In Vitro Anticancer Potential Of Hydroethanolic Extract Of Canthium Coromandelicum Against Neuroblastoma SHSY5Ycell Line

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Abstract- *Cancer is an uncontrollable growth of the living cells and it is a major public health problem in worldwide and affects the functioning of many parts of the body. Plant based medicine are used in cancer prevention and treatment in many countries. The in vitro anticancer assay was measured by MTT assay against neuroblastoma SHSY5Y cancer cell line. The hydroethanolic extract of Canthium coromandelicum moderately inhibit neuroblastoma SHSY5Y cancer cell line growth. Measurements were performed and the concentration required for 50% inhibition of viability (IC50) was determined graphically. The effects of the samples on the proliferation of neuroblastoma SHSY5Y cell line was expressed as the % cell viability. From the graphs the concentration of hydroethanolic extract yields the values of LC50 (46% mortality) as 50µg/ml respectively for Canthium coromandelicum. In the same way the concentration at which 99% mortality occurs for the hydroethanolic extract are obtained from the graphs and the values have been found to be 5µg/ml for Canthium coromandelicum.*

Keywords- Hydroethanolic extract, Canthium coromandelicum, MTT Assay, Neuroblastoma SHSY5Y cell line.

I. INTRODUCTION

A natural plant has high medicinal properties that are used for pharmaceutical industries (Yaacob *et al.,* 2010 and Suresh *et al.,* 2011). Many of the plant based product used in traditional medicine because they are easily available in rural area and cheaper compared to modern therapeutic drugs (Moglad *et al.,* 2014). Around 250,000 plant species approximately exist on earth; thousand plant species are having anticancer properties (Latif *et al.,* 2014). The chemotherapeutic drugs are active against tumor cells and harmful side effects, but a plant drug also has less side effects. India has many plant species that are used for various types of cancer (Unnikrishnan *et al.,* 1990 and Babu *et al.,* 1995). *Canthium coromandelicum* (*Canthium parviflorum* belongs to family: Rubiaceae) is bushy thorny herb and shrub. It's widely available in India, Indo china, West, South China, Malaysia and Sirlanka. Leaf are very simple, short and ovate, small flowered arises in leaf axils (Karthick *et al.,* 2014). The leaves are used for the treatment various ailments such as diarrhoea, strangely fever, leucorrhoea, intestinal worms, tonic, anthelmintic, constipating and general debility (Warrier *et al.,* 1996). *Canthium* as herbal medicine is used for the treatment of diabetes in South Tamil Nadu (Mohideen *et al.,* 2003). *Canthium coromandelicum* are present as bioactive substances such as alkaloids, tannins, flavonoids and phenolic compounds that are active against tumor cells (Karthick *et al.,* 2014). Hence the present study was investigated for the *in vitro* anticancer activity against neuroblastoma SHSY5Y cell line.

II. MATERIALS AND METHODS

Collection of plant materials and authentication

Leaves of *Canthium Coromandelicum* was collected from Nambiyur and authenticated (No: BSI / SRC/5/23/2017/Tech/912) at Botanical Survey of India (BSI), Coimbatore, Tamilnadu Agricultural University, Coimbatore.

Preparation of the extract

Fresh leaves of *Canthium Coromandelicum* were washed, shade dried and then powered using the mixture grinder and stored in air tight container. To 10g of the powered leaves, 100ml of Aqueous, Hydroethanolic, Petroleum ether, Chloroform, Ethyl acetate were added in separate conical flaks and the mixture was subjected to cold maceration for 72 hours with intermittent shaking. After 72 hours, the solution was filtered using muslin cloth and the solvent present in the filtrate extract of each solvent was stored at 4^{o}C for further use.

Cell Line and culture

Neuroblastoma SHSY5Y cell line was obtained from National centre for cell science, Pune, India. Stock cells are routinely cultured in DMEM medium supplemented with fetal bovine serum, Mitomycin and streptomycin (100µg/ml) at 37°C in incubator containing 5% CO_2.

***In vitro* assay for cytotoxicity activity (MTT assay)**

An *in vitro* cytotoxicity test was performed for the given test sample as per ISO 10993:5. The culture medium from the SH-SY5Y cells was replaced with fresh medium. Test sample in triplicates were added on the cells. After incubation at 37±1°C for 18 hours, MTT (mg/ml) were added in all the wells and incubated for 4 hours. After incubation, DMSO were added in the wells and read at 570nm using photometer. Cytotoxicity and cell viability were calculated by the below formula.

Cytotoxicity = [(Control-Treated)/ Control] *100
Cell viability = (Treated/ Control)*100

Trypan blue assay

The cells were grown in DMEM medium supplemented with 10% FBS. Equal volume of Trypsin (0.25%) and versene (0.1%) were used to detach the cells and observed them under a microscope to confirm complete dissociation of the cells. Cells (~ 10^5) were seeded in the well and incubated at 37°C for 24 hours. Samples were added at different concentration in duplicate and the cells without sample served as control. The plate was incubated for 24hours. After incubation, the medium was completely removed and rinsed with PBS. Trypan blue stain (0.4%) was added in each well and under inverted phase contrast microscope.

III. RESULT AND DISCUSSION

Medicinal plants possess cytotoxic potential and used to eradicate cancer cells and many researches are finding number of plant based therapeutics drug (Yaacob *et al.,* 2010). In the present study, the cytotoxicity activity of the hydroethanolic extract of *Canthium coromandelicum*against SHSY5Y neuroblastoma cancer was investigated in MTT as given in Table 1.

Table1 Anticancer effect of hydroethanolic extract of *Canthium coromandelicum* on SHSY5Y cell line

Concentration (µg)	Cell viability (%)
100	6
75	6
50	46
25	54
5	99

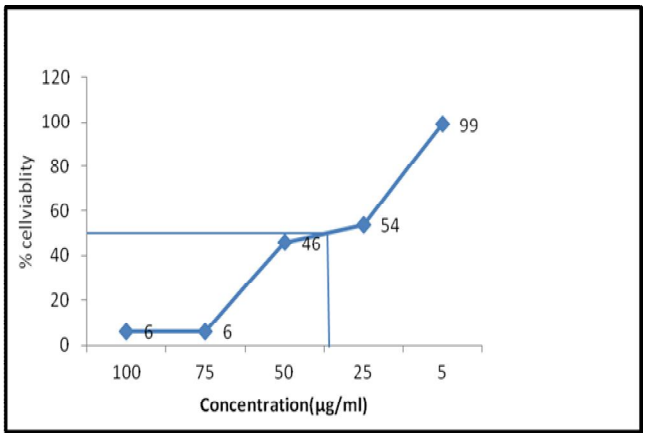

Figure 1 Cell viability of *Canthium coromandelicum*

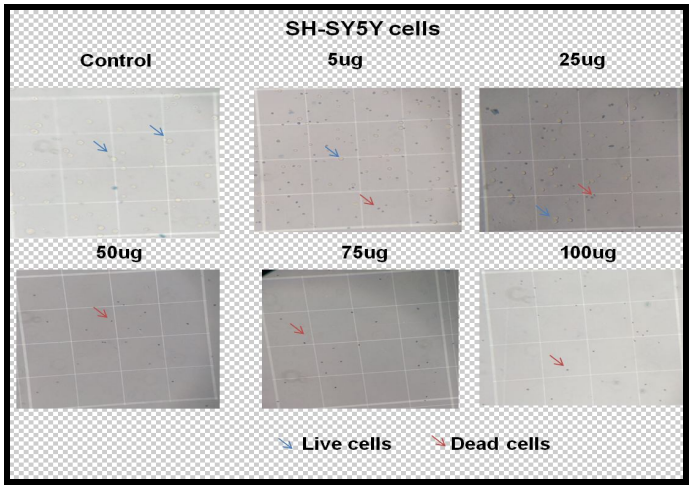

Figure 2 Trypan blue assay of *Canthium coromandelicum*

The above figure represents the percentage of cell viability for five different concentration of hydroethanolic extract of *Canthium coromandelicum*. The cell viability for five solvent extract concentrations was 100µg/ml, 75µg/ml, 50µg/ml, 25µg/ml, 5µg/ml respectively. The hydroethanolic

extract of *Canthium coromandelicum* at 100µg/ml produces 6% cell viability, 75µg/ml produces 6% cell viability, 50µg/ml produces 46% cell viability, 25µg/ml produces 54% cell viability and 5µg/ml produces greater than 99 % cell viability. Similar results were given by Al-Kalaldesh *et al.,* 2010.

From the graphs the concentration of hydroethanolic extract were evaluated as the values of LC50 (46% mortality) as 50µg/ml respectively for *Canthium coromandelicum*. In the same way the concentration at which 99% mortality for the hydroethanolic extract are obtained from the graphs and the values have been found to be 5 µg/ml for *Canthium coromandelicum* respectively. Hydroethanolic extract of the plant exhibiting anticancer activity against SHSY5Y cell line. This study suggests that the crude extracts caused cytotoxic activity may due to the presence of secondary metabolites.

IV. CONCLUSION

Canthium coromandelicum is a valuable ayurvedic medicine. The plants contain rich in bioactive compound that activate many pharmaceutical industries. The present study reveals the *in vitro* cytotoxicity activity of hydroethanolic crude extract of *Canthium coromandelicum was* active against neuroblastoma SHSY5Y cell line. Therefore our studies support to use active constituents of *Canthium coromandelicum* in treating cancer.

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