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Article

Antioxidant and cytotoxic activity of *Tecoma stans* against lung cancer cell line (A549)

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Human have been constantly using plants and plant products to overcome many diseases. The antioxidant property of the plant sources is studied to obtain an efficacious drug against cancer. The objectives of the present study is to evaluate the antioxidant and cytotoxic activity of the *Tecoma stans* extracts against lung cancer cell line in comparison with vincristine drug. The antioxidant activity was studied using the standard DPPH assay and the cytotoxic activity using MTT assay. DPPH assay results show that methanolic extract of *T. stans* in higher concentration show better antioxidant potential than the standard L-ascorbic acid. They exhibited strong antioxidant potential at 20 µg/mL concentration. The absorbance at 517 nm showed that in the range of 0.201-0.0203 compared to that of absorbance of ascorbic acid at 0.023.Cytotoxic activity was studied using MTT assay which showed that the increase in concentration of extract increases the cell death. At 100µg/mL concentration there is an increased cytotoxic activity, i.e., 99% of cell inhibition. The results of antioxidant and anticancerous activity may be positively correlated.

UNITERMS: *Tecoma stans*/extract/cytotoxicity. *Tecoma stans*/extract/antioxidant activity. Free radical scavenging. Plant extracts. Lung cancer cell line/study/drugs.

INTRODUCTION

Cancer is one of the disease that occurs in both developed and developing countries and is the leading cause of death. An extensively used treatment for cancer is chemotherapy and one of the major drawbacks is the toxicity that is caused to the normal cells due to the inability of the chemical drugs to differentiate between normal and cancerous cells (Balamurugan *et al.,* 2014). Traditional medicines have been tested and researched upon to obtain an effective drug against cancer. Plant derived compounds are widely studied for their holistic value. These plant derived compounds have clinical significance which can be further developed into effective drugs against cancer. Bio active compounds have received the attention of researchers to overcome the burden of chemotherapy related problems. Oncovin is the brand name for vincristine, which is used as a chemotherapy drug for many types of cancer. Vincristine is also used to treat neurodegenerative disease (Chun-Fai *et al*., 2013). The mode of action of vincristine is that it acts on the tubulin protein, thereby inhibiting the metaphase stage, thus leading the cell to apoptosis (Jordan, 2002).

Tecoma Stans is an ornamental tree that has its origin from the Americas belongs to the family Bignoniaceae. Presently the plant is widely cultivated throughout India for its flowers which bloom throughout the year. Presence of alkaloids tecomine and tecostamine which are potential hypoglycemic agents present in the leaves of *T. stans*. In addition to that presence of also anthranilic acid in the roots of plant which is an antidiabetic agent (Khare, 2007). There are a number of compounds from the fruits and flowers of the *Tecoma,* which had antioxidant activity and anti proliferative effect against cancer cell lines (Marzouk *et al*., 2006). Antioxidants play a major role in deciding the pharmaceutical effect of plants and to make them potent drugs against the chronic diseases. These antioxidants are taken in our dietary from the plant compounds, which is a rapid and simple method. The DPPH assay is the simple method to evaluate the presence of antioxidants in any source based on the principle of radical scavenging activity. In this assay the (2, 2-diphenyl-1-picryl-hydrazyl-

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hydrate) i.e., DPPH is reduced to DPPHH, the odd element is formed is absorbed at 517nm (Tailor, Goyal, 2014). The cytotoxic activity of the plant extract is studied using the 3-(4,5-dimethylthiazol-2-yl)-2,5- diphenyltetrazolium bromide (MTT) which forms formazan (insoluble) crystals, by interacting with the mitochondria of the cells and the insoluble formazan that is formed is solubilized using isopropanol solvent which is studied spectrophotometrically at 540 nm. MTT is reduced only by metabolically active cells, thus in, turn it helps to study the viability of the cell.

MATERIAL AND METHOD

Collection of plant material

The fresh plant of *Tecoma stans* was collected from Paramathi velur, Namakkal district of Tamil Nadu, India. The collected plants were further surface sterilized using tween 80 and it was shade dried for future investigation.

Preparation of plant extract

10 g of both fresh and dried leaves, flowers were chopped into fine pieces and macerated in 100 mL of methanol. The plant material was extracted at room temperature for 3 days in a shaker. The extract was filtered and the filtrate was concentrated in a rotary evaporator under reduced pressure to dryness. The extract obtained was stored at 4 ^{o}C until use.

Antioxidant assay

DPPH assay

The percentage of antioxidant assay was determined using the free radical scavenging activity (2, 2-diphenyl-1-picryl-hydrazyl-hydrate). About 1 mg of fresh and the dried plant extracts was dissolved in 1ml of methanol. The standard procedure for DPPH assay was performed based on Ochuko *et al.* (2012). About 10 mL of 0.1 mM of DPPH was prepared in methanol and stored in cool dark condition until use. Accurately, 1 mL of DPPH was added to different concentration (20, 40, 60, 80, 100 µg/mL) of *T.stans* extract. The mixture of DPPH and extract was shaken and incubated at room temperature in the dark for 30 minutes, then the absorbance was measured at 517 nm in the UV spectrophotometer (Tailor, Goyal, 2014). Ascorbic acid was used as a reference and DPPH without the extract served as negative control. The IC_{50} value of the sample was calculated based on the absorbance. The percentage of inhibition was calculated using the formula,

DPPH scavenging effect (%) or
Percent inhibition = (Absorbance of sample-absorbance of blank)/Absorbance of Control X 100

Lung Cancer (A549) cell line

Lung cancer cell line (A549) cell line was procured from NCCS, Pune and maintained in Dulbecco's minimal essential medium (DMEM) with 10% FBS, and antibiotic mixture (Penicillin, streptomycin and ampicillin 100 units/mL) under defined conditions of temperature at 37 ^{o}C, 95% humidity and 5% CO_2.

Cell viability assay

The cytotoxic activity of the fresh and dried extracts of leaves and flowers of *Tecoma stans* was determined using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (Terry *et al*., 1992). *Tecoma stans* extract concentrations were prepared in the range of 100-20µg/mL from the stock solution by serial dilution using Dimethyl sulphoxide (DMSO). Vin-Cristine wide range anti-cancer drug was used as the standard (0.1 g/mL). Lung cancer cell line (A549) was trypsinised and the cells were counted using haemocytometer following standard procedure.100µl of the lung cancer cell line at 1 X 10^4 cells/mL was added to poly L-lysine coated 96 well plate and incubated at 37 ^{o}C in a humidified 5% CO_2 incubator. After 24 hours of incubation, the old medium was replaced with fresh medium and 50 µL of the extract was added and incubated for 48 hours at 37 ^{o}C in a humidified 5% CO_2 incubator. 30µl of 0.5% w/v MTT was added and incubated at room temperature for 4 hours. After incubation, 50 µL of acid-isopropanol was added to dissolve the formazan formed and incubated at room temperature for 30 minutes. Then absorbance was taken at 554nm using Bio-Rad micro-titer plate reader. The assay was performed in triplicates.

RESULTS AND DISCUSSION

Antioxidant property plays an important role in reducing chronic diseases like cancer and cardiovascular (CAD) diseases. Crude plant extracts are screened on cell culture, to determine their efficiency as a potential alternate drug and also to check their efficiency in clinical application as suggested by Balamurugan *et al.* (2014). Plants are primary resources for antioxidants like carotenes, phenolic acids, etc., the phytochemical analysis *Tecoma stans* revealed that the plant has alkaloids, steroids, glycosides and carbohydrates as suggested by

(Prajapati, Patel, 2010). A number of flowers and fruits having anti oxidant activity have been identified in the flowers and fruits of *Tecoma* their effective anti-cancer activity was studied against cancer cell line (Marzouk *et al.,* 2006).

DPPH assay is performed to study the radical scavenging activity, the scavenging activities in the present investigation were similar to that as reported by Erukainure *et al.* (2012), Where the scavenging activity follows a dose dependen pattern i.e., increase in activity to increase in concentration. The alcoholic and aqueous extract of *T.stans* on phytochemical analysis has revealed the presence of polyphenolic,ß- sitosterol and flavonoids. In earlier reports by Hamburger and Hostettmann (1991), Beltrame *et al.* (2002) and Suffredimi *et al.* (2004), these compounds have exhibited anti-bacterial, antiviral, immunological and cytotoxic property on different cancer cell lines.

The current study was carried out to report the antioxidant activity of the fresh and dried leaves and flowers of *Tecoma stans* using the DPPH assay. 1,1-diphenyl-2-picrylhydrazyl reacts with the anti-oxidants present in the plant extract and accepts the hydrogen atom, thus addition of hydrogen atom converts it to 1, 1-diphenyl-2-picrylhydrazine (Ochuko *et al.,* 2012). The chemical 1, 1-diphenyl, 1-2- picrylhydrazyl changes from purple color to yellow color. In case of *T stans* the antioxidant is higher from 20-100 µg/mL when compare to the standard of L-ascorbic acid at absorbance 517 nm in UV visible spectrophotometer. Table I shows the radical scavenging activity of *T.stans* against stable DPPH.

The scavenging activity values were compared with that of ascorbic acid (concentrations of 20,40,60,80 and 100 µg/mL) the values were dose dependent and their percentage of inhibition show that at 20 µg/mL concentration the percentage inhibition was 99.98%, which increase as 99% (40 µg/mL), 99.33% (60 µg/mL), 99.67% (80 µg/mL) and 99.79% (100 µg/mL) for dried leaves and flowers while for fresh flowers and leaves its 99.98% (20, 40 µg/mL) and 99.99% (60,80,100 µg/mL) respectively. These results were compared to standard ascorbic acid favorably.

From Figure 2 it is observed that cancer cells have undergone certain morphological changes, like cellular shrinkage and blebbing, which are characteristic features of apoptosis. These morphological changes are less observed in standard (vincristine) treated cells and these changes seem increasing with increase in concentration of

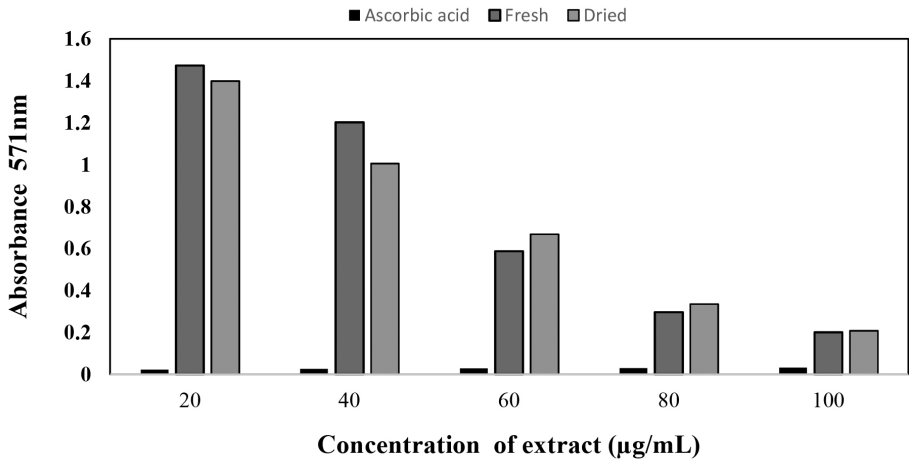

FIGURE 1 - Antioxidant activity of fresh and dried leaves and flowers of *Tecoma stans* in comparison with ascorbic acid at 517 nm.

TABLE I - Absorbance and percentage of inhibition of Fresh and Dried extract of *Tecoma stans*

Control	Ascorbic acid (517nm)	Fresh (517 nm)	Percentage of inhibition	Dried (517 nm)	Percentage of inhibition
2.203±0.01	0.023±0.01	1.472±0.02	98.60 %	1.398 ±0.02	99.98 %
2.205±0.01	0.025±0.02	1.202 ±0.01	99.00 %	1.005 ±0.01	99.98 %
2.206±0.01	0.028±0.01	0.587±0.02	99.33 %	0.668±0.01	99.99 %
2.208±0.01	0.03±0.02	0.297±0.02	99.67 %	0.335±0.02	99.99 %
2.21±0.01	0.032±0.01	0.201±0.01	99.79 %	0.208±0.02	99.99 %

the extract. At 100 µg/mL the morphological changes are higher, followed by 80µg/ml concentration.

The antioxidant assay results further, directed the study towards cytotoxic assay. The phytochemical and antioxidant assay which showed the expression of carotenes and phytophenols, hence lung cancer cell line was chosen for cytotoxic activity. The results of MTT assay in Figure 3 show that the methanolic extract of *Tecoma stans* has high cytotoxic activity against the cancer cell line from 20µg/mL concentration i.e., 99.3%. Earlier reports confirm the better cytotoxic activity of the plant (Pusapathi *et al.,* 2015).

Presence of phenolic compounds and its congers have shown to induce a cascade based apoptosis in cancer cells, thus inducing cytotoxicity (Owen *et al*., 2000). The cell viability of the lung cancer cell line decreased with increase in concentration of the plant extract and it was found to be the highest in 100 µg/mL concentration. The decrease in cell viability with increased concentration of the plant extract of *Tecoma stans* suggests the ability of the extract as an effective anti-cancer medicine. Antioxidant and free radical scavenging activity of the extract may be the reason behind its anti-cancer property**.**

CONCLUSION

The present investigation revealed that *T. stans* can act as a potential alternative remedy for lung cancer. The extract of *T. stans* can be used as an effective ingredient in drug recipe cancer. Further investigation is undertaken to identify the active compound behind the cytotoxic activity of the plant. The study in the future is to be extended to other cancer cell lines and there is a need to carry out in vivo studies to further authenticate the anti-oxidant potentials of this species.

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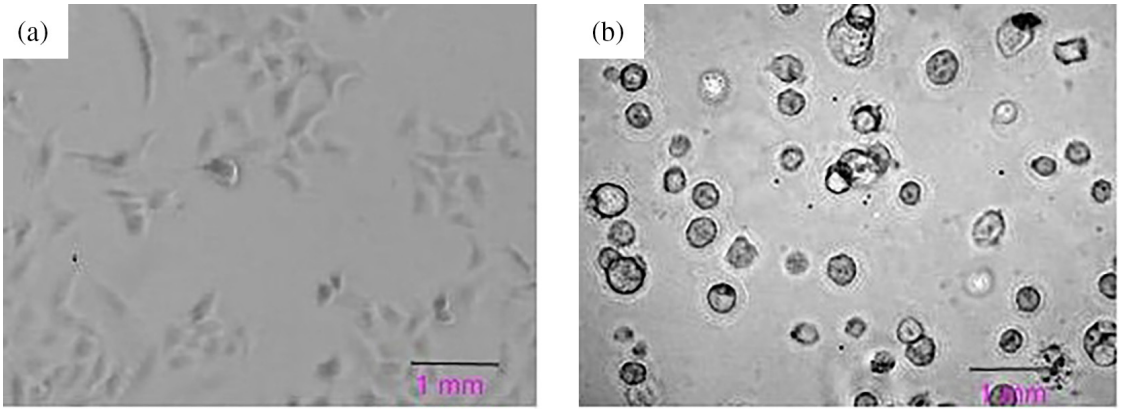

FIGURE 2 - Morphological changes of lung cancer cell lines after plant extract treatment a) Untreated lung cancer cells; b) *T. stans* treated lung cancer cells (after 24 hours).

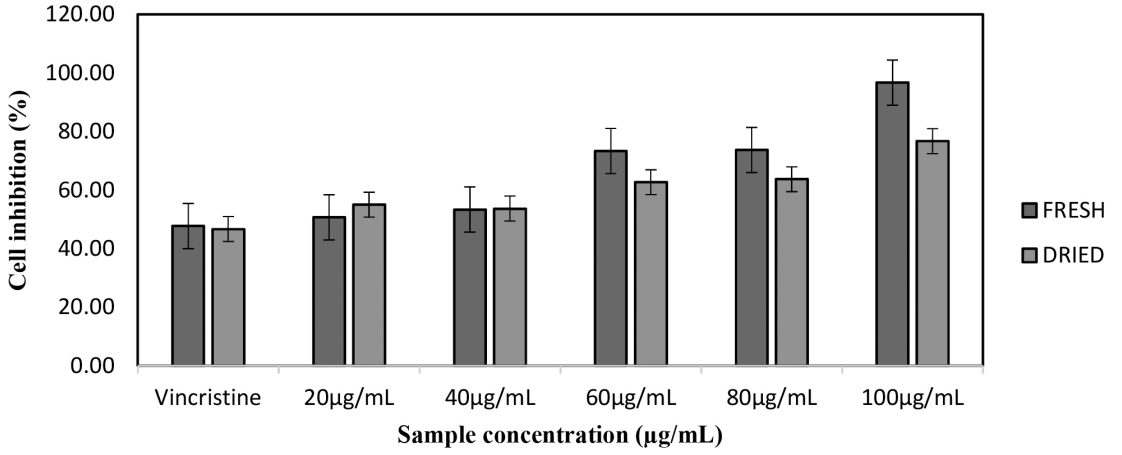

FIGURE 3 - Cytotoxic activity of *Tecoma stans* at different concentrations against Lung Cancer cell (A549).

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