Inhibitory Effect of Hesperidin on Kidney Type L-Glutaminase

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Abstract

Citrus limon was extracted with 80% methanol, Then HPLC analysis for Citrus limon methanolic extract represent on hesperidin peak at retention time 14.80 ppm. Invitro inhibition assay for standard KGA with purified hesperidin gave an inhibition of 65.33 % compared to CB-839 positive conttrol. Finally, the cytotoxic effect of purified hesperidin was assessed against breast cancer cell line (AMJ13) and normal cell line (HBL-100) via MTT assay. Demonstrated the strongest and significantly higher cytotoxic activity against AMJ13 especially at 500 µg/mL of 80.67±1.45%, with IC50 value was 22.45µg/ml.while compound showed a variation in toxicity on normal cell line HBL-100.

Keywords: Hesperidin; KGA; breast cancer; HPLC

1. Introduction

The vast majority of people on this planet still rely on their traditional materia medica (medicinal plants and other materials) for their everyday health care needs. It is also a fact that one quarter of all medical prescriptions are formulations based on substances derived from plants or plant-derived synthetic analogs [1]. Therefore, the medical plants have chemical active compounds possessing role in improve health [2].

Cancer is a group of diseases characterized by uncontrolled development and spread of abnormal cells and could lead to death [3]. Cancer cells undergo a reprogramming of metabolism in order to maintain bioenergetics, redox status, cell signaling and biosynthesis, metabolic characteristic of many cancer cells is a dependence on an exogenous supply of glutamine, despite this being a non-essential amino acid (NEAA) that mammalian cells can synthesize de novo, glutamine serves as an important source of reduced nitrogen for biosynthetic reactions, and as a source of carbon to replenish the tricarboxylic acid (TCA) cycle, produce glutathione, serve as a precursor to nucleotides and lipid synthesis via reductive carboxylation, indeed, an inhibitor of the mitochondrial enzyme glutaminase, which converts glutamine to glutamate, a precursor of the TCA cycle intermediate α-ketoglutarate is currently being evaluated in clinical trials for treatment of a range of malignancies [4].

The genus Citrus limon have high flavonoids content, as a class of plant secondary metabolites, due to their broad range of pharmacological properties, citrus flavonoids have reduction and treatment of chronic diseases such as bowel, gastrointestinal disorders, obesity ,diabetes, cardiovascular disease, cancer [5]. The Citrus have anticancer activity is act on cell cycle arrest, suppression of proliferation and proapoptosis, combined chemotherapy, anticancer metastasis and antiangiogenesis [6].

2. Materials and Methods

Preparation of Plant Extract

Methanolic extract of all plant tested were prepared according to [7]. Fifty grams of each plants powder were extracted with 80% methanol (250 ml) at 65°C for 3 hours using the soxhlet apparatus. The plant extract solution was concentrated to dryness under 37°C for 24-48 hours in incubator then stored in clear place until use to prepare the required concentrations [6].

HPLC sample preparation

To analyse the phytochemical contents of Citrus peels methanol extract, Fifty mg of Citrus peels methanol crude extract was diluted in 10 ml of (80:20) v/v methanol: water). The extract was subjected to ultra-sonicator for 25 minutes at 60% duty cycles at 25°C. Then centrifuged for 15 minutes at 7500 rpm. The clear supernatant were subjected to charcoal treatment to remove pigments prior to evaporation under vacuum. Dried samples were re-suspended in 1.0 ml HPLC grade methanol by vortexing, and the mixture was filtrated via 0.22 or 0.45 µm disposable filter and stored at 4°C for next steps. Then, 20 µl of the sample were lunched into HPLC system based on the same optimum separation conditions that were previously fixed with authentic pure standard (Sigma, U.K) [8].

Glutaminase (GLS1) Inhibitor Screening assay

BioVision's Glutaminase (GLS1) Inhibitor Screening Kit is a plate-based fluorometric kit for screening human GLS1 inhibitors. The fluorescence (FLU, λex = 535nm / λem = 587nm).

3. Statistical Analysis

The MiniTab18 software and excel Microsoft are used to analysis all results. ANOVA was used to test for significant variation. Results and Discussion

HPLC of Citrus limon peels methanolic extract

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HPLC analysis for Citrus limon, that showed presence of the highest content hesperidin in peel at 14.20 ppm of sample (Table 1). Hesperidin was detected in peel of analyzed citrus fruits by comparing their retention times and UV spectra with standards (figure 1).

Glutaminase Inhibitor Screening

The results showed that 65.33% of enzyme activity was inhibited by 5% hesperidin when compared to CB-839 as standard (figure 2). [7]. Supresssion of glutaminase enzyme with either small molecule inhibitors has antitumor activity across a variety of tumor types, including lymphoma, glioma, breast, pancreatic, non–small celllung and renal cancers [9].

Cytotoxic Evolution of Hesperidin on AMJ13 Cell Line

3-(4, 5 dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) Assay

The cytotoxic effect of hespirdin against breast cancer cells (AMJ13 cell line) and normal cell (HBL-100 cell line) were studied. The results showed hesperidin possessed cytotoxic effect against the AMJ13 cell line in a concentration dependent manner. The viability of cell decreased by increasing the concenterations of hesperidin.

The cell viability of cell treated with hesperidin ranged from 80.67±1.453 to 6±1.155 for 50 to 3.1 µg/ml respectively within IC50= 22.45µg/ml for hesperidin. Statistically, significany diffrences ($p \leq 0.01$) between growth inhibition on cancer cell for hesperidin on AMJ 13 and HBL-100 cell line table (2).

[10].reported that hesperidin considered anti-proliferative and cytotoxic agents against different cell lines, due to the Pharmacokinetics by enhancing the permeability and retention (EPR) effect and easily enter into cells by transport mechanisms or by accumulation in the extracellular space within the basal plasma membrane. Hesperidin was able to inhibit AMJ13 cells growth by inducing apoptosis and necrotic phenotypes (figure 3). The insignificant cytotoxic effect of hesperidin on normal HBL-100 cells may be due to the regular expression peptide receptors on the surface of HBL-100 cells relative to that of tumor cells [11].

Tables and Figures

Table (1): HPLC for hesperidin that explained detector Ach1 250nm.

Peak#	Ret.Time	Area	Height	Area %	Height
1	2.569	2134	181	0.070	0.118
2	3.203	3016207	152109	99.193	99.212
3	3.978	3561	379	0.117	0.247
4	8.456	18841	647	0.620	0.422
Total		3040744	153316	100.000	100.00

Table (2): Cytotoxic effect of hesperidin on AMJ13 cancer cell lines and HBL-100 normal cell lines.

Concenteration (µg/ml)	Cytotoxic effect of Hesperidin in AMJ13 and HBL-100 cells. (Mean ± SD) N=3	
	Hesperidin on AMJ13	Hesperidin on HBL-100
50	80.67±1.45a	11.67±1.453 a
25	62.00±2.646b	9.667±0.8819 b
12.5	38.00±2.646c	6.333±0.8819 c
6.25	18.00±2.309d	4.667±1.202 d
3.1	6.000±1.155e	1.333±0.3333e

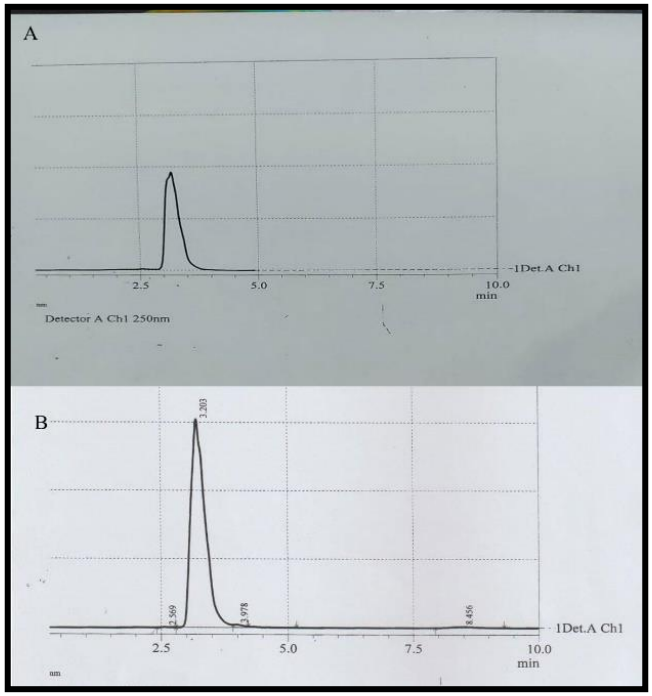

Figure (1): HPLC chromatogram of standard and extracted hesperidin

Standard hesperidin

The Peel in Citrus limon

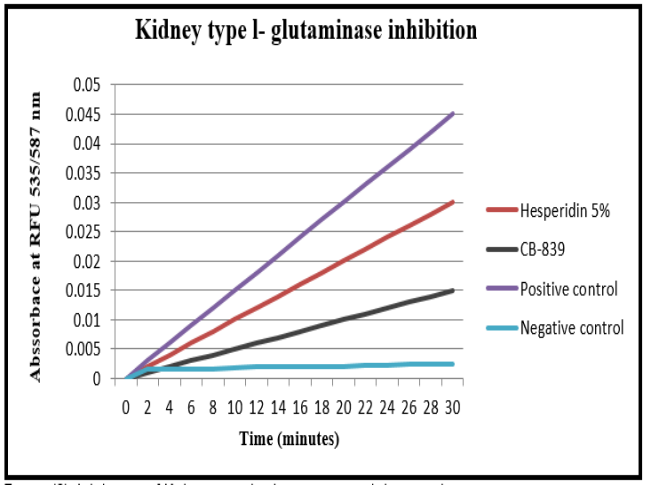

Figure (2): Inhibition of Kidney type l- glutaminase with hesperidin.

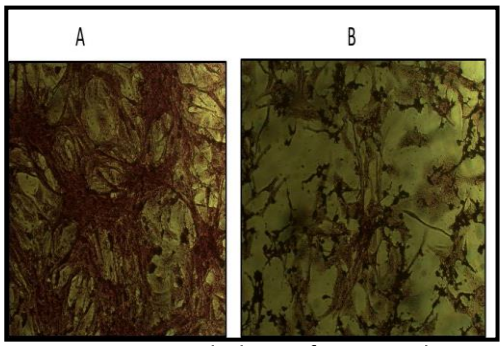

Figure (3): Morphology of AMJ13 where:

A: Control untreated AMJ13 cells.

B: Morphology of AMJ13 cells after treatment with Hespiridin.

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