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# Potent anticancer activity of *Nigella Sativa* Seeds

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## Abstract

In present investigation, anticancer activity of methanolic extract of the seeds of *Nigella Sativa* was investigated on different cancer and normal cell lines such as HL-60, U-937 and HEK 293T.. The extract was prepared by the cold maceration technique, and its antifungal activity was assessed by MTT based cytotoxicity assay. We found that methanolic extract of plant exhibits potent inhibition of cancerous cell growth against HL-60 and U- 937 with $IC_{50}$Value 13.70 µg/ml, and 28.31 µg/ml respectively.

**Keywords:** *Nigella Sativa*, MTT, Anti cancer.

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## Introduction

*Nigella Sativa* (Family: *Ranunculaceae*) is an annual flowering plant, also known as Kalijiri in Hindi. It has a pungent bitter taste and faint smell of strawberries. It is mainly used as a spice and also in the preparation of candy and liquor. *Nigella Sativa* has a potential anti-inflammatory, anti-microbial, anti-fungal, anti-parasitic, and anti-cancer activity.

Seeds of the *Nigella Sativa* contain 37% oil and 4.1 % ash (calcium salts), protein (16-19.9%), carbohydrates (33-34%), fibre (4.5-6.5%), saponins (0.013%), moisture (5-7%). The oil has chemical constituents like thymoquinone, dihydrothymoquinone, thymodihydroxyquinone, alpha-pinene, 4-terpineol. Other constituents are also present in the seeds like Linoleic acid, lonolenic acid, Arachidic acid. Most of the biological activity of *Nigella Sativa* plant showed by the oil constituents like, Thymoquinone. [1]

The antifungal activity of Thymoquinone from *Nigella Sativa* had been evaluated by the standard agar plate method. [2]

## Materials and Methods

**Chemicals**:
Methanol (Finar Chemicals,), Trypan blue, Triton X100, DMSO cell culture grade, Sodium bicarbonate, HYQ Antibiotic/Antimycotic solution, 100X (10000 U/ml Penicillin G, 10000µg/ml Streptomycin, 25 µg/ml Amphotericin B), EDTA, HYQ DPBS/modified 1X (Dulbecoo's phosphate buffer saline without Ca+ & Mg+), 0.25% Trypsin 1X,Cell proliferation kit (MTT) 2500 tests, Cyclophosphamide monohydrate as a control.

**Media**
DMEM (Dulbecoos Modified Eagles medium, low glucose with glutamine), FBS (Fetal Bovine Serum, South American origin, 500 ml), HYQ SFM HEK-293 (Serum Free Media, 500ml)

**Cell lines**
U-937 (Human myeloid lymphoma cell line), HL60 (Human leukemia cell line), HEK-293T (Human erythrocytes kidney normal cell line) All cell lines purchase from NCCS, Pune Job. No.669-671

**Collection of plant material**:
The plant material (*Nigella Sativa* seeds) was collected from the Ms Lallubhai Vrajlal Gandhi and sons, (Ahmedabad, Gujarat, India) and authenticated by Dr. Ritesh Vaidya, Bio-science department of Ganpat University.

**Extraction Preparation**:
**Methanolic extract:**
Methanolic extract of the *Nigella Sativa* seeds was prepared by socking 150 gm finely grounded powder of *Nigella Sativa* seeds in 150 ml of methanol for 7 days. After 7 days, the extract was filtered through Whatman filter paper and evaporated till dryness. [4,5]

**Preparation of media DMEM (1X Liquid Medium with FBS & antibiotic):**
10.7 gm of DMEM powder was added in 1litre of distill water and then it was stir continuously until clear solution formed. To this, $NaHCo_3$ was added to maintain pH 7.0-7.2 and then solution was filtered using membrane filtration assembly. DMEM, Antibiotic, and FBS (Fetal bovine Serum) were prepared. 10% FBS and 5 ml antibiotics were added into the DMEM.

**Subculture of cell lines Subculture of adherent cell lines (HEK 293T):**
Cultures were viewed using an inverted microscope to assess the degree of confluency and the absence of bacterial and fungal contaminants was confirmed. Cell monolayer was washed with PBS without $Ca^{2+}/Mg^{2+}$ using a volume equivalent to half the volume of culture medium. Trypsin/EDTA was added on to the washed cell monolayer using 1ml per 25cm2 of surface area. Flask was rotated to cover monolayer with trypsin. Flask was returned to the incubator and left for 2- 10 mins. The cells were examined using an inverted microscope to ensure that all the cells were detached and floated. The cells were re suspended in a small volume of fresh serum

containing HEK-293 medium. 100-200µl was removed to perform a cell count. The required number of cells were transferred to a new labeled flask containing pre-warmed HEK-293 medium and incubated as appropriate for the cell line.

**Subculture of suspension cell lines (U- 937 and HL 60)**

Cultures were viewed using an inverted phase contrast microscope. Centrifugation for subculture was not performed unless the pH of the medium was acidic which indicated the cells overgrown and were not recovered. Cells were centrifuged at 150 rpm for 5 minutes, re-seeded at a slightly higher cell density and 10- 20% of conditioned medium added to the fresh media. Small sample of the cells from the cell suspension was taken. Cells/ml was calculated and the desired number of cells re-seeded into freshly prepared flasks without centrifugation just by diluting the cells.

**MTT assay**

MTT assay was employed to assess cell proliferation. Viable cells were seeded into 96- well microtitre plates at $5 \times 10^4$ cells/well in DMEM (Dulbecco's modified Eagle's medium) supplemented with FBS (fetal bovine serum), 100units/ml penicillin, 100 µg/ml streptomycin, and were cultured in a humidified atmosphere of 5% $CO_2$ and 95 air at 370C. 180 µl of cell suspension was cultured with 20µl of various concentrations of the extract (0.005-100µg/ml) dissolved in 2% DMSO (dimethylsulphoxide) solution. Control cells were incubated in culture medium only. Wells containing only media were considered as a blank. All extract dilution doses were tested in duplicates. The cell proliferation is based on the ability of the mitochondrial succinate-terazolium reductase system to convert 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) to a blue colored formazan. The test denotes the survival cells after toxic exposure.

10 µl of MTT labeling mixture was added and incubated for 4 h at 37°C and 6.5% CO2. After 4 h, 100 µl of solubilization solution was added in each well. After 48h incubation at 370C temperature and 5% CO2, the absorbance of soluble formazan product produced by viable cells was measured at 540nm using ELISA plate reader. Reference wavelength used was 650 nm [6]**.** Percentage inhibition of the extract against all cell line was calculated using the following formula.

$$\textbf{\% cell survival} = \frac{\textbf{(At − Ab)}}{\textbf{(Ac − Ab)}} \times \textbf{100}$$

At = Absorbance of Test,
Ab= Absorbance of Blank (Media),
Ac= Absorbance of control (cells)

$$\textbf{\% cell inhibition = 100 - \% cell survival}$$

The effects of extracts were expressed by $IC_{50}$ values calculated from dose response curves.

## Results and Discussion

The effect of methanol extract of *Nigella Sativa* seeds (test) and cyclophosphamide (control) on the growth of U-937, HL60 and HEK-293T cell lines were examined by the MTT assay. Dose response curves constructed between the range 0.005 – 100µg/ml and 0.005 - 100µM for *N.sativa* and cyclophosphamide (control) respectively, express decreasing number of viable cells with increasing concentration of extract as well as cyclophosphamide. Calculation of $IC_{50}$ and was done using graphs generated from Microsoft excel 2003 edition. (Figure 1) The susceptibility of cells to the extract exposure was characterized by $IC_{50}$ values (Table 1).

Results indicate that the antiproliferative effect strengthens with increase in the concentration of extract.

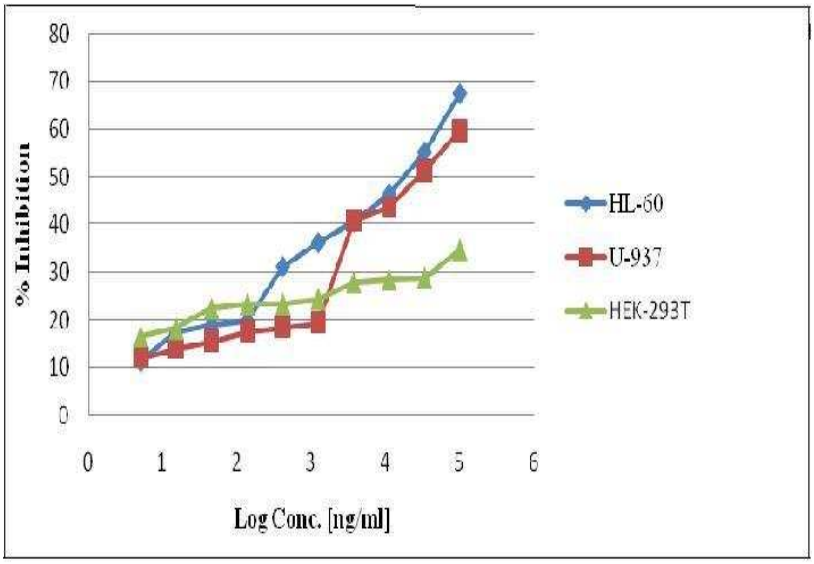


**Figure 1: Activity of Methanolic extract of *N. sativa* seeds against various cell lines**

Highest cytotoxicity of methanolic extract was found against HL60 cell line, which is more potent than that of cyclophosphamide, which shows that the extract was proven more active against the HL60 than the cyclophosphamide. However, butanolic extract was found to have lower activity against HEK293T (normal) cell line, and U-937 cell line but cyclophosphamide was found active against same cell lines.

**Table 1: $IC_{50}$ values of Methanolic extract of *N. sativa* seeds and Cyclophosphamide against various cell lines**

| $IC_{50}$ (µg /ml) | HL-60 | U-397 | HEK-293T |
|---|---|---|---|
| Methanolic extract | 13.70 | 28.31 | >100 |
| Cyclophosphamide (µM) | 41.54 | 11.86 | 27.06 |

## Conclusion

Plant flora has been a great source of therapeutic agents. [5, 6] Methanolic extract of *Nigella Sativa* seeds exhibited potent inhibition of both cancerous cell lines. The extract was found to be more potent than standard drug Cyclophosphamide. The plant can be a source of an important pharmacophore in future.

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