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# *In vitro* anticancer screening of ethanolic extracts of Macaranga peltata leaves

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**Abstract**
Cancer is a group of diseases caused by loss of cell cycle control. Cancer is associated with abnormal uncontrolled cell growth. Cancer is caused by both external factors (tobacco, chemicals, radiation and infectious organisms) and internal factors (inherited mutations, hormones, immune conditions, and mutations that occur from metabolism). Medical plants contain numerous bioactive phytochemicals or bionutrients. Various studies carried out during the past few decades on these phytochemicals reveal their important role in preventing chronic diseases like cancer, diabetes and coronary heart disease. The MTT *in vitro* cell proliferation assay is one of the most widely used assays for evaluating preliminary anticancer activity of both synthetic derivatives and natural products and natural product extracts. The highly reliable, colorimetric based assay is readily performed on a wide range of cell lines which gives an indication of whole cell cytotoxicity. In the present work the MTT *in vitro* cell proliferation assay of ethanolic extract of Macaranga peltata was carried out on HeLa cell lines and SKMEL 28 cell lines. Ethanolic extract of the plant drug produced an cytotoxic effect on both cell lines with an $IC_{50}$ of 156.9 µg/ml on cervical cell lines and 256.4µg/ml on skin carcinoma cell lines.

**Keywords:** Macaranga peltata, cytototoxic, MTT, cell lines, *in vitro*

## Introduction
Cancer is one of the most prominent diseases in humans and currently there is considerable scientific and commercial interest in the continuing discovery of new anticancer agents from natural product sources. Studies of the pharmacological activities of the genus Macaranga indicate the potential of extracts and pure compounds to display specific medical effects. Previous investigation on the chemistry and pharmacology of this genus showed that its crude extracts and compounds displayed interesting bioactivity profiles, possessing various bioactivities including anticancer, antioxidant, antmicrobial, anti-inflammatory and other different types of biological activities [1]. Cytotoxicity property of extracts of leaves of Macaranga peltata was carried out by MTT method against Sk-Mel-28 and HeLa cell lines.

## *In vitro* cytotoxicity by MTT assay
The MTT, MTs *in vitro* cell proliferation assay is one of the most widely used assays for evaluating preliminary anticancer activity of both synthetic derivatives and natural products and natural product extracts [2]. The highly reliable, colorimetric based assay is readily performed on a wide range of cell lines. This is one of the most useful anticancer activities against various human cancer cell lines. In the MTT assay, 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide is bioreduced by dehydrogenase inside living cells to form a coloured formazan dye.
The MTT assay requires the addition of solubilising agents to dissolve the insoluble formazan product formed. The number of viable cells is measured through colorimetry and works on the principle that the mitochondrial dehydrogenase enzymes which produces NADH or NADHP, reduces the colourless tetrazolium salt into a colored aqueous soluble formazan product by the mitochondrial activity of viable cells at $37^0$ C. The quantity of the coloured product is directly proportional to the number of live cells in the culture since the MTT reagent can only be reduced to formazan by metabolically active cells.

## Materials and methods
### Cell and cell culture process
Cancer cell lines have been widely used for research purposes and proved to be a useful tool in the genetic approach, and its characterizations shows that they are, in fact, an excellent model

for the study of the biological mechanisms involved in cancer [3].

**Sk-Mel-28**

Human melanoma cells (from NCCS) were grown and maintained in 25cm$^3$ tissue culture flasks in a humidified atmosphere (95% air/5% $CO_2$ ) at 37$^0$ C in RPMI- 1640 medium, containing 10% FBS, glutamine (2mM), and antibiotics (100U/ml penicillin, 100 µg/mL streptomycin, and 250ng/mL amphotericin B).

**HeLa**

Cervix adenocarcinoma cells (from NCCS was maintained in Dulbecco's modified eagles media supplemented with 10% FBS and grown to confluency at 37$^0$ C in 5% $CO_2$ in a humidified atmosphere in a $CO_2$ incubator. The cells were trypsinized (500µl of 0.025% trypsin in PBS/0.5 mM EDTA solution (Himedia)) for 2 minutes and transferred to T flasks.)

Macaranga peltata leaves were collected from Calicut district, Kerala, India and authenticated by Dr. Minoo Diwakar, HOD, Dept. of Botony, Providence Women's College, Calicut. Shade dried leaves of Macaranga peltata was extracted with 95% ethanol in soxhlet and the solvent was evaporated to get the extract. The extracts were pooled and concentrated by distillation under reduced pressure till a syrupy consistency was achieved. 2mg of each leaf extract was dissolved in 200µl DMSO then 100µl of this solution was diluted to 10ml with DMEM to obtain the stock solution which was diluted to varying concentrations used in the assay.

Cells were placed in 1ml of medium/well in24-well plates. 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$^0$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-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) solution was added. After 4h incubation, 0.04M HCl/ is isopropanol were added. Different concentrations of ethanolic extracts prepared from diluated stock was used and the assay was performed in HeLa and Sk-Mel-28 cells in tetrads. Cell control containing drug free medium and neat drug controls were included. After 3 days of incubation at 37$^0$ C under 5% $CO_2$, the absorbance ws determined by spectrophotometer. The concentration of drugs showing complete cycotoxic effect was recorded. Viable cells were determined by the absorbance at 570nm. Measurements were performed and the concentration required for a 50% inhibitionof viability ($IC_{50}$) was determined [4].

$$\text{percent cell viability} = \frac{A_{570} \text{ of control cells} - A_{570} \text{ of treated cells}}{A_{570} \text{ of control cells}} \times 100$$

**Results and discussion**

MTT assay is a sensitive method for evaluating cytotoxic activity against cancer and non-cancerous cell lines. Phytochemical screening of the leaves of Macaranga peltata confirmed the presence of phenols, flavonoids, alkaloids and glycosides. MTT assay of the ethanolic extracts of the leaves were carried out using Sk-Mel-28 and HeLa cell lines. The present study demonstrated the cytotoxic action of extracts of Macaranga peltata against the two cell lines namely Sk-Mel-28 and HeLa. Concentration required to produce inhibition of viability of 50% cell were calculated. Ethanolic extract of the plant drug produced a cytotoxic effect on both cell lines with an $IC_{50}$ of 156.9 µg/ml on cervical cell lines and 256.4µg/ml on skin carcinoma cell lines.

Cytotoxic actions of thee extract is due to the presence of specific phytochemicals present in the extract. The extracts produced a dose dependent inhibitory action. Presence of flavonoids and alkaloids in the ethanolic extracts exerted antiproliferative activity by inducing apoptosis in cancer cell lines. The association between flavonoids and reduced cancer risk has been reported in earlier studies [5].

**Conclusion**

The ethanolic extract of Macranga peltata leaves were subjected to MTT assay using HeLa and SK-Mel-28 cell lines. The extract gave promising result for the anticancer activity on both the cell lines with better effectiveness against the cervical cell cancer. A thorough study to reveal the active cytotoxic principles and their mechanism would be promising prospective to develop an effective drug in management of cancer.

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