



## Cytotoxic Potential of *Phyllanthus emblica* on Oral Cancer Cell Lines

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### ABSTRACT

This study aims at evaluating the cytotoxic potential of *Phyllanthus emblica* on oral cancer cell lines. MTT assay for evaluating the cytotoxicity potential of the extract on oral cancer cell lines was performed. The extract exhibited increased cytotoxicity with increased concentration. This study is conducted to see if *Phyllanthus emblica* is effective in treating oral cancer in a natural way rather than harmful treatments.

**Keywords:** *Phyllanthus emblica*, Cytotoxicity, Oral cancer cell lines.

### INTRODUCTION

Cancer which can affect any part of the body is a group of disease caused by loss of cell cycle control. It is associated with abnormal and uncontrolled cell growth.<sup>1</sup> Oral cancer is the sixth most common cancer affecting mankind, which also presents itself with a low survival rate. More than 90% of oral cancers are histopathologically squamous cell carcinomas (SCC).<sup>2</sup> Oral SCCs typically affect males over 40 years of age with a history of regular exposure to etiological risk factors, like tobacco, alcohol, betel quid or micronutrient deficiency. However, today even younger patients with lower cumulative tobacco or alcohol exposure are increasingly present with OSCC.<sup>3</sup>

These early onset of oral squamous cell carcinoma are often located at the base of the tongue, tonsils and oropharynx and are associated with human papilloma virus. The current standard approach of western medicine for treatment of oral cancer consists of attempts to eradicate established tumours with combined treatment, like surgery, chemotherapy and radiation. However, these therapies have failed in many aspects making human life miserable and reducing the life span of patients. Also after surgical treatment there will be distortion of face, trouble of breathing, swallowing and speaking. Patients remain sick, due to toxic effect of radiation and chemotherapies as these calls not only kill cancer cells, but also kill normal cells. This makes the patient prone to various diseases along with opportunistic infections.<sup>4</sup>

Therefore the need of the hour is to develop treatment modalities by using plant derivatives which obscure potent side effects and act as effective therapeutic agents. Plants have been used as an age old remedy of cancer history of use in the treatment of cancer.<sup>5</sup>

*Phyllanthus emblica* is a tree indigenous to tropical regions of Southeast Asia. The tree produces a fruit

commonly known as Indian Gooseberry or Amla. The *Phyllanthus emblica* fruit (also known as *Embilca officinalis*) or extract from these fruits has been used in traditional medicine for generations to treat symptoms ranging from constipation to the treatment of tumors. Most commonly, the gooseberry was employed as a gentle laxative. However, the potential of *Phyllanthus emblica* extract to be utilized as an anticancer agent has been scrutinized using modern medical techniques over the past two decades. To date, there is substantial evidence that these extracts contain small molecules with both cancer-preventative and antitumor activity.<sup>6</sup> Studies have also shown that, along with being an exceptional free radical scavenger, Amla can protect your DNA against damage from certain carcinogens and dangerous heavy metals.

The utility of cell lines acquired from tumors allows the investigation of tumor cells in a simplified and controlled environment. There are specific advantages and disadvantages to exploit cancer cell lines over animal models. These then dictate the nature of the experiment that can be organised. Firstly, the cost involved with sustaining them is significantly less than maintaining animal subjects. They are promptly available and research studies can be implemented relatively quickly.<sup>9</sup>

Cytotoxicity is the degree to which an agent has specific destructive action on certain cells. It is the possession of destructive action, particularly in reference to lyses of cells by immune phenomena. Cell proliferation rates or viability levels are good indicators of cell health. Proliferation or viability analysis which is crucial for cell growth and differentiation studies, and are often coupled with metabolism analysis. Assessing compound cytotoxicity is also a critical step in pharmaceutical development. These assays in oncological settings are also used to evaluate both compound toxicity and inhibition of tumor cell growth during drug development.<sup>7</sup>



## MATERIALS AND METHODS

### Preparation of plant extract

Due to its high content of vitamin C and polyphenols, *Phyllanthus emblica* extract is a potent antioxidant. The aqueous extract of *Phyllanthus emblica* was made. *Phyllanthus emblica* weighing 25g were thoroughly washed in distilled water, dried, cut into fine pieces and were crushed with 100 ml sterile distilled water and filtered through Whatman No.1 filter paper (pore size 25 µm).<sup>8</sup> The aqueous extract was refrigerated.

### Maintenance of cell lines

The vial containing the KB cell lines were acquired from ATCC (CCL -17) was removed from liquid nitrogen freezer and immediately placed in a 37°C water bath. It was agitated continuously until the medium thawed. Then it was centrifuged for 10 min at 150 to 200 × g, room temperature. Supernatant was discarded and cells were washed with fresh medium to remove residual DMSO. The cell pellet was re-suspended in 3ml of DMEM with 10% FBS. It was then incubated in a CO<sub>2</sub> incubator and humidified at 37°C. The medium was changed every 2 to 3 days or when pH indicator (e.g. phenol red) in medium changed colour. The culture was kept in a medium with 10% FBS until cell line were re-established.<sup>7</sup>

### MTT assay for cell viability

The MTT assay (Mossmann, 1983) is based on the ability of live but not dead cells to reduce a yellow tetrazolium dye to a purple formazan product. Cells were maintained in DMEM medium, supplemented with 10% Fetal Bovine Serum, at 37°C in humidified atmosphere with 5% CO<sub>2</sub>.

The cells were plated in 96 well flat bottom tissue culture plates at a density of approximately 1.2X 10<sup>4</sup> cells /well and allowed to attach overnight at 37°C. The medium was then discarded and cells were incubated with different concentrations of the samples (100, 200 & 300 µg) for 24 hours. After the incubation, medium was discarded and 100µl fresh medium was added with 10µl of MTT (5mg/ml). After 4 hours, the medium was discarded and 100µl of DMSO was added to dissolve the formazan crystals. Then, the absorbance was read at 570nm in a microtitreplate reader. Cyclophosphamide was used as a positive control.<sup>10</sup>

Cell survival was calculated by the following formula:

$$\text{Viability \%} = (\text{Test OD} / \text{Control OD}) \times 100$$

$$\text{Cytotoxicity \%} = 100 - \text{Viability\%}$$

## RESULTS AND DISCUSSION

Cytotoxicity analysis by using varying concentration of *Phyllanthus emblica* extract (100,200 and 300µg) was done. As seen in Table 1 the viability of the KB cell lines show a gradual increase as the concentration of the extract is increased. In Table 2, *Phyllanthus emblica* exhibited increasing cytotoxicity with increasing

concentration. This is also evident from the visual representation of the same data in graph 1 and graph 2.

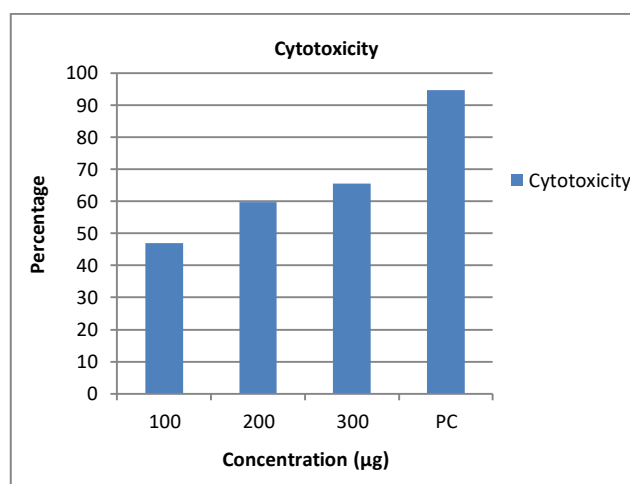
Table 1 shows the cytotoxic potential of samples and PC.

Graph 1 shows the cytotoxic potential of samples and PC.

Table 1

Concentration (µg)	OD	Cytotoxicity
100	0.7268	47.05
200	0.4261	59.66
300	0.4726	65.57
PC	0.0726	94.71

Graph 1



## CONCLUSION

Natural products are widely used nowadays to avoid the various side effects caused by carcinogenic drugs.<sup>15</sup> This study reveals the cytotoxic potential of *Phyllanthus emblica* on oral cancer cells. The potential to develop *Phyllanthus emblica* an anti-cancer drug is a thrust area for future research in the drug designing industry.

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