Available online on www.ijppr.com
International Journal of Pharmacognosy and Phytochemical Research 2015; 7(6); 1217-1221

ISSN: 0975-4873

Research Article

Cyto-Toxic Potential of Fucosterol Isolated from *Turbinaria Conoides* Against Dalton's Lymphoma Ascites

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Available Online:21st November, 2015

ABSTRACT
This is a first report of cytotoxic activity of fucosterol isolated from *Turbinaria conoides*, a brown seaweed collected from the coast of Gulf of Mannar against Dalton's lymphoma ascites**.** Fucosterol, 3β-Hydroxy-5,24(28)-stigmastadiene, a prominent sterol in brown sea weeds has been shown to posses various biological activities. Chemical structure of the compound was verified using GC-MS mass fragmentation data. Trypan blue viability assay was performed on Dalton's lymphoma ascites **(**DLA cell lines) to evaluate the cytotoxic potential of the compound. Fucosterol proved to be effective against these malignant cells. The potential of fucosterol to inhibit cell proliferation against *in vitro* DLA cells at sublethal doses were significant. Survival rate percentage of DLA cells was 0% in both 100 and 200 µg/ml of fucosterol. IC50 value of fucosterol for DLA cell lines is 9.6 µg/ml only. This result highlights the potential role of fucosterol in developing safe medicines against malignant lymphoma.

Keywords: Fucosterol, cytotoxicity, *Turbinaria conoides*, Dalton's lymphoma ascites cell.

INTRODUCTION

Screening for safe theraputical agents from natural sources is a hot area of research, especially to identify compounds with potential cytotoxicity, as various cancer forms are projected to claim cumulatively about more than 9 million human life's in 2015 worldwide[1]. Hodkings and non-Hodkings lymphoma two different forms of blood cancer, which is diagnosed positive every three minutes, have been a major cause of cancer mortality across the world annually[1]. Pathophysiology of this cancers are very complex, however, with the aid of advanced diagnostic tools developed, vital factors leading to the genesis of cancer have been unveiled. Inheritance of hereditary immune deficiency diseases along with viral and bacterial infections and free radical generating agents such as organic solvents, pesticides, herbicides have been identified as major players developing and promoting lymphoma cancer in general[2]. To an extent, early stages of the progression of this diseases can be easily retarded by the early diagnosis and ameliorating the malignant cells, reduces the fatality rate of cancer[3]. In this regard, marine resources have proved to be very effective in the treatment of cancer[4-6]. Even though, marine sources such as seaweeds, corals, sponges, microbes, are bio-factories of numerous biologically safe cytotoxic compounds. Credit for the identification of numerous cytotoxic compounds from these sources goes to the development of advanced techniques for sampling in non hostile environments, and fast screening cell models coupled with sophisticated spectroscopic tools to identify the structural features responsible for the activity[7]. Majority of the Secondary metabolites isolated from brown seaweeds have been found to play diverse role in stimulating various key biological activities[4]. For example, expression of MMP-2 and secretion of vascular endothelial growth factor in A549 lung cancer cells were effectively suppressed using Fucoidans, a sulpfated polysaccharides present in brown sea weeds[8,9]. Fucoidan along with fucoxanthen, a carotenoid molecule abundant in brown sea weeds are very effective in scavenging free radicals [10] and thereby mitigating the lethality of cell proliferation[11]. Turbinaria Conoides, a common brown sea weed along the coasts of India is a pool of cytotoxic compound. Recent investigations using oxygenated sterols isolated from *Turbinaria conoides* provides adequate evidence for significant activity against different cell lines[12], along with prominent anti-microbial and anti-inflammatory activities[13]. These results along with cytotoxic activity of sterols isolated from these sea-weeds supports their potential in developing treatment methods to retard human ailments. This study aims to investigate the cytotoxic potential of fucosterol against Dalton's lymphoma ascites. Fucosterol is a characteristic sterol of brown seaweeds and have been proved safe for human use[14-16]. Cytotoxicity study by Sheu, et al 1999 using its oxygenated forms have shown prominent activity against A-549, HT-29, KB, and P-388 cancer cell lines. The isolation of fucosterol from *Turbinaria conoides* is characterized using mass spectroscopy.

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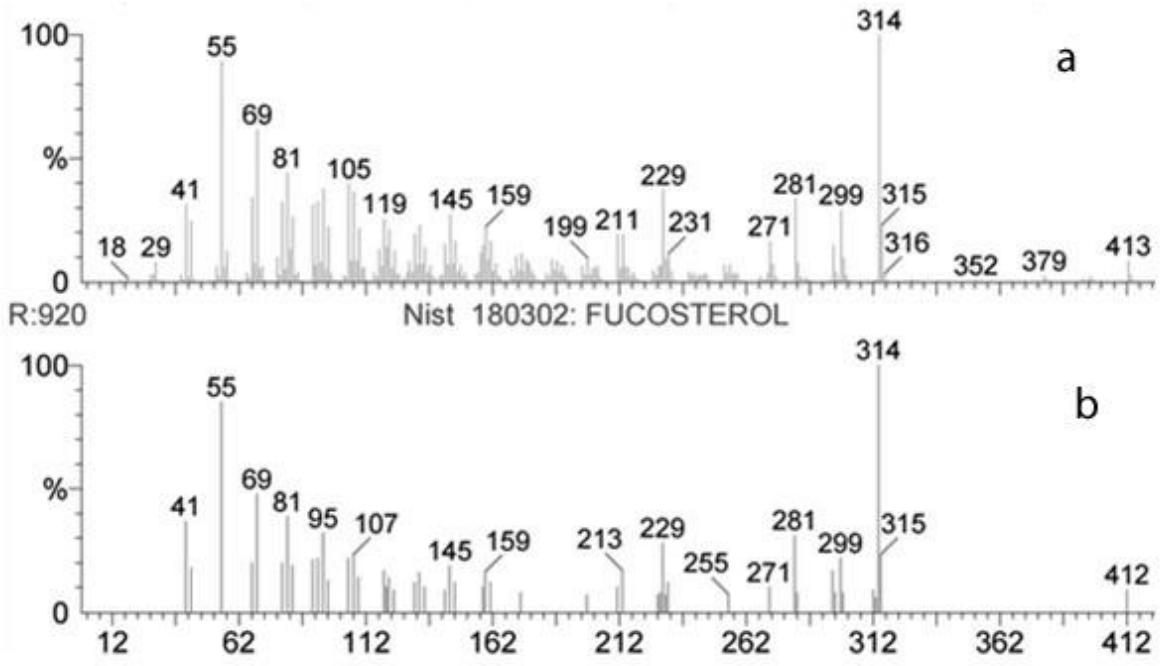

Figure 1. (a) Corresponds to mass spectra of the compound isolated from *Turbinaria conoides* and (b) corresponds to mass spectra of fucosterol from NIST library.

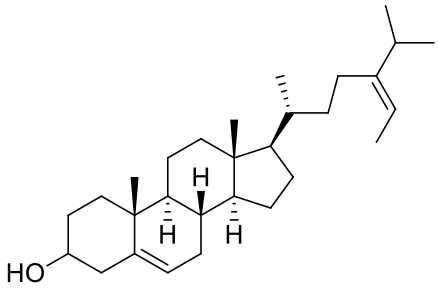

Figure 2. Structure of fucosterol.

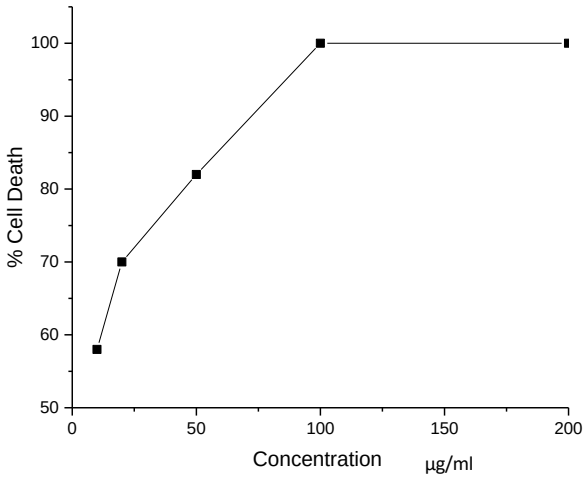

Figure 3. Fucosterol induced cell death percentage in DLA cells.

MATERIALS AND METHODS

Isolation and characterization of fucosterol

All the chemicals used for extraction and purification were purchased from Merck. Neutral lipid fraction containing fucosterols were recovered using hexane from the total extract after saponification. These fractions were subjected to further chromatography purification using homogeneously packed activated Silica-gel (60-120 mesh) column bed having 0.5 mm internal diameter and 1.5 m height, to remove alkanes and other neutral lipids. Silica gel were activated in oven over night, at 120^0C. Fucosterols were eluted from the colum using Hexane Ethylacetate solvent system in the ratio 85:15. Fine purification of the fucosterols were performed using TLC Silica gel 60 F254, Merck.

GC-MS characterization of fucosterol

GCMS analysis was performed using gas chromaotography (Perikin Elmymer Clarus 680) coupled with mass spectrometer (Perikin Elmymer Clarus 600). 1µl of sample was injected to GC equipped with non polar HP ultra double fused silica capillary column. Initially oven temperature was programmed at 70 oC for 4 minute, there after rosed to 280^{o}C at the rate of 10 ^{o}C. This temperature was maintained for another 20 minutes. Mode of operation followed by MS during analysis was EI at 70 eV and inlet and source temperature of the instrument was maintained at 200^{o}C

Cytotoxic activity of fucosterol isolated

In order to investigate the cytotoxic activity of fucosterol against blood cancer, Dalton's Lymphoma Ascites (DLA) cells were used. These tumour cell lines were maintained

at Amala Cancer Research Centre, Amala Nagar, and Thrissur, India.

In vitro cytotoxicity Assay

Trypan blue exclusion method was used to calculate the cytotoxicity of fucosterols A method which differentiate intact live cells cell membranes from the dead cells using a light microscope. Latter, which due to the retention of dye in the cytoplasam are visualized as blue colored. All the chemicals used for the assay were purchased from HIMEDIA. Prior to the viability assay DLA cells aspirated from the intra peritoneal cavity of mice with tumor were washed thrice with PBS. Different concentration of fucosterol (10µg/ml, 20µg/ml, 50µg/ml, 100µg/ml, 200µg/ml) dissolved in DMSO were uniformly mixed with 0.8 ml of PBS add 0.1 ml of cell suspension containing 1 x 10^6 cells and incubated for 3 hours at 37^{o}C. Soon after incubation, a drop of cell mixture treated with 0.1 ml trypan blue dye for 2-3 minutes were transferred to a hemocytometer to count the non-viable (stained) and viable (unstained) cells using microscopic. The total count of viable and non-viable cells was done separately.

RESULTS AND DISCUSSION

The mass fragmentation of the test compound is shown in (Figure 1) the [M]+ at m/z 413, which is in agreement with the molecular formula C29H48O with calculated molecular weight 412.69, indicating six degrees of unsaturation. The mass spectrum showed the base peak at m/z 314 is an outcome of McLafferty" type of rearrangement (a → b), in this case results from the cleavage at 22-23 bond together with a hydrogen transfer from C-20 carbon atom. Presence of low intense ions at m/z 271, corresponding to the loss of two hydrogen atoms from the steriod nucleus along side chain[17] A feeble ion peak at m/z 255 corresponds to the loss of water molecule which supports the presence of hydroxyl group in the sterol ring of fragmented molecule. These mass fragmentation of compound isolated from *Turbinaria conoides* was similar to fucosterol sterol skeleton as shown in (Figure 2)[17]. This was further confirmed by comparing the mass fragmentation data with NIIST library. The trypan blue dye exclusion assay is the viability assay used to evaluate the efficiency of test compound against the malignant cells. This assay measures the non-viable cells, which results from extracellular or intracellular cell damage mediated by fucosterol. The outcome of fucosterol on DLA cell lines after trypan blue exclusion assay are shown in (Figure 3). Only 9.6 µg/ml was required to induce 50% cell death which corresponds to IC 50 of fucosterol. Potent toxicity towards DLA cells showcased by fucosterol is clear indication of its capacity to suppress the progression of rapidly multiplying cells and supports its anti-proliferative activity. Choice of drugs for lymphoma treatment are interlinked to multiple factors, such as type , Hodkings and non-Hodkings lymphoma and their sub-classes, its stage, age of the person under treatment and their overall conditions[18-20]. Special combination of treatments is often required for patients suffering from both lymphoma and Acquired Immune Deficiency Disease[21]. Even though, radiation therapy, chemotherapy or both combined are effective in the treatment of malignant cells that are formed in the lymph system[19,22-24]. Secondary oncologic repercussions along with toxic cardiac complications resulting from these treatments have forced the scientific community to find alternative medicines that are safe and devoid of side effects[25,26]. Numerous in-vitro and in-vivo investigations have established the anti-tumorigenic potential of phytochemicals isolated from seaweeds and other marine sources against DLA. Fucosterol in this regard, is of particular interest in developing drugs for the treatment of cancer, as they posses anti-cancer and anti-oxidant potential, and are safe for human consumption[16]. Fucosterol dose ranging between 100-300 µgm have been proven safe and capable of reducing the risk of cardiovascular diseases through blood pressure regulation[27]. Their potential to suppress hyperglycemic effect is effectively used in manufacturing anti-diabetic agents[28]. Fucosterol also find applications in developing antioxidants as they enhance the activity of superoxide distumulate, catalase and glutathione peroxidase and thereby suppressing the free radical stress[29]. Safety of fucosterol coupled with their strong potential to suppress the DLA cells, observed in this investigation highlights the importance of their role in developing treatment methods to effectively cure the patients suffering from lymphoma cancer without any repercussions. However, further molecular level investigations are required to understand the mechanism by which fucosterol suppress these malignant cells. As these vital information are necessary to predict cumulative outcome of fucosterol on gene expressions and their products prior to their in-cooperation in drugs.

CONCLUSION

So far there have been no data on the cytotoxic potential of fucosterol against lymphoma cancer. This investigation using fucosterol isolated from *Turbinaria conoides* performed on DLA cells provides primary data for their potential against these malignant cells. Structure of fucosterol was confirmed using mass spectral data. Suppressing the activity of maligant DLA cells using fucosterol highlights their potential in developing safe therapeutical agents.

ACKNOWLEDGEMENTS

We thank for the chemicals, facilities, and financial aid, provided by the Department of Chemical Oceanography and Inter University Center for Development of Marine Biotechnology, Cochin University of Science and Technology, to complete the research work. We are thankful for facilities provided by Amala Cancer Research Center, Thrissur for performing cytotoxicity study.

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