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EVALUATION OF ANTICANCER ACTIVITY OF SQUALENE ISOLATED FROM CANTHIUM COROMANDELICUM LEAVES

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ABSTRACT

Squalene is a triterpene, which is widely distributed in nature, with reasonable amounts found in olive oil, palmoil, wheat-germ oil, amaranth oil, and rice bran oil. Triterpenes are compounds of natural origin, which have numerously biological activities: anti-cancer properties, anti-inflammatory, anti-oxidative, anti-viral, anti-bacterial and anti-fungal. These substances can be isolated from plants, animals or fungi. Nowadays, when neoplasms are main cause of death, triterpenes can become an alternative method for treating cancer because of their cytotoxic properties and chemo preventive activities. The present study was to evaluate the anticancer activity of squalene isolated from the leaves of *Canthium coromandelicum*. Squalene structure was elucidated using spectroscopic methods. This is the first report of the isolation of squalene from *Canthium coromandelicum*.

KEYWORDS: Squalene, Canthium Coromandelicum, HepG2 Cells.

INTRODUCTION

Neoplasms are the main cause of death worldwide. Each year tumors are diagnosed in about 11 million people, ending with death in 7.6 million; the number forecasted for 2030 reaches 13.1 million. The major ways of cancer treatment are chemotherapy and radiotherapy, which unfortunately proved toxic to other living cells of the body.[1] Therefore, numerous studies have focused on application of natural products to prevent and to treat cancer. Among bioactive compounds, an important group is that of triterpenes, which show cytotoxic properties against tumor cells at low activity toward normal cells.[2] Triterpenes are naturally occurring alkenes of vegetable, animal and also fungal origin, classified among an extensive

and structurally diverse group of natural substances, referred to as triterpenoids.[3-8] Their structure includes 30 elements of carbon and they are constituted by isoprene units.[9] Taking into consideration the structure, triterpenes may be divided into linear ones-mainly derivatives of squalene, tetracyclic and pentacyclic, containing respectively four and five cycles, as well as two- and tricyclic ones.[10] Representatives of those show anti-cancer properties as well as anti-inflammatory, anti-oxidative, anti-viral, anti-bacterial and anti-fungal ones.[11-18] The aim of this study was to evaluate Cytotoxic activity activity of squalene isolated from *Canthium coromandelicum*.

MATERIALS AND METHODS

The ethanolic extract of leaves of *Canthium coromandelicum* was subjected to column chromatographic separation using normal phase silica gel column. The 20g of ethanolic extract of *Canthium coromandelicum* was adsorbed on silica gel (20g) and transferred to a column of silica gel (200g equilibrated with benzene). Squalene (200 mg) was isolated with a solvent system of ethyl acetate: ethanol 70:30 v/v from ethanolic extract of *Canthium coromandelicum*. This is the first report of squalene from the genus *Canthium*.

Cell culture: HepG2 cells were obtained from National centre for cell science (Pune, India) was maintained and grown in a humidified incubator at 37°C with 5% CO_2. Cells were grown as a monolayer in plastic tissue culture flasks in Dulbecco's Modified Eagle's Medium (DMEM) (Gibco, Grand Island, New York, USA). The medium was supplemented with 10% fetal bovine serum (FBS) (Gibco, Grand Island, New York, USA) and antibiotics (penicillin 50 IU/mL, streptomycin 3.5 µg/mL and gentamycin 2.5 µg/mL) (Gibco, Grand Island, New York, USA).

Cell Viability assay: Cells were seeded in 96-well plates at a density of 5×10^3 cells/well in 200 µL DMEM containing 10% FBS overnight. Non-adherent cells were removed by gentle washing after 24 h. Cells were replaced with serum-free medium with varying concentrations of squalene (5, 10, 50, 100,150,200.250µg/ml). A negative control containing serum-free medium with DMSO was also evaluated. After 72 h of treatment, the plates were incubated with 20 µL 3-(4, 5 dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) solutions (5 mg/mL) for 3 h at 37°C. The formazan was dissolved in 150 µL/well dimethyl sulfoxide (DMSO) and the absorbance was detected at 590 nm using a microplate reader (Bio-Rad, USA). Cell viability was expressed as percentage of untreated cells, which served as the negative control group and was designated as 100%. The results were expressed in

percentage of the negative control. The median inhibitory concentration (IC_{50}, defined as the drug concentration at which cell growth was inhibited by 50 per cent) was assessed from the dose-response curves.[19]

RESULTS AND DISCUSSION

The biochemical structure of squalene is $C_{30}H_{50}$, a 30-carbon compound (polyprenyl, holding 6 prenyl groups, better known as isoprenoid or isoprene) (Fig. 1). Due to a double bond structure of six CH_3 groups, the isoprenoid has a strong natural antioxidative effect. Squalene has a similar structure to other isoprenoids as β-carotene, lycopene, vitamin A, vitamin E, and coenzyme Q10 (ubiquinone).[20] Until today, shark liver oil is considered the richest source of squalene, with squalene accounting for at least 40% of its weight. It is also widely distributed in nature, in lesser proportions in amaranth oil (6-9%), in wheat germ oil, and in olive oil (usually from 0.4% up to 1% in extra virgin olive oil).[21,22]

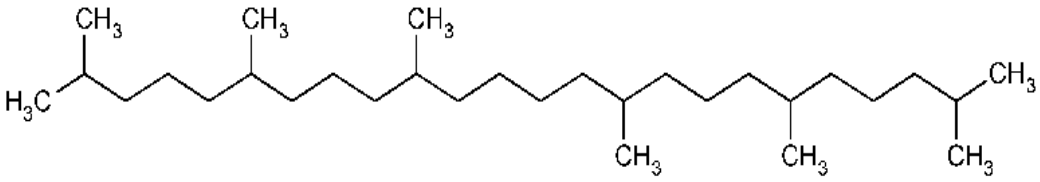

Fig.1: Structure of Squalene.

Squalene (200 mg) was obtained directly from the fraction eluted with ethyl acetate: ethanol (70:30) as a colourless oil. IR (KBr) ν_{max} 2920, 1450, 1390 cm^{-1}. Mass spectrum (Fig.2) showed molecular ion peak at m/z 410 with major fragments of m/z = 391,367, 341, 325, 299, 259, 231, 203, 191, 149, 136, 121, 95, 81, 69, 55, 41, 29.

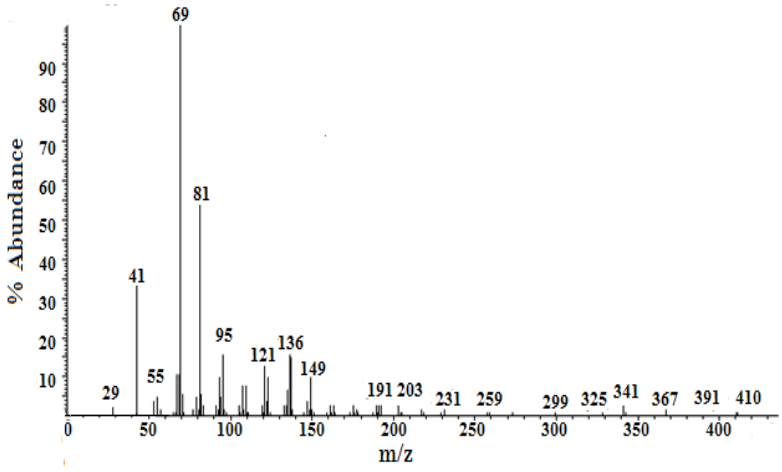

Fig. 2: Mass spectrum of Squalene

Cell viability and cytotoxicity assays are used for drug screening and cytotoxicity tests of chemicals. As the cells are removed from the living (in vivo) environment and subjected to experimental manipulations in the culture systems (in vitro), their viability assumes significance. Viability of the cells represents the capability of their existence, survival and development. Many experiments are carried out with cells in the culture rather than using the animal models. This is particularly so with regard to the determination of safety and cytotoxicity of several compounds (pharmaceuticals, cosmetics, anticancer drugs, food additives). In vitro testing for cytotoxicity and safety evaluation is in fact cost-effective, besides reducing the use of animals. Studies on cytotoxicity broadly involve the metabolic alterations of the cells, including the death of cells as a result of toxic effects of the compounds. For instance, in case of anticancer drugs, one may look for death of cells, while for cosmetics the metabolic alterations and allergic responses may be more important. There are several assays developed in the laboratory for measuring the cell viability and cytotoxicity. A majority of the cytotoxicity and viability assays are based on the measurement of membrane integrity, cellular respiration, radioisotope incorporation, colorimetric assays and luminescence- based tests.

Cell Viability Assay: In the recent years, cytotoxicity of phytochemicals has been an important research area. Many phytochemicals were investigated for their cytotoxic aspect. These phytochemicals showed no significant toxicity in HeLa cells, while marked size-dependent toxicity was observed in fibroblast, epithelial cells and melanoma cells. In this study, *Invitro* cytotoxic effects of squalene was screened against HepG2 cancer cell line. The result of MTT assay demonstrated a dose- dependent decrease in cell viability of HepG2 cells at all tested concentrations of squalene (Fig. 3). The data showed that Squalene displayed marked cytotoxic effects on HepG2 cells, and the viability of HepG2 cells was significantly reduced to 32.8%. The IC_{50} values of squalene were found to be 100µg/ml. Viability test explains the cellular response to toxicant and also provides information on cell death and survival. Our results suggested that the cytotoxic effects of squalene increased on cancer cell line. The present study has corroborated with previous reports.

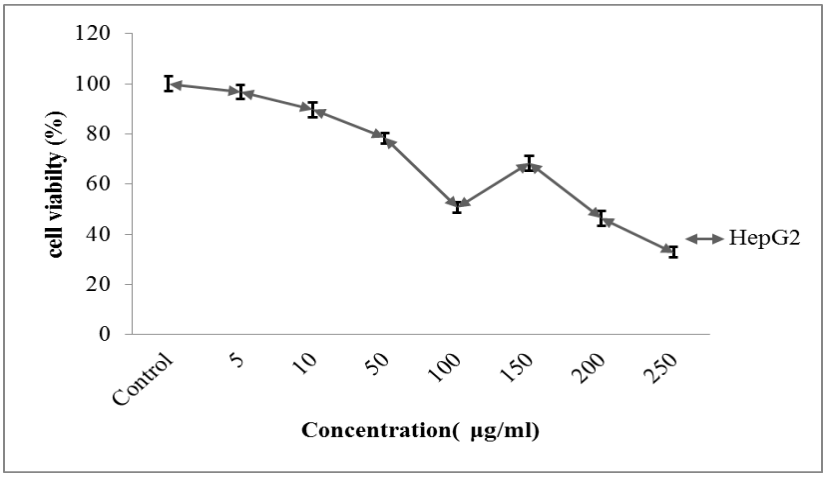

Fig. 3: The cytotoxicity assay of extracted squalene.

In previous report, cytotoxic activity has also been observed in derivatives of linear squalene. Triterpenes isolated from algae showed cytotoxic activity toward leukemia (Jurkat), sarcoma (CADO-ES-1), cervical carcinoma (HeLa) and multiple carcinoma (MM1440).[23] Apart from the inhibitory role, squalene compounds show also the capability of inducing apoptosis in many neoplastic lines: leukemia, melanoma, colon cancer, prostate cancer, ovarian carcinoma, liver cancer, breast cancer, lung cancerand peripheral nervous system carcinoma[24], therefore, may be investigated as potential, alternative agents in cancer treatment.

CONCLUSIONS

Triterpenes not only they are capable of inhibiting life of neoplastic cell lines, but also induce apoptosis of cancer cells, to cause their "suicidal"death, with no threat to normal cells of the body. Such properties, in particular the selectivity of triterpenes' activity, present them as alternatives in cancer treatment and prevention. Therefore, it is essential to gain such compounds, for evaluation of their cytotoxic properties and underlying mechanisms, through synthesis of new derivatives. On the basis of the present investigation was observed as the squalene isolated from *Canthium coromandelicum* shows a significant cytotoxic activity.

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