

Evaluation of Anticancer Activity Using Hexanic Extract of *Vitex trifolia* on Two Different Cancer Cell Lines

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ABSTRACT

The present study was designed to evaluate cytotoxicity of n-Hexane fractions of *Vitex trifolia* in two cancer cell lines viz., Hep G2 and HeLa. Different concentrations such as 20, 40, 80, 160, 320 and 640 µg/ml of extract were tested for cytotoxicity using MTT Assay. Based on our previous research findings on qualitative and quantitative analysis of phytochemicals in *Vitex trifolia*, it was evident that phytochemicals in *Vitex trifolia* had strong antibacterial activity. Hence in the current study anticancer activity was tested using *Vitex trifolia* extracts. Concentration required for a 50% inhibition of viability (IC₅₀) was determined graphically. MTT Assays showed that hexanic extract of *Vitex trifolia* were highly effective against both Hep G2 and HeLa cancer cell lines at a concentration of 80 µg/ml. This conclude that *Vitex trifolia* can be potent drug for cancer treatment and an alternative medicine.

Key words: *Vitex trifolia*, MTT Assay, Cytotoxicity, (Hep G2), (HeLa), Vero cell line, cell viability

INTRODUCTION

Cancer is a dreadful disease, which features uncontrollable cellular growth, local tissue invasion and metastasis and cause several deaths per year. In case of modern therapy there are serious side effects due to chemotherapy, surgical and radiations. Hence current pharmacological research is focused on Indian traditional system of medicine, which are natural and safe. Use of herbs has been authorized as alternative form of medicine with less toxicity and safe for patients. The phytochemicals such as alkaloids, flavonoids and others play a major role in fight against cancer cells⁸.

Vitex trifolia is a ethno medicinal plant belonging to the family lamiaceae⁶. The leaves are trifoliolate and treated for inflammation and pain, leucoderma, bronchitis fever, rheumatism, contusions, to improve memory, promote growth of hair, headache, and in many skin diseases and as insect and rodent repellent. The dried leaves are burned to deter

Mosquitoes⁶. *Vitex* is widely used in Chinese folk medicine³.and ethanolic extracts of flowers of *Vitex trifolia* Linn were effective on CCL4 induced hepatic injury in rats¹⁶. Further wound healing potency of *Vitex trifolia* L. and *Vitex altissima* L.¹⁰ have been reported. Several others reported for its antioxidant potential^{5,11,21} and antihelminthic property¹⁷

Anticancer studies against Hep-2, MCF-7 and Vero cell lines using methanol and n- hexane fractions on *Ononis hirta* showed better inhibitions in cancer cell lines²⁵. In the present study two cancer cell lines were selected. HeLa, a human immortal cell line derived from cervical

cancer cells taken from Henrietta Lacks, in 1951 used in this research³¹. Hep G2, a perpetual cell line which was derived from the liver tissue of a Caucasian American male with a well-differentiated hepatocellular carcinoma³². *Vitex* has been reported even for its anti-HIV properties^{18,13} Invitro studies on cytotoxicity were conducted by treating cells with *Vitex* toxic extract which may cause cell damage .¹⁵ Here cell integrity is an important factor and to assess we had to measure cell viability in two cancer cell lines viz., Hep G2 and HeLa.

MATERIALS AND METHODS

Collection and Extraction of Sample: *Vitex trifolia* leaves are trifoliolate palmately compound, with reticulate venation were collected from National Siddha Institute, Tambaram, Chennai. *Vitex* leaves were cleaned and shade dried for a week and fresh course powders were subjected to maceration by petroleum ether to remove fatty materials and then extracted with methanol, hexane and ethanol using soxhlet apparatus. All three fractions of the extract was then filtered and concentrated to dryness in a rotary vacuum evaporator under reduced pressure and stored in dessicators. n-Hexane fractions were selected for further studies of cytotoxicity.

In vitro assay for cytotoxicity (MTT ASSAY) of *Vitex trifolia*.

Cell line and culture: In this experiment three different cell lines viz., two cancer cell lines HeLa (Immortal cell line),

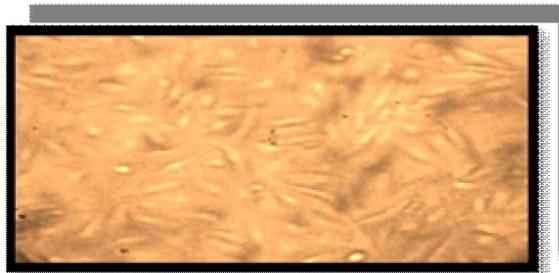


Fig. 1a Normal HeLa Cell line
Cytotoxicity effect of *Vitex trifolia* on Hep G2 cell line

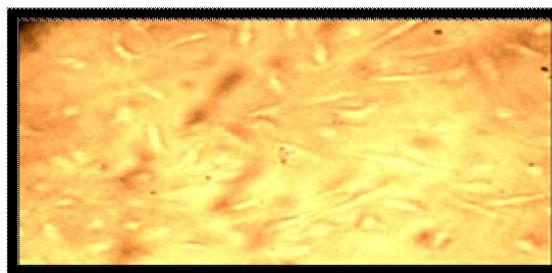
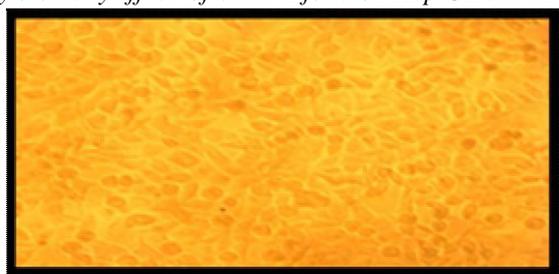


Fig. 1b. Toxicity (80 µg/ml)



1c. Normal Hep G2 Cell line

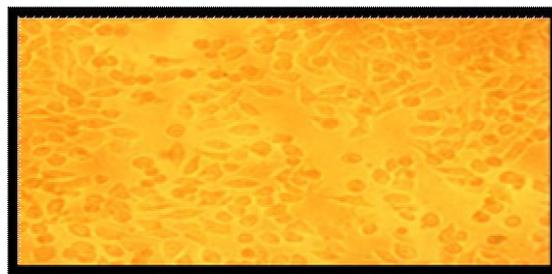


Fig. 1d. Toxicity (80µg/ml)

Fig. 1: Cytotoxicity effect of *Vitex trifolia* on HeLa cell line and Hep G2 cell lines

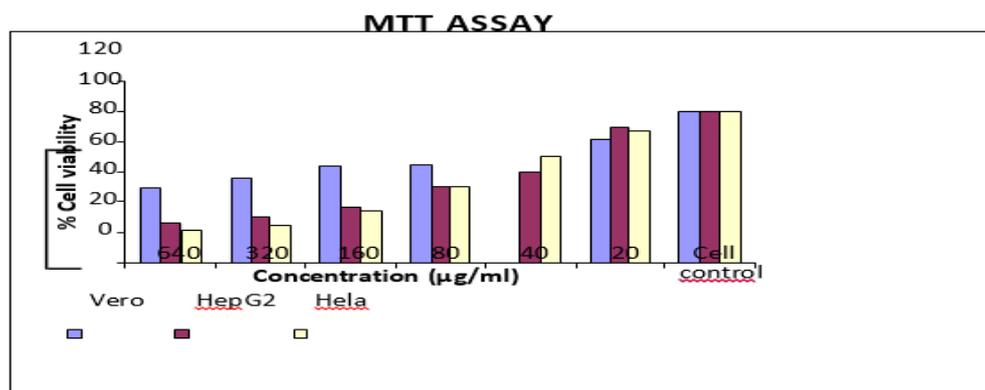


Fig. 2: MTT Assay for cytotoxicity and cell viability

HepG2 (hepato cellular liver carcinoma) and Vero normal cell line (Control) were used to test anticancer activity of *Vitex trifolia*. All the cell lines were obtained from National Centre for Cell Sciences, Pune (NCCS). The cells were maintained separately in Minimal Essential Media supplemented with 10% FBS, penicillin (100 U/ml), and streptomycin (100 µg/ml) in a humidified atmosphere of 50 µg/ml CO₂ at 37 °C.

Reagents: MEM was purchased from Hi Media Laboratories Fetal bovine serum (FBS) was purchased from Cistron laboratories Trypsin, methylthiazolyl diphenyl- tetrazolium bromide (MTT), and Dimethyl sulfoxide (DMSO) were purchased from (Sisco research laboratory chemicals Mumbai). All of other chemicals and reagents were obtained from Sigma Aldrich Mumbai.

In vitro assay for cytotoxicity (MTT assay) using *Vitex trifolia*

The Anticancer activity was determined by MTT assay²⁴. The assay detects the reduction of MTT by mitochondrial

dehydrogenase to blue formazan product, which reflects the normal function of mitochondria and cell viability. Cells (1 × 10⁵/well) were plated in 1ml of medium/well in 24-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 samples in 0.1% DMSO for 48h at 37°C. After removal of the sample solution and washing with phosphate-buffered saline (pH 7.4), 200µl/well (5mg/ml) of 0.5%

3-(4, 5-dimethyl-2-thiazolyl)-2, 5-diphenyl--tetrazolium bromide cells (MTT) solution was added. After 4h incubation, 0.04M HCl/ isopropanol were added. Different concentrations of the hexanic extract (1:2 to 1:32 dilutions - 20 µg to 640 µg) prepared from the diluted stock (1mg /ml), and the assay was performed in HeLa and Hep G2 cells in tetrads. Cell Control containing drug free medium and neat drug controls were included. After 3 days of incubation at 37°C under 5% CO₂, the absorbance was

determined by spectrophotometer. The concentration of drugs showing complete cytotoxic effect was recorded. Viable cells were determined by the absorbance at 570nm. Measurements were performed and the concentration required for a 50% inhibition of viability (IC50) was determined graphically. The Minimum concentration nontoxic in Vero but completely inhibiting the cancer cells (HeLa or Hep G2) is considered as the "effective drug concentration". The effect of the samples on the proliferation of cell lines HeLa and Hep G2 were expressed as the percent cell viability, using the following formula: Percent cell viability = A570 of Control cells - A570 of treated cells / A570 of control cells × 100. Graphs are plotted using the percentage of Cell Viability at Y-axis and concentration of the sample in X-axis. Cell control and sample control is included in each assay to compare the full cell viability in cytotoxicity activity assessments.

RESULTS AND DISCUSSION

The present study suggests high toxicity of *Vitex trifolia* against two cancer cell lines viz., Hep G2 and HeLa. MTT Assays showed inhibition at a concentration of 80 µg/ml in both Hep G2 (Fig. 1a, 1b) and HeLa (Fig. 1c, 1d). Anticancer activity more than 50 percent was found to be significant; Cell counting and cell viability tests confirmed anticancer activity in both cell lines by *Vitex trifolia*. Concentration required for a 50% inhibition of viability (IC50) was determined graphically (Fig. 2).

In the present study, Vitex extract were effective against both cancer cell lines. This is in support with the study on *V. trifolia* inhibiting proliferation of mammalian cancer cells⁸ and another report which stated *Vitex negundo* to be cytotoxic to human breast cancer cells (MCF-7)²³. Further observations on IC50 values of n-hexane fraction of *Ononis hirta* in a study conducted in Jordan recorded 72.06 µg/ml for MCF-7 and 90.30 µg/ml for Hep2²⁵.

This selectivity was due to the sensitivity of the cell line to the active compounds in the extract or to tissue specific response³⁵. Similarly ethanolic extract of *Vitex negundo* showed cytotoxicity to Hep G2 cells in a dose and time-dependent manner³³. In the present study n-hexanic fractions of *Vitex* were cytotoxic to liver and cervical cancer cell lines in accordance to the above mentioned work. Antiproliferative activities of plant extracts against human cancer cell lines is novel and may be strongly correlated with plants used against a wide variety of afflictions.

Our previous work on qualitative and quantitative analysis of phytochemicals revealed that phytochemicals like alkaloids, flavanoids and other phenols in *Vitex trifolia* to be a potent antibacterial agent^{13,21,22}. Further antiproliferative activity of total flavonoids and alkaloids isolated from different plants were reported earlier³⁰. Presence of flavonoids and terpenoids in active plants and alkaloids in *Ononis hirta* and *Inula viscosa* extracts exerted antiproliferative activity by inducing apoptosis in cancer cell lines^{7,15,25}. The association between flavonoids and reduced cancer risk has been reported in two different studies that showed a decrease in cancer risk with

consumption of vegetables and fruits rich with flavonoids^{28,15}. Our previous study on *Vitex* phytochemical screening showed the presence of flavonoids¹³ and also in a similar study at Jordan on active *Onis* plant extracts²⁵.

Numerous groups with antitumor properties are plant derived natural products including alkaloids, phenylpropanoids, and terpenoids^{27,29,28}.

CONCLUSION

We conclude that phytochemicals might have played a major role in anticancer activity. Hence *Vitex trifolia* can be recommended for cancer treatment especially for liver and cervical cancers. Further in-depth study need to be initiated for finding out bioactive compounds responsible for anticancer activity.

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