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RESEARCH ARTICLE

# Anticancer and Antioxidant activity of *Tephrosia calophylla* against cancer cell lines

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**Abstract**

To evaluate anticancer and antioxidant potentials of activity of methanol extract of *Tephrosia calophylla*. The methanol extract of *Tephrosia calophylla* was prepared and tested for *in vitro* anticancer activity by using human MCF 7, HCT-116, HEP-$G_2$, A-549 cancer and vero normal cell lines. The antioxidant activity for the extract was evaluated by Superoxide scavenging, Lipid peroxidation and DPPH methods. $IC_{50}$ values of extracts were parameters in both studies. The results of the study have shown that methanol extract of *Tephrosia calophylla* has shown significant IC50 values against MCF 7, HCT-116 and A-549 cell lines which shows its anticancer potentials but inhibitory effect of methanol extract against HEP-$G_2$ and vero cell lines was not significant. The results also suggest that the methanol extract of *Tephrosia calophylla* has significant antioxidant properties against Superoxide scavenging, Lipid peroxidation and DPPH methods.The present study shows anticancer potentials of methanol extract of *Tephrosia calophylla* against MCF 7, HCT-116 and A-549 cell lines.

**Keywords:** Anticancer activity; Tephrosia calophylla; IC50 value; MTT assay; antioxidant activity.

## Introduction

Cancer is pathological condition which has been characterized by excessive proliferation of abnormal cells and always been a major threat to developing and developed countries [1]. It is most common and serious complication as it slaughters societies more than malaria, tuberculosis, and HIV [2]. About 12.5% of deaths worldwide reported every year are due to cancer. Among 15.6 million cases of cancer were reported 2016, 45% were from developed and the remaining 55% were from developing countries [3]. Breast, lung, liver and colorectal cancers are most common cancers in the world [4] Moreover, the incidence of this disease has increased steadily in recent years [5]. Despite advances in medicinal field over the past few decades, the mortality rate of patients diagnosed with cancers remains more than 60% mainly due to ineffectiveness and toxicity of presently available cytotoxic drugs [6]. Chemotherapy of cancer usually involves lots of adverse effects like alopecia, vomiting, bone marrow deression, diarrhoea, anorexia, imuune suppression, reproductive toxicity and etc. Hence till now there is no truly satisfactory anticancer agent for the effective treatment of cancers with the least side effects [7, 8].

Medicinal plants have been used since ancient time in Ayurveda and other traditional medicine system and their utility has been increasing day by day throughout world. Natural compounds obtained from herbs are considerably safe and effective than synthetic compounds. Moreover, the problem of development of drug less compared to synthetic drugs. is also reduced. The herbal drugs comprise a major source of anticancer medicine due to presence of various phyto-constituents such as vincristine, vinblastine, taxanes, camptothecines and in developed countries and developing countries [9]. The free radicals are the main agents which induces mutation by damaging cellu-

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lar DNA ultimately leads to development of cancers in the body. Hence much attention has been given on the development of anticancer agents that possess antioxidant property due to their free radical scavenging potential which plays an important role in protection of DNA free radical mediated damage [10].

The Tephrosia is a genus of plant which is of Indian and several species of Tephrosia are medicinally important and have used in ayurveda for the treatment of ailments [11]. The *Tephrosia calophylla* belongs to this genus frequently used as traditional and folklore medicine for the management various health complications such as ulcer, diabetes, urinary disorders, liver diseases and cancer [12]. Though this plant used essentially in traditional medicine for cancer, it has lack of scientific evidence. Hence, it was necessary to provide a clear background proof for the beneficial property of the plant in cancer. In this attempt, the study had been conducted to determine *in vitro* antioxidant and anticancer potentials of methanol extract of *Tephrosia calophylla*.

# Materials and methods

## Plant material

The areal parts of plant *Tephrosia villosa* have been collected from the surroundings of Sri Venkateshwara University, Tirupati, India and dried under shade. The plant material was identified and authenticated by Dr. Madhava Chetty Asst.Prof., Department of Botany Sri Venkateshwara university, Tirupati and specimen herbarium was preserved at institute herbarium library. The aerial parts of plant were separated from other parts, washed, cleaned and dried under shade for further use.

## Preparation of extract

The shade dried plant material was pulverised into powder and passed through sieve No. 22 mesh. About 350 g (appx.) of coarse powder was subjected to successive solvent extraction using petroleum ether and methanol in soxhlet's apparatus [13]

## Preliminary phytochemical investigation

The preliminary phytochemical investigation for the methanolic extract of *Tephrosia villosa* had been conducted as per procedure prescribed by Khandelwal [14].

## Drugs and chemicals

All reagents and chemicals used in the study were obtained commercially and were of analytical grade. The standard drugs tamoxifen and paclitaxel were obtained as gift samples from Strides laboratories, Bangalore.

## Evaluation of antioxidant property

The evaluation of antioxidant activity of ethanolic extract of *Tephrosia calophylla* was carried out by the following two methods:

### *Lipid peroxidation method*

Lipid peroxidation inhibition was estimated by the formation of colored product in the reaction mixture. The assay mixture contained the ethanolic extract in various concentrations, to which were added 0.1 ml of potassium chloride (30 mM), 0.1 ml of ascorbic acid (0.06 mm) and 0.1 ml of ammonium ferrous sulphate (0.16 mM) in succession. Later the reaction mixture was treated with 0.2 ml of sodium dodecyl sulphate (8.1%), 1.5 ml of thiobarbituric acid (0.8%) and 1.5 ml of 20 % acetic acid (pH 3.5) and then 5 ml of 15:1 v/v butanol-pyridine mixture was added. The absorbance of the organic layer containing the thiobarbituric acid reactive substances (TBARS) was measured at 532 nm [15].

### *DPPH radical scavenging activity*

The free radical scavenging activity was measured in terms of hydrogen donating or radical scavenging ability, using the stable radical, DPPH. About 0.1 mM solution of DPPH in methanol was prepared and 1 ml of this solution was added to 3 ml of the different concentration of ethanolic extract (50-400 $\mu$g/ml) in different test tubes. The mixture was shaken and allowed to stand at room temperature for 30 min and the absorbance was measured at 517 nm using a spectrophotometer. Decrease in absorbance of the reaction mixture indicates higher free radical scavenging activity [16].

### *Superoxide scavenging activity*

This method carried out by using Nitro blue tetrazolium (NBT) reagent, the method is based on generation of super oxide radical (O2-) by auto oxidation of hydroxylamine hydrochloride in presence of NBT, during the reaction the NBT is reduced to nitrite. In brief, aliquots of 0.1 to 1.0 mL to ascorbic acid solution were taken in a test tube, to which 1 mL of sodium carbonate, 0.4 mL of NBT and 0.2 mL of EDTA were added and zero minute reading was taken at 560 nm. The reaction was initiated by the addition of 0.4 mL of hydroxylamine hydrochloride to the above solution. Reaction mixture was incubated at $25^{0}$C for 5 mins; the reduction of NBT was measured at 560 nm. A parallel control was also treated in the similar manner. The methanol extract was treated in the similar manner, absorbance was recorded and $IC_{50}$ values was calculated [16].

### Evaluation of anticancer activity

*Procurement of cell lines*

*In-vitro* anticancer activity for methanol extract was evaluated by MTT assay against MCF-7, HCT-116, HEP-G2, A-549 cells and vero cell lines. The following cells were obtained from NCCS, Pune and subcultured under suitable conditions [17, 18].

*MTT assay*

The Cytotoxicity of TCME against MCF-7, HCT-116, HEP-G2, A-549 and vero cells was determined by the MTT assay [17, 18]. Exponentially growing cells were harvested from 25mL flask and a stock cell suspension was prepared. Cells (1 ï¿Ů105/well) were plated in 100ï¡ţl of medium well in 96-well plates (Costar Corning, Rochester, NY). After 48 hours incubation the cell reaches the confluence. Then cells were incubated in the presence of various concentrations of the TCME and standard drugs in 0. 1% DMSO for 48 h at 37 ï¡řC. The tamoxifen was used reference drugs for the breast cancer cell lines and paclitaxel was used standard drugs for other cell lines. After removal of the sample solution and washing with phosphate-buffered saline (pH7 .4), 20ï¡ţl/well (5 mg/ml) of 0.5% 3-(4, 5-dimethyl-2-thiazolyl)-2, 5-diphenyl-tetrazolium bromide cells (MTT), phosphate-buffered saline solution was added. After 4h incubation, 0.04M HCl isopropanol was added. Viable cells were determined by the absorbance at 450 nm. Measurements were performed and the concentration required for a 50% inhibition of viability was determined graphically. The absorbance at 450 nm was measured with a UV- Spectrophotometer using wells without sample containing cells as blanks. The effect of the samples on the proliferation of cells was expressed as the % cell viability, using the following formula:

% cell viability = A450 of treated cells / A450 of control cells ï¿Ů 100%.

The percentage growth inhibition was calculated using the following formula.

% growth inhibition =

$$100 - \left(\frac{mean\ abs\ of\ individual\ test\ grp}{Mean\ abs\ of\ control\ grp}\right) x\ 100$$

The IC50 value obtained is the concentration of sample required to inhibit the growth of 50% of viable cell population.

## Results & Discussion

### Preliminary phytochemical investigation

The percentage yield of the TCME was found to be 8.19 % w/w. The preliminary phyto-chemical investigation for the methanol extract of *Tephrosia calophylla* reveals the presence of poly phenols, flavonoids, tannins, steroids, alkaloids and carbohydrates.

### Determination of *in-vitro* antioxidant activity

*DPPH method*

In DPPH assay, the methanol extract of *Tephrosia calophylla* significantly decreased the absorbance produced by the DPPH and it was found to possess significant $IC_{50}$ value which was comparable to that of standard drug ascorbic acid ( See Table No.1).

*Lipid Peroxidation*

The antioxidant property of methanol extract of *Tephrosia calophylla* and *Tephrosia pumila* was compared with the standard Ascorbic acid. In lipid peroxidation assay, the TCME and ascorbic acid have significantly inhibited of lipid peroxidation by decreasing the absorbance of the supernatant, The $IC_{50}$ value of TCME was comparable to that of ascorbic acid (See Table No.1)

*Superoxide scavenging activity*

In superoxide free radical scavenging activity, the ascorbic acid TCME has offered good free radical scavenging activity by decreasing the absorbance due to NBT and exhibited significant IC50 value which was comparable to ascorbic acid (See Table No.1).

**Table 1** Effect of methanol extract of Tephrosia calophylla (TCME) on, DPPH, Lipid peroxidation and Superoxide scavenging methods

| Sample ($\mu$g/ml) | I.C50 ($\mu$g/ml) | | |
|---|---|---|---|
| | DPPH method | Lipid Peroxidation | Superoxide Scavenging |
| Ascorbic acid | 6.603 ± 3.528 | 7.0367 ± 1.0707 | 38.332±0.451 |
| TCME | 29.24 ± 3.060 | 23.51 ± 3.659 | 53.112±1.03 |

All the values are expressed as Mean± SEM, n = 6.

### Evaluation of in-vitro anticancer activity by MTT assay

Cancer is a dreadful disease and combating this disease is of great importance to public health [19]. The free radical are major cause that damage the genetic material either by inducing abnormalities in chromosomes (numerical and structural abnormalitites) and/or by damaging DNA results in mutation. The mutations in somatic cells ultimately lead to various cancers which are the leading causes of death in the human population and the antioxidants are agents that neutralize free radicals and proved effective in treating cancers [20, 21]. There is a necessity for search of new compounds with effective cytotoxic activity with least side effects as the present treatment availability is not truly unsatisfactory due to the problem associated with

adverse effects. Medicinal plants have long been used to prevent and treat many diseases, including cancer due to their antioxidant potentials and thus they are good candidates for the development of anti-cancer drugs. In this regard the study was performed to evaluate the anticancer potentials of the methanol extract of *Tephrosia calophylla* against human normal, breast, colon, liver and lung cancer cell lines [22, 23].

In the present study, we used 4 types of human cancer cells for the evaluation of *in-vitro* cytotoxicity potentials; they are MCF-7 (breast cancer cell lines), HCT-116 (colon cancer cell lines), HEP-G2 (liver cancer cell lines) and A-549 (lung cancer cell lines). All are tested by using trypan blue exclusion method which is based on decrease in viable tumor cell count and increased nonviable tumor cell count.

### *MTT assay against MCF-7 cells*

Human breast cancer cells are estrogen receptor (ER)-dependent and carries the wild type tumour suppressor p53 gene [24]. The Tamoxifen is an estrogen receptor antagonist used to treat the breast cancer was used in this study as a reference standard drug. The study revealed that the methanol extract prepared from the *Tephrosia calophylla* obtained was effective in attenuating the viable tumor cell count in dose dependent manner and shown significant IC50 value which was comparable to results of standard.

In present study, standard drug Tamoxifen and TCME significantly reduced the growth of breast cancer cell lines and the percentage of inhibition at was XYZ. In study conducted to determine the anticancer property of TCME against breast cancer cell lines, the methanol extract of *Tephrosia calophylla* has shown significant cytotoxicity the percentage of inhibition at 500$\mu$g/ml was 68.366. The TCME as it exhibited the significant $IC_{50}$ values (Figure No.1 and Figure No.2 and Table No.2)

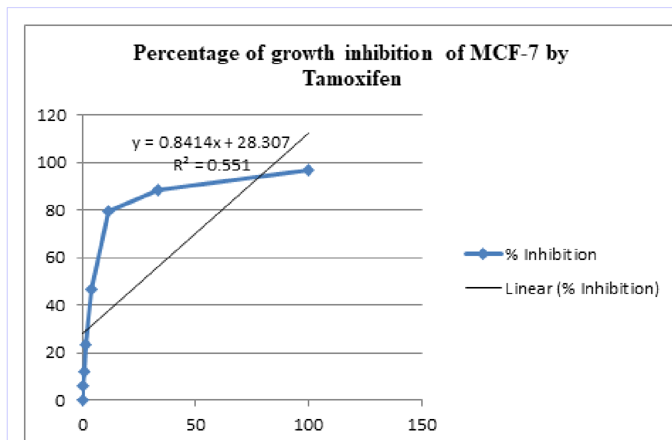


**Figure 1** The percentageinhibition of growth of MCF-7 by Tamoxifen

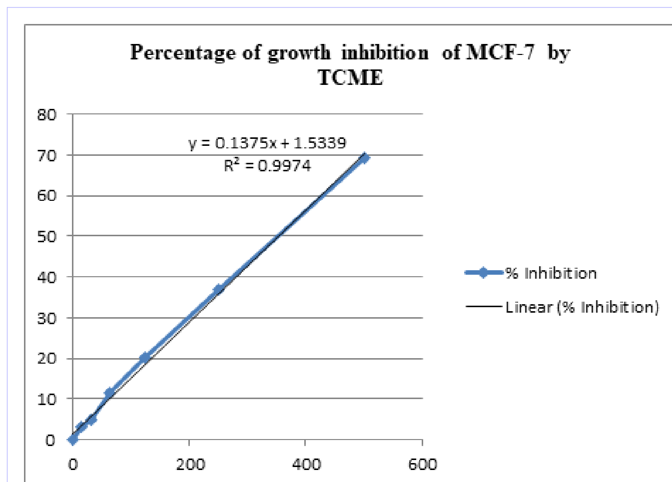


**Figure 2** The percentageinhibition of growth of MCF-7 by TCME

**Table 2** $IC_{50}$Values of methanol extracts of Tephrosiapumila, Tephrosia villosa and Tephrosiacalophylla against cancer and normal cells

| Treatment | $IC_{50}$ Values | | | | |
|---|---|---|---|---|---|
| | MCF-7 | HCT-116 | HEP-G2 | A-549 | Vero |
| Tamoxifen | 25.802 | – | – | – | – |
| Cyclophos-phamide | – | 41.182 | 33.565 | 38.479 | 89.235 |
| TCME | 353.773 | 302.754 | 263.726 | 297.75 | 1540 |

### *MTT assay against HCT-116 cells*

MTT assay was performed to determine in-vitro anticancer potentials of methanol extract against colon, hepatic and lung alveolar cancer cells using Cyclophosphamide as reference standard. We found that the TCME significantly caused cytotoxicity against all the three cancer cell lines probably by inducing apoptosis. The $IC_{50}$ values of TCME against all three, i.e, HCT-116, HEP-G2 and A-549 were significant and results comparable to standard. The standard drug Cyclophosphamide and TCME significantly reduced the growth of colon cancer cell lines and the percentage of inhibition at 500$\mu$g/ml was 79.773. The $IC_{50}$ value of methanol extract of against HCT-116 cell lines was significant was comparable to standard drug Cyclophosphamide (See Figure No.3, 4 and Table No.2).

### *MTT assay against HEP-G2 cells*

In this study, for the determination of anticancer activity of TCME against human liver cancer cells extract has shown significant inhibition of the cell growth the percentage of inhibition at 500$\mu$g/ml was 87.62. The samples treated with methanol extract

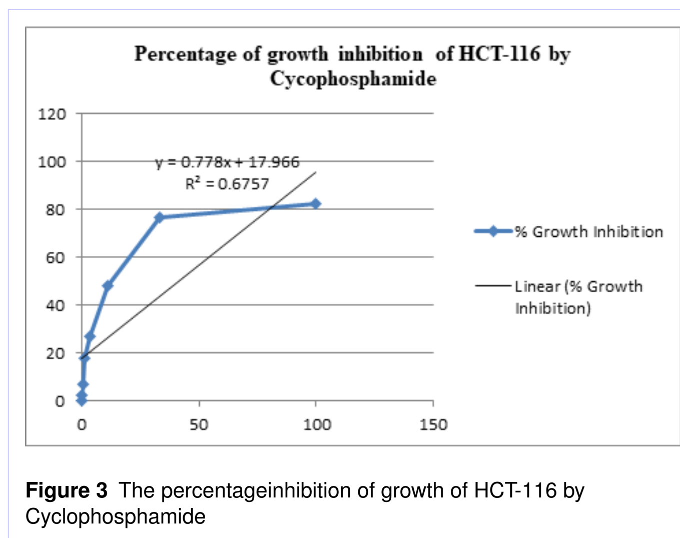


**Figure 3** The percentageinhibition of growth of HCT-116 by Cyclophosphamide

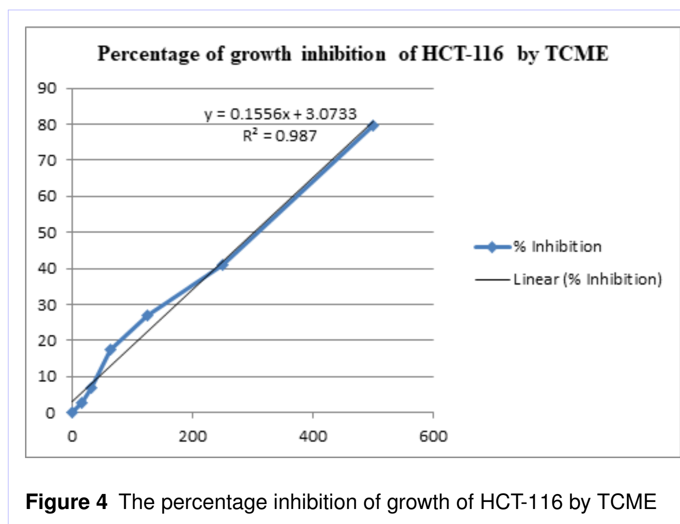


**Figure 4** The percentage inhibition of growth of HCT-116 by TCME

of Tephrosia calophylla and standard drug Cyclophosphamide have shown significant $IC_{50}$ values which indicates the cytotoxic potentials of the TCME against liver cancer (See Figure No.5, 6 and Table No.2).

*MTT assay against A-549 cells*

The methanol extract of *Tephrosia calophylla* has also shown significant cytotoxic potentials against lung cancer cells and percentage of inhibition of cell growth observed at 500$\mu$g/ml was 77.589 and significant $IC_{50}$ value in the present study. The results of TCME were comparable to that of Cyclophosphamide a reference standard used in the study. This also shows usefulness of the extract in treating lung cancer (see Figure No.7, 8and Table No.2).

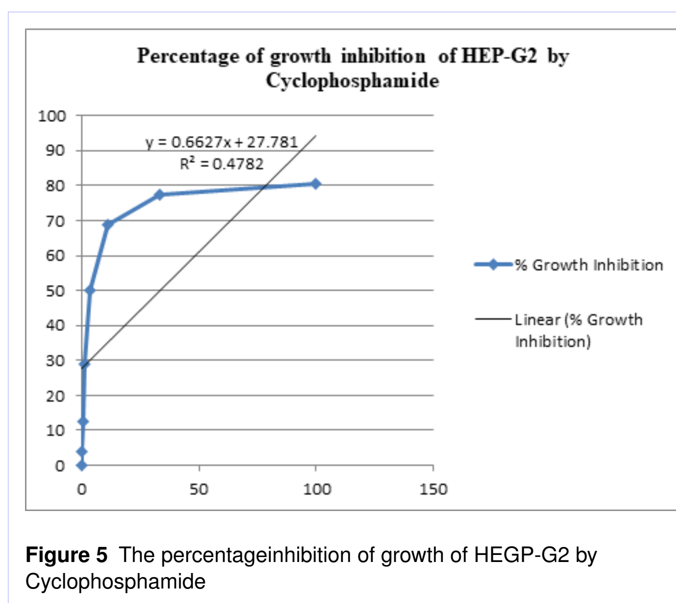


**Figure 5** The percentageinhibition of growth of HEGP-G2 by Cyclophosphamide

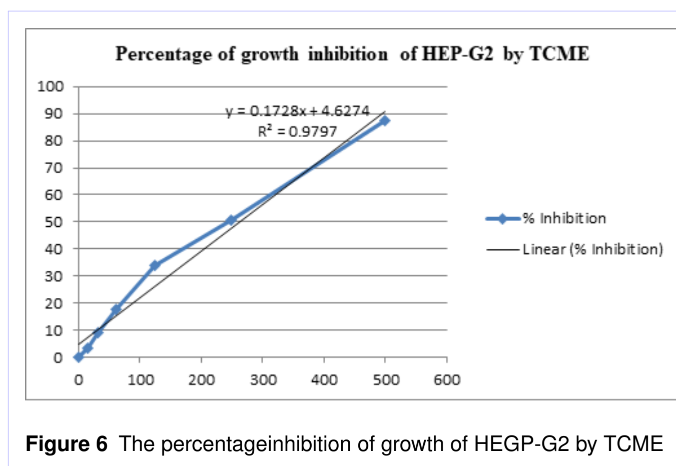


**Figure 6** The percentageinhibition of growth of HEGP-G2 by TCME

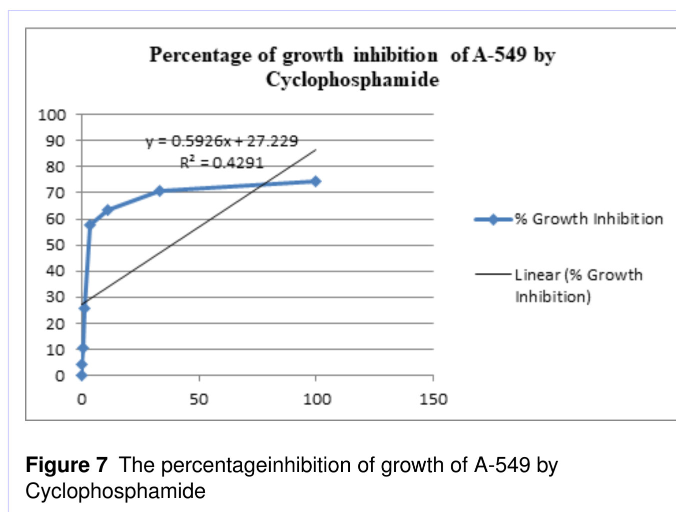


**Figure 7** The percentageinhibition of growth of A-549 by Cyclophosphamide

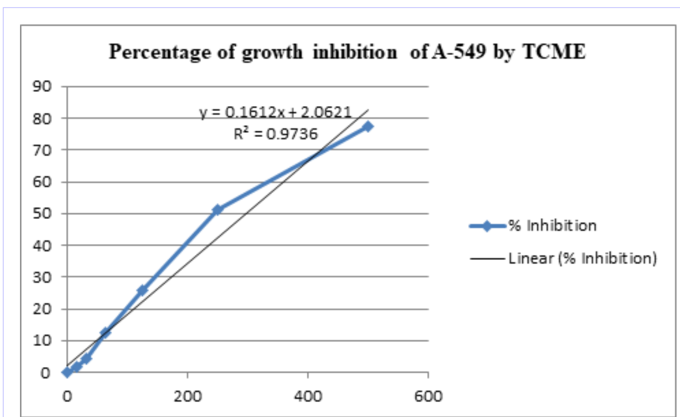


**Figure 8** The percentageinhibition of growth of A-549 by TCME

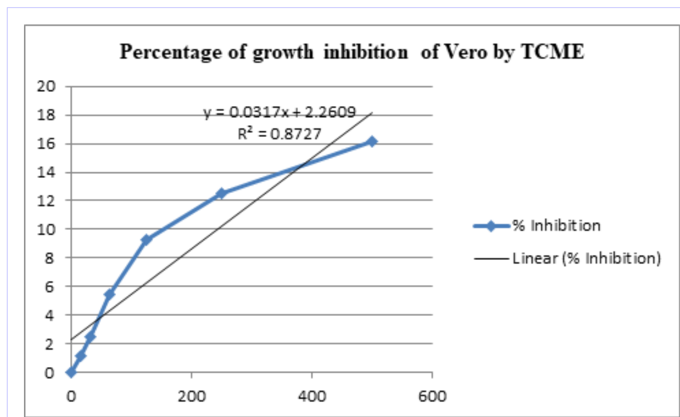


**Figure 10** The percentageinhibition of growth of Vero by TCME

*MTT assay against Normal human cells (Vero)*

In this study the methanol extract of Tephrosia calophylla have not shown significant cytotoxicity against normal cells and the maximum inhibition observed at500$\mu$g/ml was 16.137. The $IC_{50}$ value was more than 500$\mu$g/ml (see Figure No.9, 10 and Table No.2). But In MTT assay for the methanol extract against human normal cells, the treatment with TCME could not show significant cytotoxicity as the IC50 value is more than 1000$\mu$g/ml. This indicates the advantage of the extract compared to presently available anticancer drugs which possess serious adverse effects.

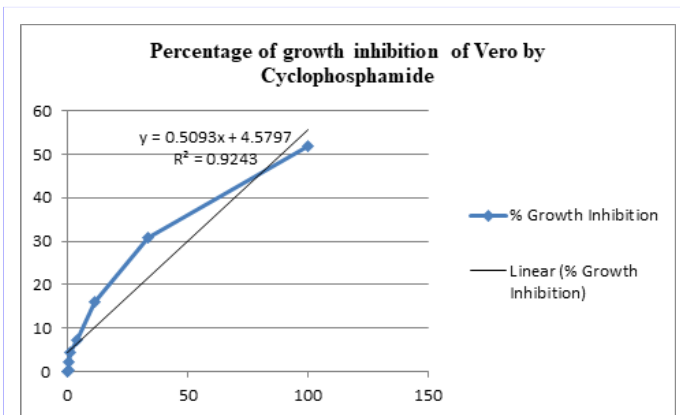


**Figure 9** The percentageinhibition of growth of Vero by Cyclophosphamide

The *Tephrosia calophylla* was essential component of Ayurveda a traditional medicinal system of medicine due to presence of various phyto-constituents for the treatment of various health complications such as diabetes mellitus, ulcers, liver diseases, urinary disorders, cancers and etc. In the present study the methanol extract was proven for its effective anti-oxidant potentials which is the important mechanism required for the anticancer activity [25, 26]. The methanol extract also shows the significant cytotoxicity against various cancer cells while normal cells were not affected by the extract. But further detailed study is necessary to correlate the anti-oxidant and cytotoxic effects of the extract.

# Conclusion

The results of the present study suggests that, methanol extract of *Tephrosia calophylla* posses *in-vitro* cytotoxic potentials against breast cancer, colon cancer, liver cancer and lung cancer cell lines. Further study is required to explore the in-vivo anticancer property of the plant and also to isolate and evaluate the specific phyto-constituents from the extract which are responsible for this beneficial pharmacological properties.

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