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# *In Vitro* Antitumor Activity of Alcoholic Extract of Piper Betel Leaf

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## ABSTRACT

*Piper betel* leaf extract **(**alcoholic) was screened for its possible cytotoxic activity on proliferation of HT29 colon cancer cell line. The activity was determined by microculture tetrazolium viability (MTT) assay. The cells were exposed to different concentrations (100, 50, 25, 12.5, 6.25and 3.125 µg/ml). The % cytotoxicity produced in MTT assay at100µg/ml was 76.41 with IC50 of 24.37. From these results it was observed that alcoholic extract of Piper betel leaf has significant cytotoxic activity.

**Keywords:** Piper betel leaf, MTT assay, cytotoxicity, IC50.

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## INTRODUCTION

Cancer is a leading cause of death in today's era. It is the uncontrolled cell proliferation. Carcinoma of the large bowel is equally distributed between men and women. [1] Cases of colorectal cancer are increasing and it made to develop a more effective preventive measure. Natural products/various medicinal plants are rich source of medicinal chemical constituents which are having potential pharmacological activities. There is always a hope that natural products may provide potent and safe medicines. The *Piper betel Linn*. A perennial dioecious root climber, useful in asthma, rheumatism, dyspepsia and laryngitis. [2] The leaves contain good amount of vitamin B, ascorbic acid and carotene. [3] The essential oil and extracts of the leaves possess antimicrobial activity, antioxidant activity, cytotoxic activity [4] and anti-inflammatory activity. [5] It also shows ulcer healing property. The present study aimed to evaluate the possible cytotoxic activity of *Piper betel* leaf extract on HT-29 Human colon cancer cell line.

## MATERIALS AND METHODS

Plant material Collection: Fresh leaves of Piper betel were collected from nearby field and are identified and authenticated in RMRC, Belgaum. Ethanolic extract is prepared using soxhlet apparatus.

**Cell Culture:** HT-29 human colon cancer cells were obtained from NCL, Pune, India. The cells were maintained in laboratory. MTT reagent was purchased from Sigma. The solution was preserved at 4ċ away from light.

**MTT solution preparation:** 10 mg in 10 ml of Hank's balanced solution.

**Cell culture:**

The cell line were maintained in 96 wells micro titer plate containing Minimum Essential Medium (MEM) media supplemented with 10% heat inactivated fetal calf serum (FCS), containing 5% of mixture of Gentamycin, Penicillin ( 100 Units/ ml) and Streptomycin (100µg/ml) in presence of 5% $Co_2$ at 37ºC for 3-4 days. After 3-4 days remove the supernatant and replace MEM media with Hank's balanced solution supplemented with Gentamycin, Penicillin and Streptomycin. Incubate overnight. *In-vitro* growth inhibition effect of test compound was assessed by calorimetric or spectrophotometric determination of conversion of MTT into "Formazan blue" by living cells. Remove the supernatant from the plate and add fresh Hank's balanced salt solution and treated with different concentrations of extract or compound appropriately diluted with DMSO. Control group contains only DMSO. After 24 hrs incubation at 37ºC in a humidified atmosphere of 5% $Co_2$, the medium was replaced with MTT solution (100µl, 1mg per ml in sterile Hank's balanced solution) for further 4 hr incubation. The supernatant carefully aspirated, the precipitated crystals of "Formazan blue' were solubalised by adding DMSO (200µl) and optical density was measured at wavelength of 570nm. The test

denotes the survival cells after toxic exposure. Percentage inhibition of the extract against all cell line was calculated using the following formula:

$$\text{Surviving cells (\%)} = \frac{\text{Mean OD of test compound}}{\text{Mean OD of control}} \times 100$$

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

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

**Calculations and statistics:**

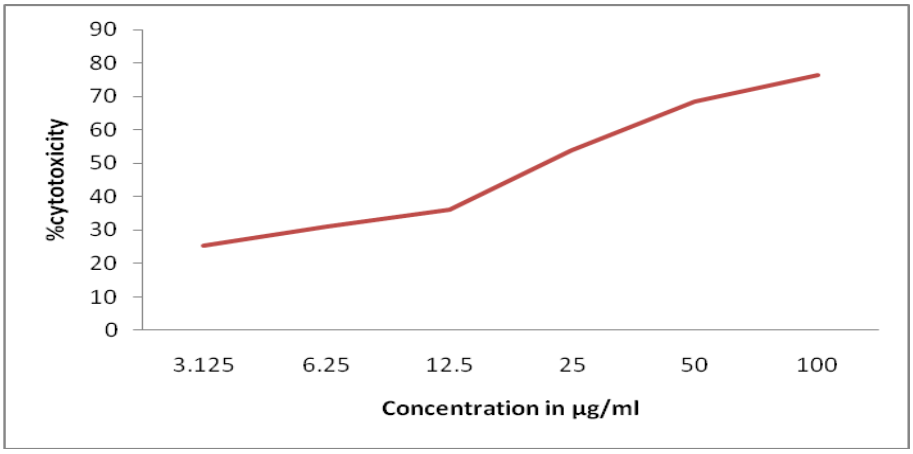
Results were expressed by calculating percentage growth inhibition of control. $IC_{50}$ values were derived from a nonlinear regression model (curve fit) based on sigmoidal dose response curve (variable) and computed using Graph pad Prism version 5.00.

## RESULTS

**Table No: 01 Effect of *Piper betel* leaf extract on HT-29 Cell lines**

| Sl.N0 | Concentration (µg/ml) | Cytotoxic activity (%) | $IC_{50}$ (µg/ml) | $R^2$ value |
|---|---|---|---|---|
| 1 | 100 | 76.41 | 24.37 | 0.997 |
| 2 | 50 | 68.25 | | |
| 3 | 25 | 53.91 | | |
| 4 | 12.5 | 36.11 | | |
| 5 | 6.25 | 31.04 | | |
| 6 | 3.125 | 25.37 | | |

**Figure No: 01**



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## DISCUSSION

The *in vitro* cytotoxity against human colon cancer cell lines showed potential cytotoxic activity. The results obtained are showed in table 1.The results clearly indicates the concentration dependant inhibition of HT29 cells, with an IC 50 value of 24.37 .Antioxidants may offer resistance against the oxidative stress by lipid peroxidation and by other mechanisms and thus prevent disease. [6]Antioxidants have shown good results in prevention of many diseases. The present activity of *Piper betel* may be because of antioxidant principles present in the plant.

## CONCLUSION

*Piper betel* leaf extract showed selective *in vitro* cytotoxicity against human colon cancer cell lines. The activity might be depended upon the morphology of cell lines and mechanism of action of the plant extract etc, predictive as a anticancer activity and $IC_{50}$ value calculated for extract was below 50 µg/ml, which indicates that a extract bears a potential cytotoxic activity. Therefore it is suggested that the isolation of active constituent to obtain a good therapeutic agent.

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