Research Article

# Phytochemical Investigation and Anti-cancer Activity of *Vitex negundo*

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**ABSTRACT**

Evaluation of anticancer activity of chloroform, ethanol and aqueous extract of *Vitex negundo* by trypan blue dye exclusion assay against Daltons ascites lymphoma cell lines. *In vitro* anticancer activity of chloroform, ethanol and aqueous extract of *Vitex negundo* was evaluated on selected cancerous cells lines trypan blue dye exclusion assay. Trypan blue assay is based on staining of cells. Cells are then counted using heamocytometer under the microscope, non-viable cells were stained blue, viable cells remain unstained. The potent anticancer activity was shown by the methanol extract of *Vitex negundo* on Daltons ascites lymphoma cell lines. The medicinal plant *Vitex negundo* was studied by *in vitro* evaluation methods trypan blue exclusion assay. The chloroform, ethanol and aqueous extract of *Vitex negundo* have shown potent anticancer activity on selected cancerous cell lines. More efforts are needed to explore potent anticancer plants from the mother earth and save humans around the world from cancer.

**Keywords:** Anticancer activity, chloroform, ethanol and aqueous extract of *Vitex negundo*, Trypan blue exclusion assay, Daltons ascites lymphoma cell lines.

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## INTRODUCTION

Cell division in humans is mainly controlled by DNA of the cell. Main factors are responsible for the cause of cancer such as chemical carcinogens, viruses, chromosomal rearrangement or spontaneous transformation, and tumor suppressor genes. Cancer can be caused by any of the three ways improper diet, genetic factors, and environmental factors[1,2]. More than 35% of all cancers worldwide are caused by improper diet in the case of colon cancer; diet may account for more than 80% of the cases. Alcohol and cigarettes to their diet, the percentage cause of cancer may increase to 60%. Plants have been demonstrated clinical source for anticancer compounds. However, many of the plant products and their derivatives are approved for cancer control. Hence, the development of new drugs to play an important role in cancer control is greatly desired [3].

*Vitex negundo* Linn (Synonym: *Viteex incise* Linn, *Vitex incise Lam Var hetropylla.,* family: Verbanaceae). A large, aromatic shrub or sometimes a small slender tree, upto 4.5 m in height found throughout the greater part of India. Leaves possess anti-inflammatory, analgesic and antihistamine properties. Roots are used for leprosy, dyspepsia, rheumatism and piles. Bark is used as verminosis and opthalmopathy. Flowers are used in cholera. Fruit used as anthelmintic. The whole plant is used in inflammations, antiseptic, antipyretic and diuretic[4-10]. Earlier studies have shown that the plant possess anti-inflammatory and antihistamine[11], analgesic[12], antioxidant[13], antibacterial[14], CNS depressant[15], antifungal[16], snake venom neutralization[17], mosquito repellant activity[18], insecticidal[19], larvicidal efficacy[20], antinociceptive[21], antiandrogenic[22], Hepatoprotective[23], antifertility[24], skin aging inhibitor[25] and anti dopaminergic[26] effects. Constituents previously isolated from the plant include eight lignans[27] (negundin A, negundin B, 6-hydroxy-4-( 4-hydroxy -3 methoxy)-3-hydroxyl methyl -7 methoxy -3,4 di hydro 2 napathaldehyde, vitrofolal, (+) – iynoiresinol, (+) – iynoiresinol -3α-0-β-Dglucoside, (+)(-)(-) pinorecinol and (+) –diasyringaresinol, irridoid glycoside[28] (2-p-hydroxy benzoyl mussaenosidic acid), flavonones[29] (5,3' di hydroxyl -7,8,4' trimethoxy flavonone and (5,3' dihydroxy -6,7,4' trimethoxy flavonone), flavones[30] (vitexicarpin), β – sitosterol[31], essential oils[32] (α –pinene, linalool, terpinyl acetate, beta caryophyllene ), non diterpene[33] (vitedoin B), pentacyclic triterpenoids [34] (beutinilic acid, ursolic acid) and flavonoid glycoside[35] (luteolin, agnuside, negundoside, iso-orientin).

## MATERIALS AND METHODS

### Collection and identification of plants

*Vitex negundo* plant materials were collected from Aritapatti village near Madurai district, in December and was identified by Dr.Stephen, Professor, American college, Madurai, Tamilnadu; a voucher specimen has been deposited at the herbarium unit of the Department of Pharmacognosy, Ultra. College of pharmacy, Madurai, Tamilnadu, India.

### Extraction and phytochemical screening of plant

The powdered plant materials (500g) were extracted with petroleum ether at 40-60o C, by continuous hot percolation using soxhlet apparatus. The extraction was carried out by using solvent of increasing polarity starting from petroleum ether, chloroform, ethanol and aqueous respectively. The extraction was carried out for 72 hours. The petroleum ether extract was filtered and concentrated to dry mass by using vacumm distillation. A dark greenish brown residue was obtained. The marc left, after petroleum ether extraction was taken and then subsequently extracted with chloroform, ethanol and aqueous for 72hours. Phyto chemical screening was performed using standard procedure [36-38].

### Preliminary phytochemical investigation

The qualitative chemical test of various extracts of *Vitex negundo* was carried out using standard procedure. Terpenoids, Phenolic compounds, Carbohydrates, Tannins, Alkaloids, glycosides, Flavonoids and Phytosterols were present in all the extracts. (Table 1)

**Table 1:** Phytochemical Screening of *Vitex nigundo*

| Extracts | Petroleum Ether | Chloroform | Ethanol | Aqueous |
|---|---|---|---|---|
| Sterols | - | + | + | - |
| Terpenoids | + | + | + | - |
| Carbohydrates | + | + | + | - |
| Flavonoids | + | + | + | + |
| Proteins | + | - | + | - |
| Alkaloids | - | + | + | + |
| Glycosides | + | + | + | - |
| Tannins | - | + | + | + |
| Saponin | - | - | - | - |
| Phenolic compounds | + | + | + | + |
| Fixed Oil and Fats | + | + | - | - |

### *In-vitro* cytotoxicity assay

### Trypan Blue

Trypan blue is a vital stain used to selectively colour dead tissues or cells blue. It is a diazo dye. Live cells or tissues with intact cell membranes are not coloured. Since cells are very selective in the compounds that pass through the membrane, in a viable cell Trypan blue is not absorbed; however, it traverses the membrane in a dead cell. Hence, dead cells are shown as a distinctive blue colour under a microscope. Since live cells are excluded from staining, this staining method is also described as a Dye Exclusion Method [39-42].

## MATERIALS REQUIRED

DLA (Daltons lymphoma ascites) bearing mice. Phosphate buffered saline (PBS) contains Nacl- 4gm, $Na_2HPO_4$- 0.72gm, $KH_2PO_4$- 0.1gm, KCl- 0.1gm and Distilled water- 500ml. The dye used is Trypan blue and the cell is counted by using Haemocytometer.

## PROCEDURE

The tumour cells aspirated from the peritoneal cavity of tumour caring mice were washed thrice with normal saline and checked for ability using tryphan blue dye exclusion method. The cell suspension (1 X $10^6$ cells in 0.1 ml) was added to tubes maintaining various concentrations of the test compounds and the volume was made up to 1 ml using phosphate buffered saline (PBS) control tubes contained only cell suspension. These assay mixtures were incubated for 3 hours at $37^0$C and percent of dead cells were evaluated by trypan blue exclusion method.

$$\% \text{ Cytotoxicity} = \frac{\text{Number of dead cell}}{\text{Number of life cell} + \text{Number of dead cell}} \times 100$$

### Statistical analysis

All data were presented as means of percentage inhibition. Statistical analysis for all the assays results were done using Microsoft Excel program.

**Table 2:** Cytotoxicity of Chloroform Extract of *"Vitex negundo"* to Dalton's Lymphoma Ascites Carcinoma (DLA) Cells

| Concentration µg/ml | Percentage of Cytotoxicity % |
|---|---|
| 200 µg | 100% |
| 100 µg | 100% |
| 50 µg | 41% |
| 20 µg | 16% |
| 10 µg | 7% |

**Table 3:** Cytotoxicity of Ethanol Extract of *"Vitex negundo"* to Dalton's Lymphoma Ascites Carcinoma (DLA) Cells

| Concentration µg/ml | Percentage of Cytotoxicity % |
|---|---|
| 200 µg | 100% |
| 100 µg | 100% |
| 50 µg | 38% |
| 20 µg | 15% |
| 10 µg | 6% |

**Table 4:** Cytotoxicity of Aqueous Extract of *"Vitex negundo"* to Dalton's Lymphoma Ascites Carcinoma (DLA) Cells

| Concentration µg/ml | Percentage of Cytotoxicity % |
|---|---|
| 200 µg | 100% |
| 100 µg | 100% |
| 50 µg | 39% |
| 20 µg | 16% |
| 10 µg | 7% |

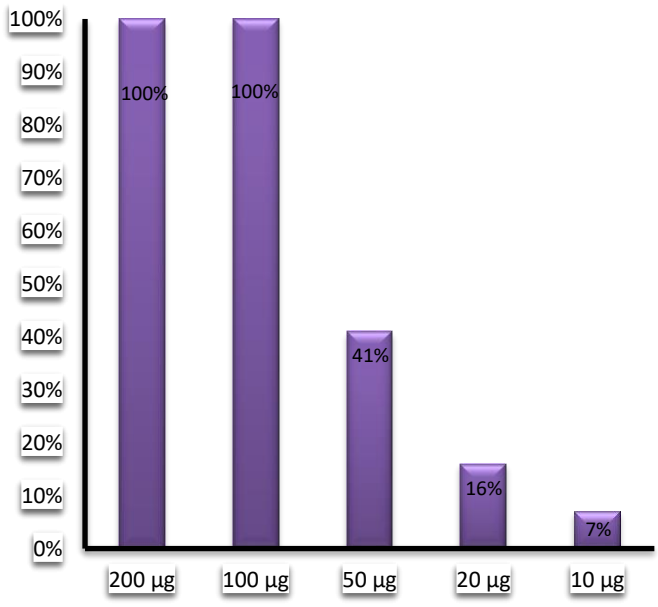


**Figure 1:** Cytotoxicity of Chloroform Extract of *"Vitex negundo"* to Dalton's Lymphoma Ascites Carcinoma (DLA) Cells

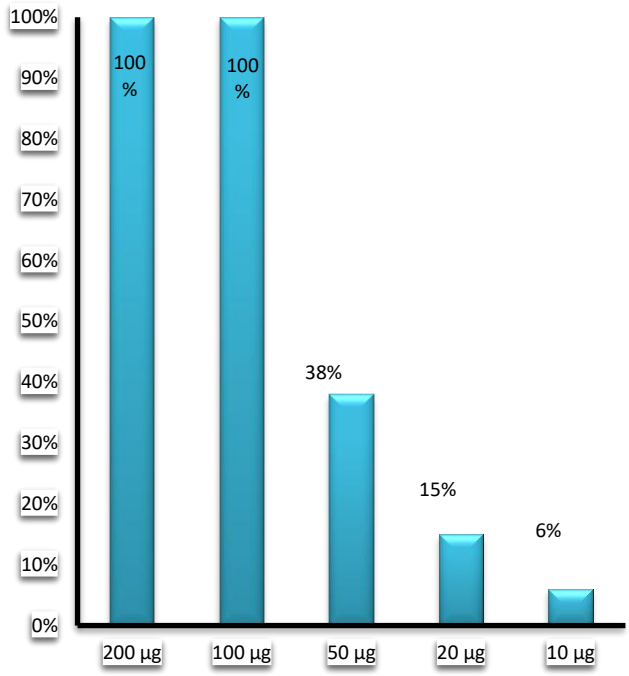


**Figure 2:** Cytotoxicity of Ethanol Extract of *"Vitex negundo"* to Dalton's Lymphoma Ascites Carcinoma (DLA) Cells

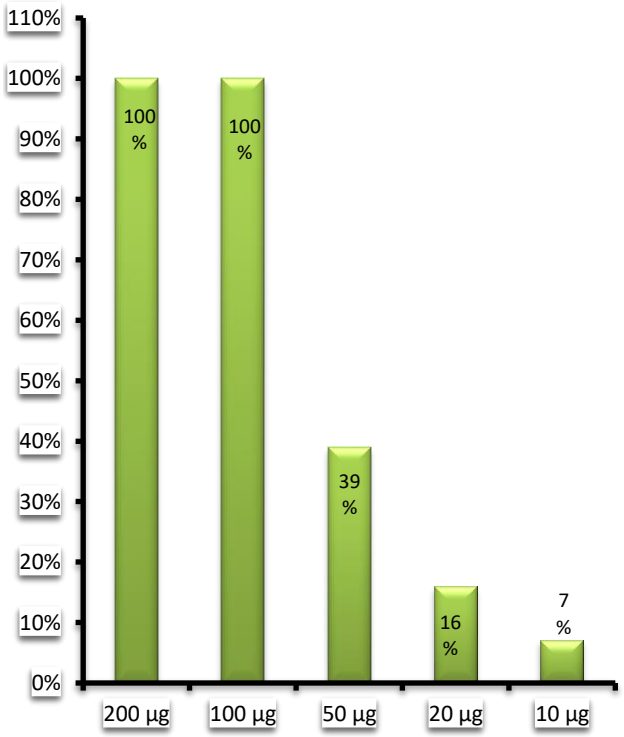


**Figure 3:** Cytotoxicity of Aqueous Extract of *"Vitex negundo"* to Dalton's Lymphoma Ascites Carcinoma (DLA) Cells

## RESULTS AND DISCUSSION

The ethanolic extract of leaves of *Vitex negundo* was tested against DAL cell lines. Different concentration of plant extract was inoculated with selected cell line and the cytotoxicity was assessed using trypan blue dye exclusive.

The test based on the principle that the dead cell accepts dye and stain with blue color. The plant drug may disturb the membrane integrity and caused the cell death, which is one of the hall marks of apoptosis. The Chloroform, Ethanol and Aqueous extracts showed 100 % of cytotoxicity against DAL cell line.

The phytochemical investigation shows the presence of Flavanoids, carbohydrates, steroids and alkaloids and phenolic compounds in the extract. The Chloroform, Ethanol and Aqueous extracts were subjected to i*nvitro* cytotoxicity studies at 50µg, 100µg, 200µg concentrations using PBS and DLA cells (Table 2,3,4). The Chloroform, Ethanol and Aqueous extracts showed 100%, 100% and 100% cytotoxicity respectively (Fig 1,2,3). The extracts of Petroleum ether, chloroform, Ethanol and Aqueous extracts have significant cytotoxicity activity.

## CONCLUSION

The plant was initially selected and tested for anticancer activity based on their historical and other traditional uses. The root extract of *Vitex negundo* Chloroform, Ethanol and Aqueous extracts were prepared and tested for their potential as anticancer activity by *in-vitro* evaluation method, i.e., trypan blue exclusion assay. This was done by closely monitoring the viability of cultured human cells exposed to the plant extracts. More efforts are needed to explore potent anticancer plants from the mother earth and save humans around the world from cancer.

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