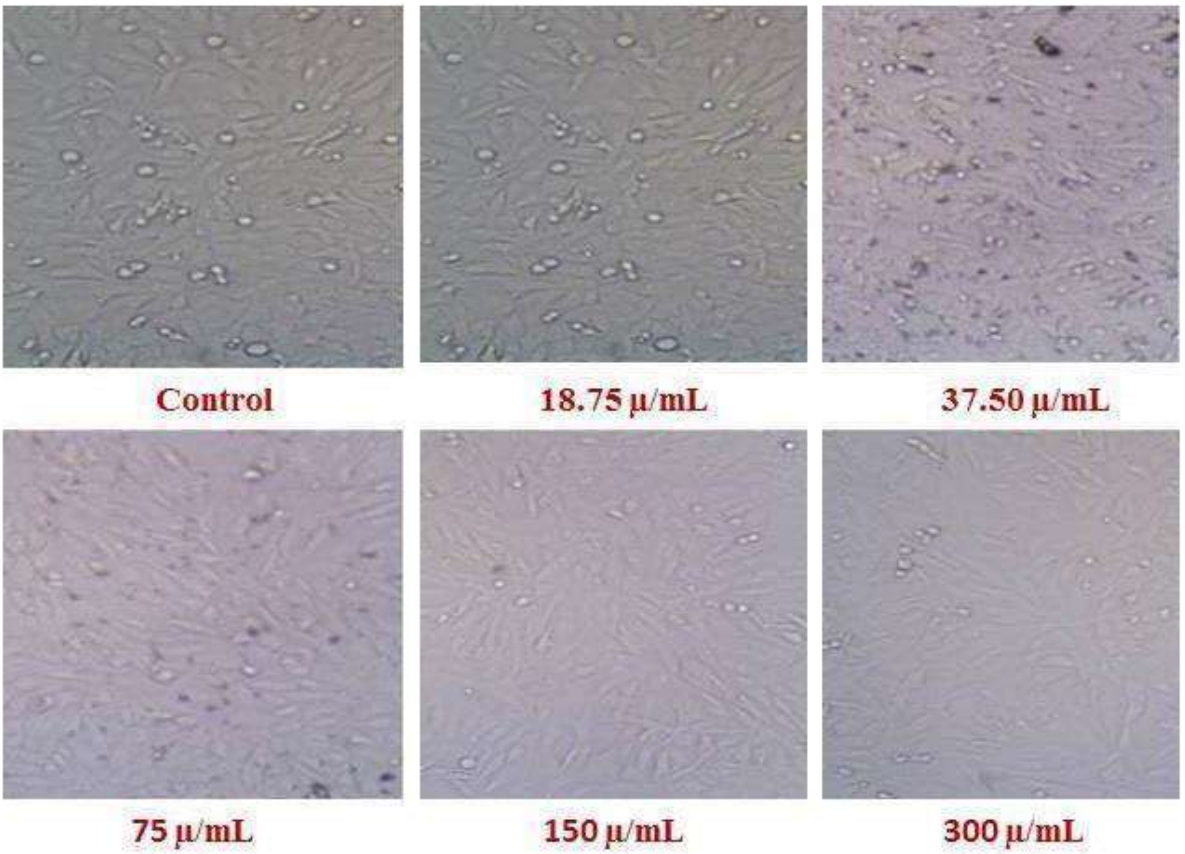

Fig. 3 E *In vitro* anticancer activity of chloroform leaf extracts of *T. procumbens*

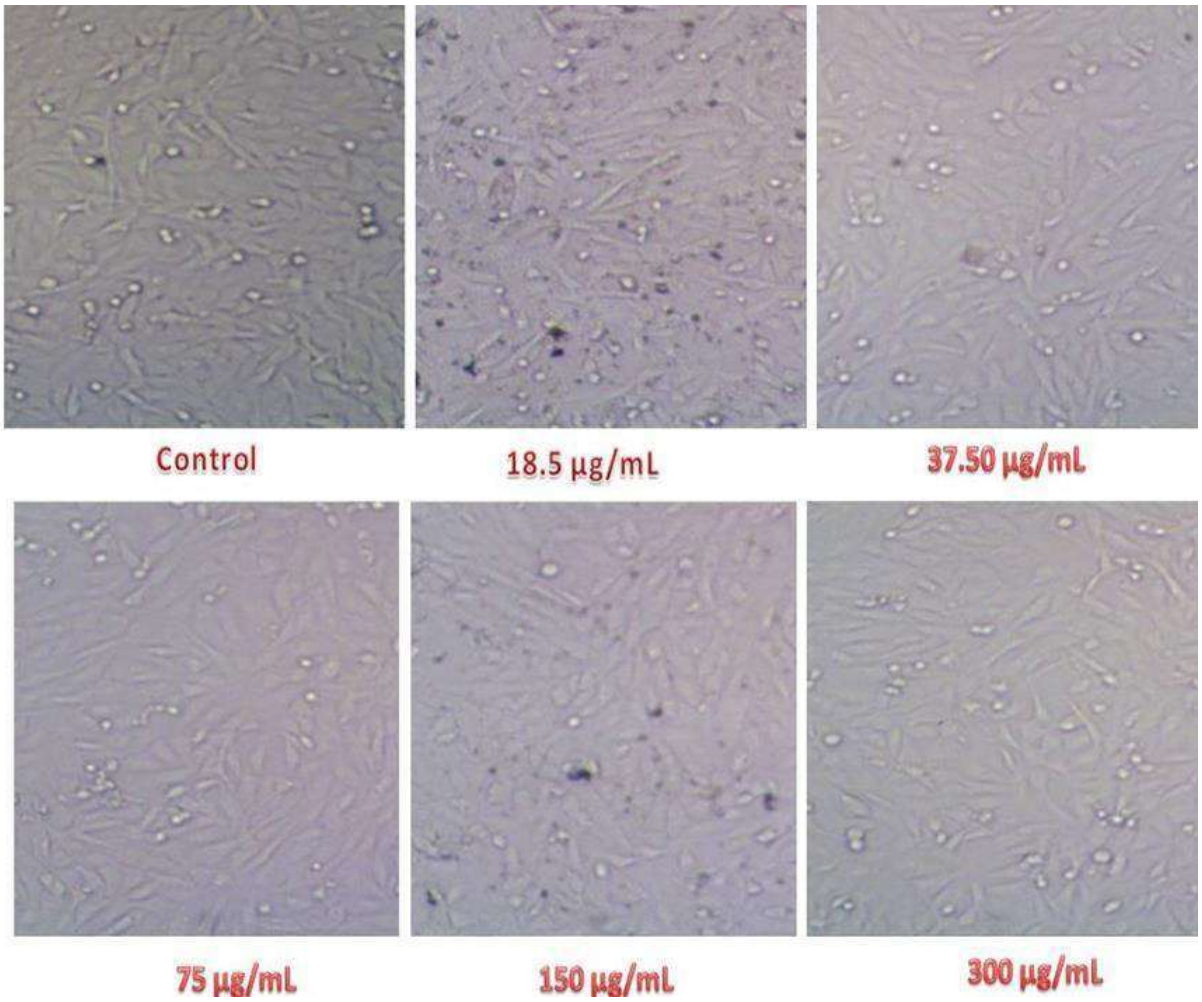

Fig: 4 *In vitro* anticancer activity of acetone leaf extracts *T. procumbens*

Conclusion

The medicinal plants which immunodultory and antioxidant properties leading to possess good anticancer activities. There is a remarkable anticancer potential was observed against the breast cancer cell lines in chloroform and acetone leaf extract of *T. procumbens.* There is a correlation was observed in the concentration and % of growth inhibition against the extract and breast cancer cells. 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. The Antibacterial activity of the acetone leaf extract of *T. procumbens* showed a varied inhibitory effects on the gram positive and gram negative strains. The Antioxidant activity of acetone and chloroform exposes decreasing antioxidant activity in increasing concentrations. The results were expressed as trolox equivalent in µ mol/g extract. There is a remarkable anticancer potential was observed against the breast cancer cell lines in chloroform leaf extract of *T. Procumbens.* There is a correlation was observed in the concentration and % of growth inhibition against the extract and breast cancer cells.

The Antibacterial activity of the acetone leaf extract of *T. procumbens* showed a varied inhibitory effects on the gram positive and gram negative strains. The Antioxidant activity of acetone and chloroform exposes decreasing antioxidant activity in increasing concentrations. The results were expressed as trolox equivalent in µ mol/g extract. There is a remarkable anticancer potential was observed against the breast cancer cell lines in chloroform leaf extract of *T. procumbens.* There is a correlation was observed in the concentration and % of growth inhibition against the extract and breast cancer cells.The present study confirms the presence valuable chemicals present in the plants further thorough studies may bring out the real potential of these widely used medicinal plants in the preparation of antibiotic,antioxidantandnti cancerdrugs.

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