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SCREENING OF ANTICANCER ACTIVITY OF METHANOL EXTRACT OF *COCOS NUCIFERA* INFLORESCENCE IN SK-MEL CELL LINES

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ABSTRACT

Cocos nucifera (L.) is an important member of the family *Arecaceae* (palm family) popularly known as coconut, coco, coco-da-bahia, or coconut-of-the-beach .The plant is originally from Southeast Asia (Malaysia, Indonesia, and the Philippines) and the islands between the Indian and Pacific Oceans. The present study was intended to screen the anticancer activity of methanol extract of *Cocos nucifera* inflorescence against SK-MEL cell lines. The percentage of cell viability showed 6.25 µg/mL, 12.5 µg/mL,25 µg/mL,50 µg/mL,100 µg/mL such as 91.63 %, 85.86 %, 74.31 %, 61.18 %, 48.05 % respectively and the cytotoxic effects of cell line showed the percentage of cytotoxicity such as 8.37%, 14.14%, 25.69%, 38.82% and 51.95% respectively. Dose dependent reduction in cell viability was observed in SK-MEL cancer cells administered with different concentrations of the sample. The IC_{50} value was obtained as 98.49µg/mL of the sample. The results showed that *Cocos nucifera* inflorescence extracts have intresting pharmacological active compounds and used in ethnomedicine for treatment of various diseases.

Keywords: *Cocos nucifera,* anticancer, SK-MEL cell lines, cytotoxicity

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1.INTRODUCTION

Globally cancer is a disease which severely effects the human population. There is a constant demand for new therapies to treat and prevent this life-threatening disease. Scientific and research interest is drawing its attention towards naturally-derived compounds as they are considered to have less toxic side effects compared to current treatments such as chemotherapy. The Plant Kingdom produces naturally occurring secondary metabolites which are being investigated for their anticancer activities leading to the development of new clinical drugs (Greenwell and Rahman.,2015).

In addition, phytochemicals that scavenge free radical species can be beneficial in the treatment of cancer. Cancer is the second most common cause of death in humans standing next to cardiovascular disorders. Of this, cervical cancer is the third most common cancer and the fourth leading cause of cancer related death among women worldwide(Jemal *et al*.,2011, Ferlay *et al*.,2010. Approximately 80 % of cervical cancers occur in developing countries (Saavedra *et al*., 2012). Chemoprevention of cancer is a way of cancer control in which the occurrence of this disease is prevented by administration of one or several chemical compounds (Jang *et al*., 1997). A large number of chemical compounds have been shown to prevent cancer by different mechanisms and appear to work at different stages in the neoplastic process. In recent years, there has been increasing interest in the potential cancer chemopreventive properties of phytochemicals with fewer side effects. According to literature, about 60 % of most effective anticancer/anti-infectious drugs already on the market and under clinical investigations are of products or compounds derived from natural products (Wang *et al*.,2007).Malignant melanoma is an invasive form of skin cancer that originates from the transformation of melanocytes. It is characterized by the highly aggressive nature and ability to metastasize to diverse distant sites. Human cell lines can provide a realistic prediction of drug responses in humans as well as information on safety and efficacy of drug candidates. The SK-MEL-28 cell line was established from the skin of a 51-year-old male patient with malignant melanoma. SK-Mel-28 cells exhibit polygonal cell morphology, and this is just one of many melanoma cell lines (also named SK-MEL). The modal chromosome number of 90 occurs nearly in 50% of cells. Also, the hypotetraploid SK-MEL-28 expresses wildtype N-Ras gene. This tumorigenic cell line could be helpful for skin cancer research as well as for other cell and molecular biology applications.

In *Cocos nucifera*, the flowers and the flower bearing branched stalk are collectively called inflorescence, which is covered by a spathe. In immature inflorescence, the male and female florets lie very close to the peduncle and the whole is so tightly packed. Thus, the individual florets cannot be distinguished. In Indian folk medicine, the fresh juice of *Cocos nucifera* inflorescence (CnI) is used in treating dyspepsia, diarrhea, dysentery, diabetes, haemoptysis and strangury. In most of these cases, scientific proof in terms of modern medicine is lacking. However, nowadays, it is essential to provide scientific evidence to justify the use of a

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plant or its active principles (Singh *et al*.,2002). In Ayurveda, the inflorescence is used for the treatment of menorrhagia and back pain (Bhandary *et al.,*1995 and Padumadasa *et al*.,2016). The fresh kernel is an ingredient of many Indian food preparations like puddings, sweets, curries, chutneys etc.

2. MATERIALS AND METHODS

2.1. Collection of the sample

The inflorescence of *Cocos nucifera* L. was collected from Vavarai Gramapanchayat (8.5678° N, 77.0189° E), a Western Ghats region of Kanyakumari District of Tamilnadu State, India. The samples were collected during the winter season.

2.2. Preparation of inflorescence extracts

The collected fresh inflorescence of *Cocos nucifera* was thoroughly washed in tap water, shade dried, powdered and was stored in air tight container. The powdered plant material (100 g) was then extracted successively with Methanol using a Soxhelet apparatus for about 24hrs each. The extracts were filtered and concentrated under reduced pressure in a rotary vacuum evaporator. The dried extracts were then stored at 0-4°C until further use. **2.3. 2.3. Cell lines and maintenance**

SK-MEL (human melanoma) cell lines were procured from the National Centre for Cell Sciences (NCCS), Pune, India.

2.4. Cell culture media and maintenance

The cells were cultured in Dulbecco's Modified Eagles Medium (DMEM-Himedia), supplemented with 10% heat inactivated Fetal Bovine Serum (FBS) and 1% antibiotic cocktail containing Penicillin (100U/ml), Streptomycin (100µg/ml), and Amphotericin B (2.5µg/ml). The cell containing TC flasks (25cm^2) were incubated at 37^{O}C at 5% CO_2 environment with humidity in a cell culture incubator (Galaxy® 170 Eppendorf, Germany).

2.5. Principle:

The MTT assay is used to measure cellular metabolic activity as an indicator of cell viability, proliferation and cytotoxicity. This colorimetric assay is based on the reduction of a yellow tetrazolium salt (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide or MTT) to purple formazan crystals by metabolically active cells. The viable cells contain NAD(P)H-dependent oxido reductase enzymes which reduce the MTT to formazan(Mosmann *et al*., 1983). The insoluble formazan crystals are dissolved using a solubilizing solution (100% DMSO) and the resulting purple colored solution is quantified by measuring absorbance at 570 nm using an ELISA plate reader.

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2.6. Procedure:

SKMEL(2500 cells/well) were seeded on 96 well plates and allowed to acclimatize to the culture conditions such as 37 °C and 5% CO_2 environment in the incubator for 24 h. The test samples were prepared in DMEM media (100 mg/mL) and filter sterilized using 0.2 µm Millipore syringe filter. The samples were further diluted in DMEM media and added to the wells containing cultured cells at final concentrations of 6.25, 12.5, 25, 50, 100µg/mL respectively. Untreated wells were kept as control. All the experiments were done in triplicate and average values were taken in order to minimize errors. After treatment with the test samples the plates were further incubated for 24 h. After incubation period, the media from the wells were aspirated and discarded. 100 µL of 0.5 mg/mL MTT solution in PBS was added to the wells. The plates were further incubated for 2h for the development of formazan crystals. The supernatant was removed and 100 µL DMSO (100%) were added per well. The absorbance at 570 nm was measured with micro plate reader. All experiments were done in triplicates. The cell viability was expressed using the following formula:

$$Percentage\ of\ cell\ viability = \frac{Average\ absorbance\ of\ treated}{Average\ absorbance\ of\ control} \times 100$$

Percentage cytotoxicity = 100 - % viability

The IC_{50} value is the half maximal inhibitory concentration of the sample. The IC_{50} values were calculated using the equation for slope obtained by plotting the % cytotoxicity of the different concentrations of the test sample (6.25-100 µg/mL).

3.RESULTS

3.1.Anticancer activity of Methanol extract in *Cocos nucifera* inflorescence of SK-MEL Cell Lines

The methanol extract of *Cocos nucifera* inflorescence in the SK-MEL cell lines showed anticancer activity. The percentage of cell viability showed 6.25 µg/mL, 12.5 µg/mL,25 µg/mL,50 µg/mL,100 µg/mL such as 91.63 %, 85.86 %, 74.31 %, 61.18 %, 48.05 % respectively and the cytotoxic effects of cell line showed the percentage of cytotoxicity such as 8.37%, 14.14%, 25.69%, 38.82% and 51.95% respectively. Dose dependent reduction in cell viability was observed in SKMEL cancer cells administered with different concentrations of the sample. The IC_{50} value was obtained as 88.49µg/mL of the sample. The results were tabulated in figure3.1, table 3.1 and plate 3.1.

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Figure: 3.1. Graphical Representation of cytotoxicity of the methanol extract of *Cocos nucifera* inflorescence

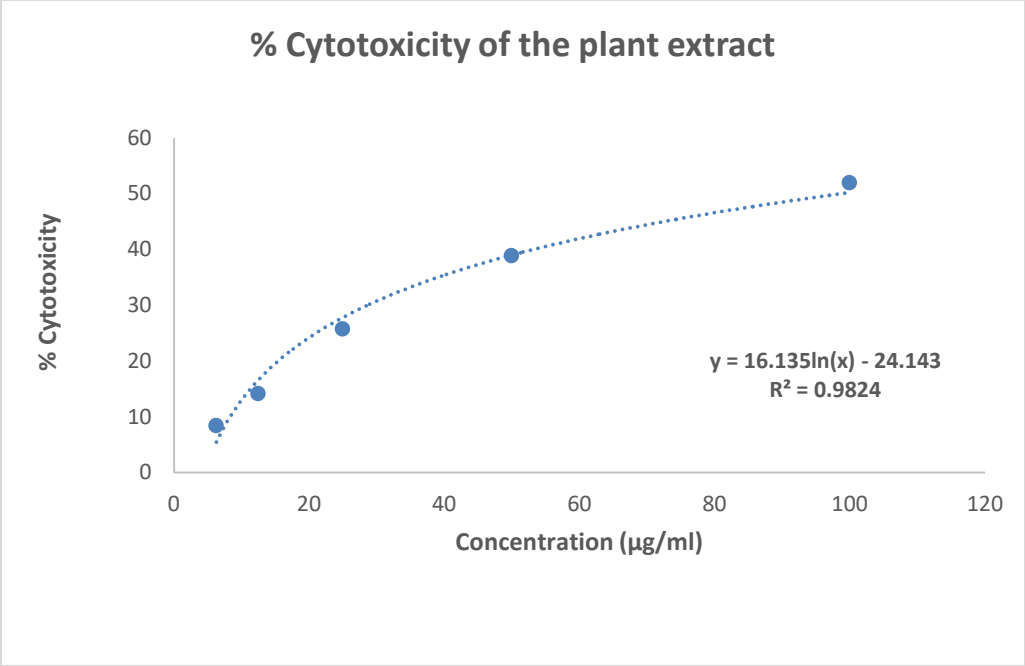

Table : 3.1. Absorbance, % viability and % cytotoxicity of the methanol extract of *Cocos nucifera* inflorescence

Samples	OD 1	OD 2	OD 3	Mean ± SD	% viability	% Cytotoxicity
Control	0.696	0.691	0.693	0.693 ± 0.002	-	-
6.25	0.635	0.638	0.631	0.635 ± 0.002	91.63	8.37

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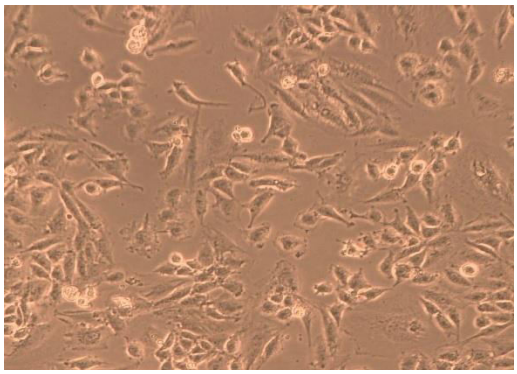
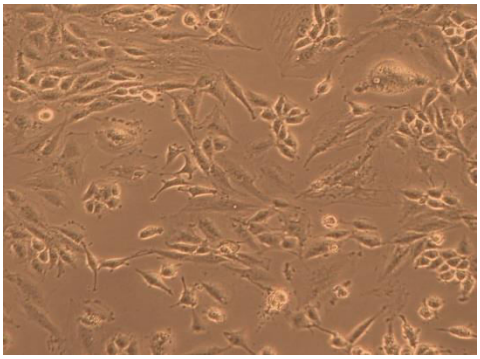
12.5	0.601	0.592	0.593	0.595 ± 0.004	85.86	14.14
25	0.512	0.518	0.514	0.515 ± 0.002	74.31	25.69
50	0.427	0.424	0.422	0.424 ± 0.002	61.18	38.82
100	0.327	0.338	0.335	0.333 ± 0.004	48.05	51.95

Plate :3.1.Anticancer activity of Methanol extract of *Cocos nucifera* inflorescence in SK-MEL Cell Lines



(a) Control



(b)Concentration at 6.25 µg/ml

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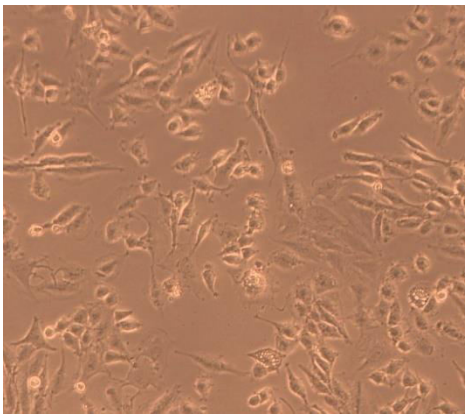
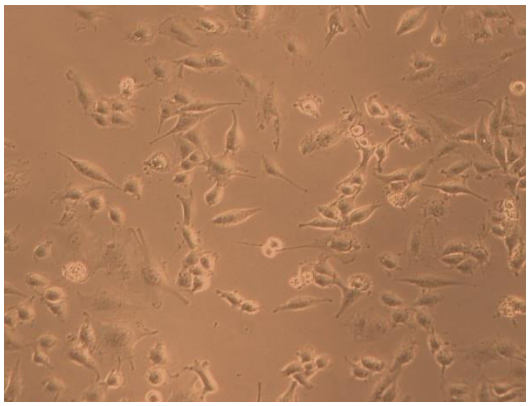
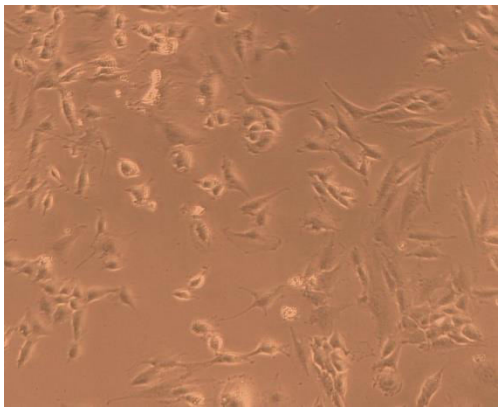


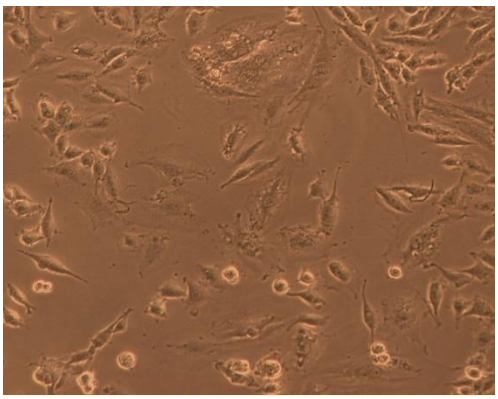
(c) Concentration at 12.5 µg/ml



(d) Concentration at 25 µg/ml



(e) Concentration at 50 µg/ml



(f) Concentration at 100 µg/ml

4. Discussion

In the present study the methanol extract of *Cocos nucifera* inflorescence in the SK-MEL cell lines showed anticancer activity. The percentage of cell viability showed 6.25 µg/mL, 12.5 µg/mL,25 µg/mL,50 µg/mL,100 µg/mL such as 91.63 %, 85.86 %, 74.31 %, 61.18 %, 48.05 % respectively and the cytotoxic effects of cell line showed the percentage of cytotoxicity such as 8.37%, 14.14%, 25.69%, 38.82% and 51.95% respectively. Dose dependent reduction in cell viability was observed in SK-MEL cancer cells administered with different concentrations of the sample. The IC_{50} value was obtained as 98.49µg/mL of the sample. Stockert *et al*., 2012 also reported the cytotoxicity of Virgin Coconut Oil extract was determined by MTT assay. The decrease in cell viability shows that the extract is toxic to cancer cells and may potentially alter or possibly halt its growth. Viable and metabolically active cells can cleave the MTT dye to produce formazan crystals, and quantified calorimetrically. Alexandra Ghiţu *et al*., 2021 revealed the MTT assay illustrates the cell viability percentage of the human melanoma SK-

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MEL-24 cells. The effect of Active Pharmaceutical Ingredient (API) was assessed on SK-MEL-24 cells after a 72 h stimulation period and compared to control. The effect of API such as 0.3, 1, 3, 10, 30, and 60 μM on melanoma cell viability. In this results indicate that in the range of tested concentrations, there is a decrease in tumor cell viability with the most significant reduction noticed at the two highest used concentrations for 30 μM, tumor cell viability was 75.08±5.5% against. control, and for 60 μM, tumor cell viability was 62.9±5.5% against control.

Stephanie *et al.*,2009 studied about the leaf extract of *Ricinus communis* L. is cytotoxic to several human tumour cell lines in a dose-dependent manner, with IC(50) values ranging between 10-40 µg/ml. SK-MEL-28 human melanoma cells at a concentration of 20 µg/ml as identified by morphological study , nuclear staining and flow cytometric analysis of DNA content. This study provides additional insight into the potential use of mixtures of terpenoids as they occur in nature of cancer cells.

Tong-Kewn Yoo., *et al* 2020 revealed the effect of the methanol extracts of *Abeliophyllum distichum* leaves, branches, and fruit on the growth of human SK-MEL-2 melanoma cells was investigated using MTT assay. The incubation with 50 µg/ml of *Abeliophyllum distichum* leaves (AL) (48.091 ± 12.741%) strongly inhibited the proliferation of SK-MEL-2 cells compared with *A. distichum* branches (AB) (84.229 ± 7.335%) and *A. distichum* fruits (AF) (86.949 ± 6.287%), even though 200 µg/ml of all extracts showed similar cytotoxic activity. Therefore *A. distichum* leaves was selected for further experiments. *A. distichum* leaves exhibited a dose- and time-dependent inhibitory effect on the viability of SK-MEL-2 cells.

Tooba Ghazanfari *et al.*, 2013 investigated that the cytotoxic effect of *Cuscuta* extract, a traditional Iranian medicinal herb, on melanoma cell line (SK-MEL-3) and human Burkitt lymphoma were studied. The cytotoxic effect of *Cuscuta* extract was performed by MTT assay. The most cytotoxic effects of *Cuscuta* extract on SK-MEL-3 and human Burkitt lymphoma cell lines were 80 and 81%, respectively, compared to a control group. *Cuscuta* extract seems to be a good applicant as an anti-cancer agent against lymphoma and melanoma cancers. Mohan *et al.*, 2022 also reported that the analysis of cytotoxic effect of the methanolic extract of *Acmella Ciliata* the cell biomass revealed that it is capable of inhibiting or reducing the growth of SK-MEL-28 cell lines and a lesser concentration i.e., 24.144 µg/mL of extract was needed to inhibit the growth by 50%.

Katrin *et al.*, 2017 revealed the strongest anticancer effect was found with the methanol extract of *chamomile* flowers against SK-MEL-2 cells (IC_{50} 40.68 ± 2.92 µg/ml). With the *marigold* flower extracts, the half maximal cell growth inhibitory concentrations exceeded the pre-determined threshold (100 µg/ml) in SK-MEL-2 cell line studied. At this concentration

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(100 µg/ml) the methanol extract of *marigold* flowers provided only 37.4 % growth inhibition on SK-MEL-2 cells.

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