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INVITRO ANTICANCER ACTIVITY OF COCONUT SHELL OIL ON BREAST CANCER CELL LINE

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ABSTRACT

The ethanolic extract of Coconut shell oil were analysed for the anticancer activity against breast cancer cell lines. Coconut shells were collected and further processed for extract preparation. Breast cancer cell line was obtained from NCCS, Pune and was maintained in DMEM High Glucose media in 10% FBS, Penecillin (100 U/ml) and Streptomycin (100 µg/ml).The antiproliferative activity of Coconut shell oil were analysed by 0.5% 3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyl-tetrazolium bromide (MTT) method. The highest non toxic concentration was observed at 125µg/ml concentration of ethanol extract of Coconut shell oil recorded in the cell line. These results showed the potential of Coconut shell oil as anticancer activity and further studies are necessary to analyse their mechanism and to isolate their anticancer compounds that is responsible for such activity.

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INTRODUCTION

Cancer is one of the important common causes of mortality in worldwide. Cancer therapy progress is not sufficient to lower the risk of annual death rate but there is an immediate need for new strategies to control cancer cells. (Lahouel *et al.,* 1987). Normal cells transformed into a cancer cells by the alteration of genes that regulate the cell growth and differentiation (Priya *et al.,* 2013). Every year there are about 11million people were diagnosed with cancer and it will be 16 million new patients by 2020 (WHO, 2008). Globally 85,000 plants were recorded for their therapeutic usage (Liu and Wang, 2008). According to WHO statement, 65% of the world population gives preference to use traditional and herbal medicines to treat disease (Gupta AK, 2004). In 1950's US Natural Cancer Institute (NCI) recognized that usage of natural products are potential for the preparation and identification of anticancer drugs (Cragg and Newmann, 2005). It is believed that the medicinal plants would contain polyphenolics, flavanoids, alkaloids, terpenoids and saponin compounds that has therapeutic properties and hinder cancer formation (Latif *et al.,* 2014).

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Consuming large quantities of vegetables and fruits in our diet have potential chemopreventive agents and prevent the growth of cancer cells (Rao *et al.,* 2004). Coconut shell found in coconut fruit to protect fruit core and located on the inner side of the fiber ranging from 3 to 6mm in thickness. It has components as hardwood and consists of lignin, cellulose and hemicelluloses with water content of approximately 6-9% (Tilman, 1981). The phytochemical screening of CSO showed the presence of many secondary metabolites that has various biological and therapeutic properties (Dorathy SJP and Karpagam, 2017a). The ethanolic extract of CSO has antibacterial and antifungal activity (Dorathy and Karpagam, 2018 b). The crude extract from the coconut shell used in the treatment of microbial infections and also cure some human diseases (Siddheshwar, 2013).

MATERIALS AND METHODS

Preparation of extract

The coconut shells were collected from the local market in Thiruvallur district, Tamilnadu, India. They were sundried for few days to remove the moisture content. Then it is broken into small pieces and ground into course powder. 250 g of

ground coconut shells were heated in the earthen pot for a span of 3 hours giving a yield of 25cc of oil. 10ml of oil were extracted with 30ml of ethanol and preserved in airtight container for the further studies.

Invitro anticancer activity

Cell line and culture: The breast cancer cellline (MCF-7) was obtained from National Centre for Cell Sciences (NCCS), Pune. The cells were maintained in DMEM- High glucose (Himedia), supplemented with 10% FBS, Penecillin (100U/ml) and streptomycin (100µg/ml) in a humidified atmosphere of 50 µg/ml CO_2 at 37^0C.

Invitro assay for cytotoxicity activity (MTT assay)

The cytotoxicity of the samples was determined by MTT assay (Mosmann *et al.,* 1983). The cultured cells (1 x 10^5 / well) were plated in 24 well plates and incubated with 5% CO_2 condition overnight at 37^oC for 24 hrs. After the cell reaches the confluence, the various concentrations of the samples were added and incubated the cells for 24 hrs. At the end of treatment, the sample was added with Phosphate - Buffered Saline (PBS) at pH 7.4. After this wash 100µl/well (5mg/ml) of 0.5% 3-(4, 5-Dimethyl-2-thiazolyl)-2, 5-diphenyl-tetrazolium bromide (MTT) was added and incubated for 4 hrs. After incubation, 1ml of DMSO was added in all the wells. The absorbance at 570nm was measured with UV spectrophotometer using DMSO as the blank. Measurement was performed and the concentration required for a 50% inhibition (IC_{50}) was determined graphically. The % cell viability was calculated using the following formula:

% Cell viability = A570 of treated cells / A570 of control cells × 100

Table 1. Anticancer effect of Coconut Shell oil using *MCF 7* Cell line

S. No	Concentration (µg/ml)	Absorbance (O.D)	Cell viability (%)
1	1000	0.217	27.43
2	500	0.282	35.65
3	250	0.343	43.36
4	125	0.408	51.58
5	62.5	0.472	59.67
6	31.2	0.533	67.38
7	15.6	0.595	75.22
8	7.8	0.662	83.69
9	Cell control	0.791	100

Graphs are plotted using the % of Cell Viability at Y-axis and concentration of the sample in X-axis. Cell control and sample control is included in each assay to compare the full cell viability assessments.

RESULTS

Anticancer activity was determined with Coconut shell oil followed by DMSO as control. The anticancer activity of Coconut shell oil was confirmed through Cell viability percentage by MTT assay. The percentage of cell survival of the control is 100 % shown in Table 1. The morphological changes and shrinkage of cells leads to death of cells induced by the coconut shell oil in the breast cancer cell lines. Anticancer activity of the Coconut shell oil was found to be concentration dependant and it works even at low doses. The viability of cancer cells treated with ethanol extract decreases as the concentration level increases. The least value of concentration of cell death was 27.43%. The IC_{50} value of Coconut shell oil was 51.58 at 125 µg/ml. Based on the cytotoxicity studies against MCF-7 cell lines the ethanolic extracts could be used as potential source for anticancer drugs (Fig. 1 and Fig. 2).

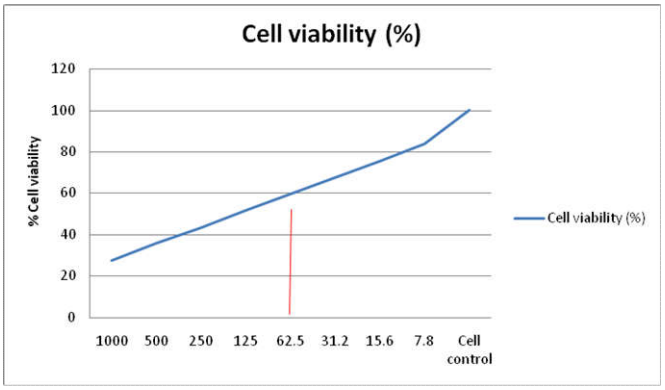

Figure 1. Determination of *In vitro* assay for cytotoxicity (MTT assay)

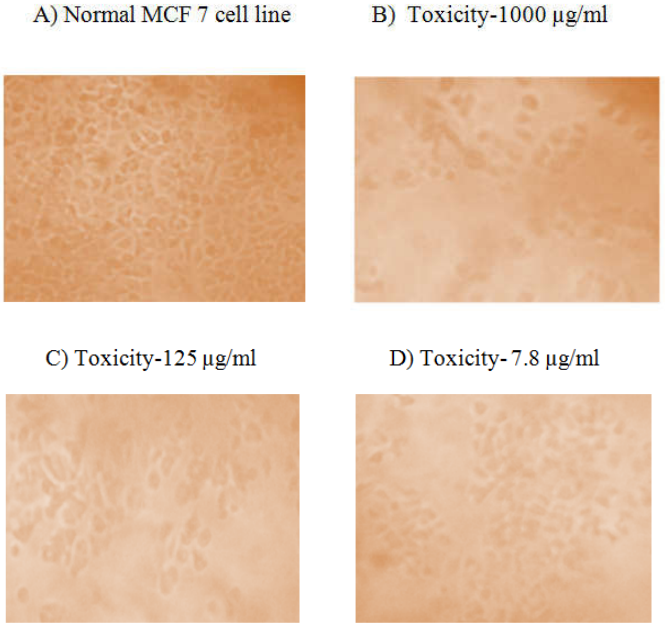

Fig. 2. Anticancer effect of Coconut shell oil on *MCF 7 cell* line

DISCUSSION

The herbal medicines were used now a days for the trearment of cancer for their various phytochemical contents with varying biological activities (Mann J. 2001). The primary task in the pharmacognosy aspects is to design new drugs to cure cancer at the same time with lesser side effects (Denny and Wansbrough, 1995). Vinblastine, paclitaxel and etoposide are some of the natural products and its derivatives play an important role in cancer chemotherapy (Schwartsmann *et al.,* 2002). In spite of this new anticancer agents various plant extracts have been isolated in invitro and invivo cancer models and the correlation of these studies helpful in the research. However, anticancer activity of this plant oil extract has not reported so far. Keeping in view, this study has been made to investigate anticancer activity of the ethanolic extract of Coconut shell oil against MCF- 7 cell lines.

Conclusion

Coconut shell oil possess anticancer activities due to the presence of phytochemicals against the cancer cell lines. Further studies are necessary to carry out the isolation of detailed profile of anticancer compounds in present in Coconut shell oil.

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