Ann. For. Res. 66(1): 392-404, 2023
ISSN: 18448135, 20652445

ANNALS OF FOREST RESEARCH
www.e-afr.org

THE EFFECT OF HESPERIDIN TO KIDNEY TYPE L- GLUTAMINASE EXPRESSION AND MOLECULAR DOCKING STUDY

Ali G. Al-Dulimi [a], ziyad k. Radeef [a], Shahrazad A. khalaf [b]

[a] Department of Dentistry, Bilad Alrafidain University College, Diyala, 32001, Iraq.

[b] Department of Pharmacy, Bilad Alrafidain University College, Diyala, 32001, Iraq.

Corresponding author: dr.alighazi@bauc14.edu.iq

Abstract

Different plant sections from (Laurus nobilis, Malus domestica, and Citrus limon) were extracted using 80% methanol. All plants were then evaluated phytochemically for total flavonoids, with different concentrations of 952.77173.47, 980.55673.57, and 341.6683.33 mg/ml, respectively. Additionally, all plant extracts outperformed trolox in terms of their ability to reduce. Additionally, plants' radical scavenging abilities are stronger than vitamin C. (positive control). Some active plant chemicals, including hesperidin, quercetin-3-rhamnoside, and Q-3-O-B-glucopyranoside, underwent molecular docking. Due to its chemical structure, hesperidin had a stronger binding affinity with the active site of human kidenny type l-glutaminase (KGA) with a binding energy of (-7.09 kcal/mol) than the reference (crystal ligand), which had a binding energy of (-6.96 kcal/mol). Hesperidin peak is shown by HPLC analysis of Citrus limon methanolic extract at retention time 14.80 ppm. Purified hesperidin provided an inhibition of 65.33% for standard KGA in an in vitro inhibitory experiment when compared to CB-839 positive control. Using RT PCR, the GLS1 gene expression levels in the AMJ13 cell line were assessed after treatment with hesperidin and CB-839. The results revealed that hesperidin suppressed GLS1 gene expression in the cancer cell line while CB-839 increased GLS1 gene expression.

Keywords: Hesperidin; molecular docking; KGA; breast cancer; GLS1gene expression

Introduction

Cancer is a category of illnesses that can be fatal and are defined by the unchecked growth and spread of aberrant cells [1]. In order to sustain bioenergetics, redox status, cell signaling, and biosynthesis, cancer cells must reprogram their metabolism. One hallmark of many cancer cells' metabolisms is their reliance on an exogenous glutamine source [2]. Despite the fact that glutamine is a non-essential amino acid (NEAA) that mammalian cells can synthesize on their own, it is an important source of reduced nitrogen for biosynthetic reactions, as well as a source of carbon to replenish the tricarboxylic acid (TCA) cycle, produce glutathione, and serve as a precursor to nucleotide and lipid synthesis via reductive carboxylation [3]. In fact, clinical trials are currently being conducted to determine the effectiveness of an inhibitor of the mitochondrial enzyme glutaminase, which transforms glutamine to glutamate, a precursor of the TCA cycle intermediate -ketoglutarate [4]. A intriguing treatment target in recent years has been kidney-type glutaminase (KGA), a mitochondrial enzyme that converts glutamine to glutamate for energy supply, was found to be overexpressed in many malignancies and was thought to be a prospective therapeutic target in recent years [5].

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 [6]. Therefore, the medica for their daily health care requirements, the vast majority of people on this planet continue to use their traditional materia medica. It is also true that 25% of all prescriptions for medicines are made up of synthetic analogues or compounds derived from plants [7]. As a result, the medicinal plants contain chemically active substances that contribute to improved health [8].

Medicinal plants with anticancer properties include some species of the genus Laurus nobilis, which are widely prized for their therapeutic properties like antibacterial, antifungal, antioxidant, antiproliferative, and anti-inflammatory effects [9]. Malus domestica possesses biological effects of these substances, including anti-inflammatory, antibacterial, anticancer, and improvements to the cardiovascular system [10]. Citrus flavonoids, a group of secondary metabolites found in plants, are abundant in the genus Citrus limon. Because of their diverse range of pharmacological properties, citrus flavonoids can be used to prevent and treat chronic diseases like cancer, diabetes, obesity, and gastrointestinal disorders. The anticancer effects of citrus include cell cycle arrest, pro-apoptosis suppression, combination chemotherapy, anti-cancer metastasis, and anti-angiogenesis [11].

Hesperidin, a flavonoid that is abundant in citrus species, particularly orange peel, and is used frequently in Chinese herbal medicine, is the primary anticancer component of citrus [12]. A number of cancers, including hepatocellular carcinoma, prostate cancer, breast cancer, and bladder cancer, are resistant to hesperidin, which is a potent anticancer drug, according to past studies [13]. According to earlier findings, hesperidin is known to have an anticancer effect via inducing apoptosis [14]. Protein misfunction is a common cause of disease, and therapies often rely on blocking or activating the target proteins. Molecular docking has become a significant common component of the drug development toolbox. Its perceived ease of use and relatively low-cost implications have encouraged an ever-increasing, it is primarily designed to anticipate the binding of tiny drug-like compounds to target proteins [15]. In the current study, hesperidin, Q-3-O-B-glucopyranoside, and quercetin-3-rhamnoside, four distinct citrus active chemicals, were molecularly docked with KGA.

Materials and Methods

Preparation of Plant Extract

All of the examined plants, methanolic extracts were prepared in accordance with Rafiq and his co-workers [16]. The soxhlet equipment was used to extract 50 grams of each plant's powder for 3 hours at 65°C in 250 cc of 80% methanol. To prepare the necessary concentrations, the plant extract solution was concentrated to dryness under 37°C for 24-48 hours in an incubator, then stored in a clear location.

Determination of Total Flavonoids

By using the aluminum chloride colorimetric method described by Senguttuvan and his co-workers [17], total flavonoids content in the methanolic extract of all plants was assessed using Spectro photochemical techniques method (flavonoids standard). The entire plant's methanolic extract (3.2 mg) was diluted in 5 ml of 50% methanol before 1 ml of a 5% (w/v) sodium nitrite solution was added. The mixture was let another 5 minutes to stand after the addition of 1 ml of a 10% (w/v) aluminium chloride solution after 6 minutes, and then 10 ml of a 10% (w/v) NaOH solution was added. With distilled water, the combination was prepared up to 50 ml and thoroughly blended. After 15 minutes, the absorbance at 450 nm was measured using a spectrometer. Six concentrations (2.5, 5, 10, 20, 40, and 80 g/ml) were subjected to a comparable technique, and a standard curve was created. With the help of a standard curve curve-fitting equation, the total flavonoids content was calculated.

Assessment of Anti-oxidant Activity In vitro

Reductive ability and DPPH radical scavenging activity were used to evaluate the anti-oxidant activity of the extract of all plants in vitro.

Reductive Ability

To assess the reductive ability, According to Jabbar and his co-workers [18] 1 ml of each concentration of the seven plant extracts (0.02, 0.04, 0.08, 0.16, 0.32, and 0.64 mg/ml) with 1.5 ml of 1% potassium ferricyanide and incubating the mixture at 50°C for 20 minutes. In order to stop the process, 1 ml of 10% trichloroacetic acid was also added to the mixture. After centrifuging the mixture for 10 minutes at 3000 rpm, 2.5 ml of the supernatant was combined with 0.5 ml of freshly made 1% ferric chloride and 2 ml of distilled water. At 700 nm after that, the absorbance was measured. The Trolox solutions underwent the same process (standards). There were three copies of each test run.

DPPH Radical Scavenging Activity

According to Ibraheem and his co-workers [19], the antioxidant activity of all plant methanolic extracts and the standard (vitamin C) were evaluated based on the stable DPPH free radical's capacity to scavenge free radicals. To 3.9 ml of DPPH solution in a test tube, aliquots of 0.1 ml of each plant extract or standard (standardized to 0.625, 0.125, 0.250, and 0.500 mg/ml) were added. A spectrophotometer was used to measure each solution's absorbance at 517 nm after 30 minutes of incubation at 37 °C. Three duplicates of each measurement were taken. The following equation was used to determine how well a compound might scavenge DPPH radicals:

$$\text{DPPH radical scavenging activity (\%)} = \left(1 - \frac{\text{Absorbance of Sample}}{\text{Absorbance of Standard}}\right) \times 100$$

Molecular docking

In a molecular docking study, the target site was chosen by the protein data bank (https://www.rcsb.org/), and several plant active compounds, including quercetin-3-rhamnoside, hesperidin, and Q-3-O-B-glucopyranoside, were tested with crystal ligands for their ability to bind with critical amino acids in a target site of enzyme. Using MOE 14.0901 Software, the molecular binding modes of the investigated substance inside the pockets of (human kidney-type glutaminase) (KGA/GLS) were used. The crystal protein's crystallized ligand served as the source of the binding sites (PDB codes: 5UQE; www.rcsb.org). The complex's initial removal of water molecules. Then, using protein report and utility and clean protein options, crystallographic disorders and unfilled valence atoms were fixed. The necessary amino acids for proteins have been identified and prepped for docking. Chem-Bio Draw Ultra16.0 was used to create 2D structures of the tested compounds, which were then stored in MDL-SD file format by the MOE 14.0901 software.

HPLC sample preparation

Fifty mg of citrus peels methanol crude extract was diluted in 10 ml of (80:20) v/v methanol: water in order to analyze the phytochemical composition of the extract. The extract was run through an ultrasonicator for 25 minutes at a duty cycle of 60% at room temperature. then a 15-minute centrifugation process at 7500 rpm. Prior to vacuum-assisted evaporation, the pigments in the clear supernatant were treated with charcoal to get rid of them. By vortexing, dried samples were re-dissolved in 1.0 ml HPLC grade methanol. The mixture was then filtered through a 0.22 or 0.45 m disposable filter and kept at 4°C for further processing. Following that, 20 ml of the sample were added to the HPLC system using the same optimal separation settings that had been previously established using an actual, pure standard. (Sigma, U.K) [20]

Glutaminase (GLS1) Inhibitor Screening assay

The Glutaminase (GLS1) Inhibitor Screening Test from BioVision is a plate-based fluorometric kit for identifying inhibitors of human GLS1. Fluorescence (ex = 535 nm/em = 587 nm; FLU).

Gene Expresssion GLS1 gene

RNA was extracted using 25 g of AMJ13 cell line that had been pretreated with hesperidin and CB-839, incubated at 37 °C for 3 days, and kept in 1 ml of trizole.

Homogenize prepared biopsy

Using a homogenizer or an equivalent, samples were homogenized in 10–20 volumes of AccuZolTM (1 ml of AccuZolTM per 50–100 mg of tissue, for example). The AccuZolTM sample volume that was homogenized. By adding 1 ml of AccuZolTM per 10 cm3 of the culture plate's volume and piping the cell lysate via a pipette multiple times, cells were immediately lysed in the dish.

RNA extraction:

The AccuZolTM reagent kit was used to extract the RNA from AMJ13 cell lines treated with hesperidin and CB-839 as well as untreated cells, and the results were kept at -80% until use.

cDNA synthesis

Using EasyScript one step gDNA removal and DNA synthesis supermix, cDNA was synthesized and amplified after RNA, primer, and water were mixed and incubated at 65°C for 5 minutes, followed by 2 minutes on ice.

Reverse transcriptase PCR (RT-PCR) technique:

BrightGreen qPCR MasterMix is used to execute RT-qPCR in a reaction solution of 20 liters by combining the following components:

1. 0.6 µL of each forward (F) primer. reverse (R) primer
2. 10µL of BrightGreen 2X qPCR mastermix.
3. ≤ 500 ng of cDNA
4. Nuclease - free H2O to 20 µl.

In order to determine the Cycle Threshold (CT) value, the produced solution was put into a real-time PCR cycler for a heat reaction.

Statistical analysis:

Microsoft Excel and the MiniTab18 program are used to analyze all results. To check for substantial variation, ANOVA was utilized [21].

Results and Discussion

Assessment of Total Flavonoids

The measurement was based on reaction with AlCl3 and spectrophotometrically method and it determined the total flavonoid content in all extracts of the investigated plants (L. nobilis, M domestica, and C. limon). A duplicate of each determination was made. The total flavonoid concentration in the extracts of the examined plants, L. nobilis, M. domestica, and C. limon, was found to be 952.77173.47, 980.55673.57, and 341.6683.33 mg/ml, respectively. The primary flavonoids in alcoholic extracts of leaves of L. nobilis are flavones and flavonols. It is also common to find apigenin, kaempferol, quercetin, and their glycosides [22]. M. domestica had the highest flavonoid concentration, which varied between cultivars and was 306.1 6.7 mg/ml [23]. Additionally, peel sample concentrations of total flavonoids were 4-32 times higher than those of other parts [24]. The C. limon displayed a high flavonoid content of 341.67–83.33 mg/ml. The most abundant types of flavonoid content in citrus were glycosides, specifically hesperidin and naringin, and another class of O-methylated aglycones of flavones, such as nobiletin, narirutin, and tangeretin, are relatively two common polymethoxylated flavones (PMFs). These were primarily found in the flavedo part of the peels and other solid residues [25].

Anti-oxidant and radical scavenging activity

Reductive Ability

The absorbance of L. nobilis, M domestica, and C. limon methanolic extract was significantly higher than trolox (vitamin E) in all concentrations tested (0.02, 0.04, 0.08, 0.16, 0.32, and 0.64 mg/ml). These results imply that all plant extracts are more effective than trolox in the reductive ability, in a concentration-dependent manner. Since 0.02 demonstrated the lowest concentration and 0.64 revealed the maximum (table 1).

However, at 0.64 mg/ml the maximum reductive capacity were recorded in all direct plants (L. nobilis, M domestica, and C. limon), rose considerably (P 0.05) at the concentration (0.02 to 0.64)

mg/ml, and were compared with trolox as (positive control). When compared to trolox, L. nobilis' reductive capacity ranged from 0.44 0.007 to 0.99 0.04 and produced the greatest results overall. M. domestica, however, possessed reductive abilities (0.270.03 to 0.730.16). The reductive ability of C. limon ranged from 0.42 to 0.82. Plant extracts' increased ability to reduce substances when compared to controls (vitamins E) can be related to their high flavonoid content, which was likely caused by their high concentration of tannins, flavonoids, xanthones, and benzophenones [26].

DPPH Radical Scavenging Activity

At the four tested concentrations (0.0625, 0.125, 0.250, and 0.500 mg/ml), methanolic extract of L. nobilis, M domestica, and C. limon was substantially more effective in DPPH radical scavenging activity than vitamin C as (positive control). (Table 2). However, the strongest vitamin C-based radical scavenging activity was seen in citrus limon. According to L. nobilis' data, DPPH radical scavenging activity ranged from (19.794.09 to 51.15 4.31) for (0.0625 to 0.500) mg/ml, respectively, and was concenteration dependent (figure 4). Our findings supported those of [27], who discovered that L. nobilis had a DPPH free radical scavenging activity of 59.2 2.3 at 500 mg/ml. Additionally, M. domestica's results were dose dependent since its free scavenging activity ranged from 0.0625 to 0.500 mg/ml (19.63-4.25 to 94.01-1.71 mg/ml). From apple fruit, various kinds of the bioactive substance have been discovered (Malus spp.). Results from earlier studies indicated that the peels contain a significant amount of phenolic chemicals, which have antioxidant and antiproliferative properties [28]. The maximum DPPH radical scavenging activity was demonstrated by C. limon, which had values of (30.421.67 at 0.0625 mg/ml and (97.531.43) at 0.500 mg/ml. Citrus EOs have antioxidant activity that can delay or prevent cell damage caused by physiological oxidants by inhibiting or preventing the initiation or propagation of excess reactive species and reduce the risk of potential health effects in humans related to oxidative stress or free radicals [29]. Plant essential oils have been reported to scavenge the free radicals that lead to cell death and tissue damage as well as the development of chronic diseases.

Molecular docking

to determine the citrus compound's inhibitory effect on kidney type l-glutaminase activity. The molecular docking approach can speed up the drug design process and save costs. Several outcomes of molecular docking simulation can make drug modeling simpler for in vivo, in vitro, and pharmacy researchers. Different active compounds (Hisperidin,, Q-3-O-B-glucopyranoside, and Quercetin-3-rhamnoside) were subjected to molecular docking utilizing lower estimated binding energies, which are thought to have stronger binding affinity to an enzyme. Hesperidin had the highest binding affinity to kidney type l- glutaminase, although the results in table (3-3) demonstrated that there were differences in the binding energies between these active chemicals and the enzyme. Figure 1 shows that the reference (crystal ligand) binding mode had an energy binding of -6.96 kcal/mol. The candidate molecule (Hesperidin binding)'s mode displayed an energy binding of -7.09 kcal/mol (Figure 2). The candidate chemical (Q-3-O-B-glucopyranoside binding)'s mechanism displayed an energy binding of -6.28 kcal/mol (Figure 3). The energy binding for quercetin-3- rhamnoside was -6.63/kcal/mol (Figure 4).

HPLC of Citrus limon peels methanolic extract

Citrus limon HPLC examination revealed the presence of the maximum concentration of hesperidin in the peel at 14.20 ppm of sample. By comparing the retention periods and UV spectra of the citrus fruits under analysis to standards, hesperidin was found in the peel of the fruits.

Glutaminase Inhibitor Screening

In comparison to CB-839 as the control, the results indicated that 5% hesperidin inhibited 65.33% of enzyme activity. [30]. Breast, pancreatic, non-small cell lung, and kidney malignancies are only a few of the tumor types that can be inhibited by small molecule inhibitors of the glutaminase enzyme. [31].

GLS1 Gene Expression in AMJ13 cancer cell line

To evaluate the GLS1 gene's function in the response of the breast cancer cell line (AMJ13), its gene expression was identified. The amount of GLS1 mRNA transcripts in the AMJ13 cell line treated with hesperidin and CB-839 was measured using (qRT-PCR) and compared to control. Measuring the fluorescence throughout each cycle led to the conclusion that the PCR cycle was related to the quantity of PCR products. The AMJ13 cell line treated with hesperidin and the AMJ13 cell line treated with CB-839 showed significantly different results in GLS1 gene expression, than the control (Figure 5), Hesperidin treatment of AMJ13 cells produced no evidence of glutaminase gene expression, which may be explained by the drug's action on DNA, which prevents or blocks the creation of mRNA and hence the enzyme [32]. On the other hand, cells treated with CB-839 displayed increased kidney type l-glutaminase expression.

Tables

Table 1: Reductive ability of (L. nobilis, M domestica and C. limon) methanolic extract and vitamin E.

Plants	Reductive Ability Absorbance (Mean ± SD)					
	0.64(mg/ml)	**0.32(mg/ml)**	**0.16(mg/ml)**	**0.08(mg/ml)**	**0.04(mg/ml)**	**0.02(mg/ml)**
L. nobilis	0.99±0.04 [a]	0.68±0.01 [b]	0.56±0.01 [c]	0.49±0.01 [d]	0.45±0.01 [d]	0.44±0.007 [d]
M. domestica	0.73±0.16 [a]	0.5±0.006 [b]	0.48±0.004 [b]	0.39±0.005 [ab]	0.38±0.03 [a b]	0.27±0.03 [c]
C. limon	0.82±0.02 [a]	0.59±0.03 [b]	0.54±0.02 [a b]	0.51±0.005 [c]	0.43±0.02 [c]	0.42±0.02 [c]
Trolox (Vitamin E)	0.1 ± 0.001 [a]	0.1 ± 0.001 [a]	0.1 ± 0.001 [a]	0.11 ± 0.004 [a]	0.13 ± 0.007 [b]	0.21 ± 0.015 [c]

Table 2: DPPH Radical Scavenging activity of (L. nobilis, M domestica and C. limon) methanolic extract and vitamin C.

Plants	DPPH Radical Scavenging Activity (Mean ± SD; %)			
	0.500(mg/ml)	**0.250(mg/ml)**	**0.125(mg/ml)**	**0.0625(mg/ml)**
L.nobilis	51.15±4.31 [a]	40.04±6.13 [a b]	31.13±3.6 [b c]	19.79±4.09 [d]

M. domestica	94.01±1.71 [a]	39.69±3.04 [b]	38.88±4.03 [b]	19.63±4.25 [c]
C.limon	97.53±1.43 [a]	64.24±3.83 [b]	47.38±3.072 [c]	30.42±1.67 [d]
Vitamin C	53.00 ± 10.53 [a]	48.33 ± 8.50[a]	41.33 ± 10.01[a]	39.66 ± 2.52[a]

Table 3 : Show (DG) kcal/mol of candidates against (Human Kidney-Type Glutaminase) target site PDB ID: 5UQE.

Compounds NO.	Interactions			Score (DG)	RMSD Value
	Dipole int.	H.B	Pi-interaction		
Hesperidin	-	3	5	-7.09	1.33
Q-3-O-B-glucopyranoside	3	2	3	-6.28	1.89
Quercetin-3- rhamnoside	-	4	7	-6.63	0.86
Crystal ligand	-	1	3	-6.96	1.36

Figures

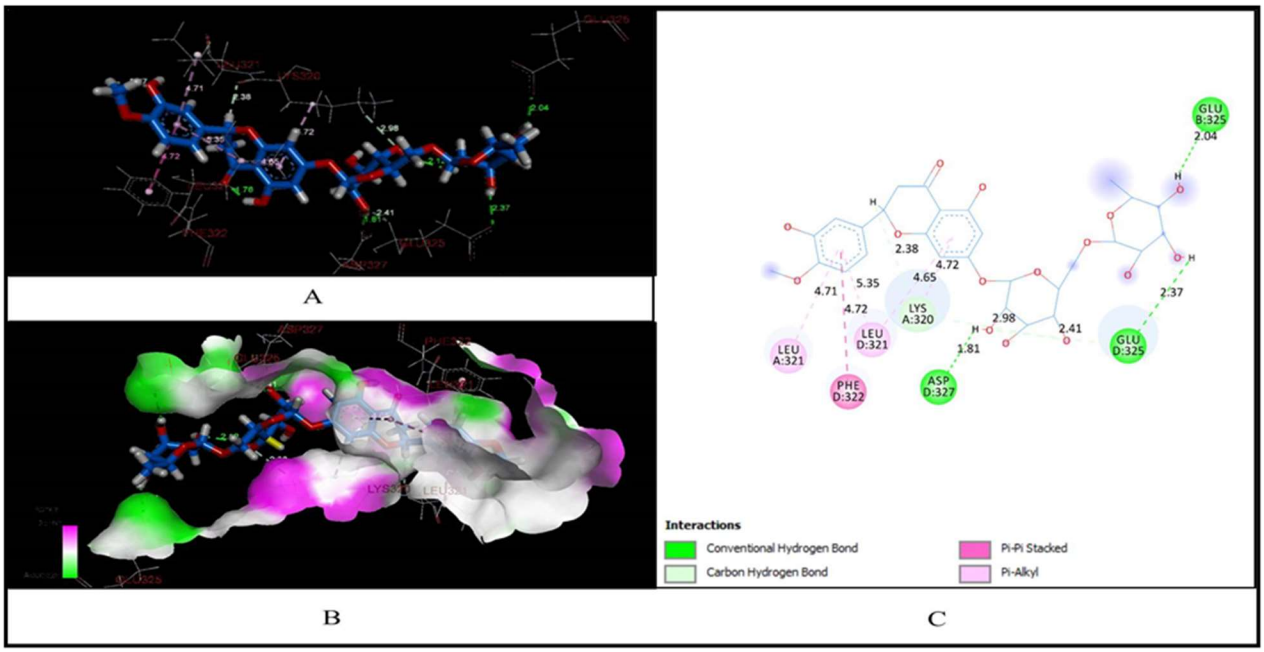

Figure 1 : A. The reference's binding mode (crystal ligand). B. Surface mapping of (crystal ligand) in the active pocket of human kidney-type glutaminase. C. Docked reference crystal ligand in human kidney-type l-glutaminase, hydrogen bonds (green) and pi interactions (purple lines).

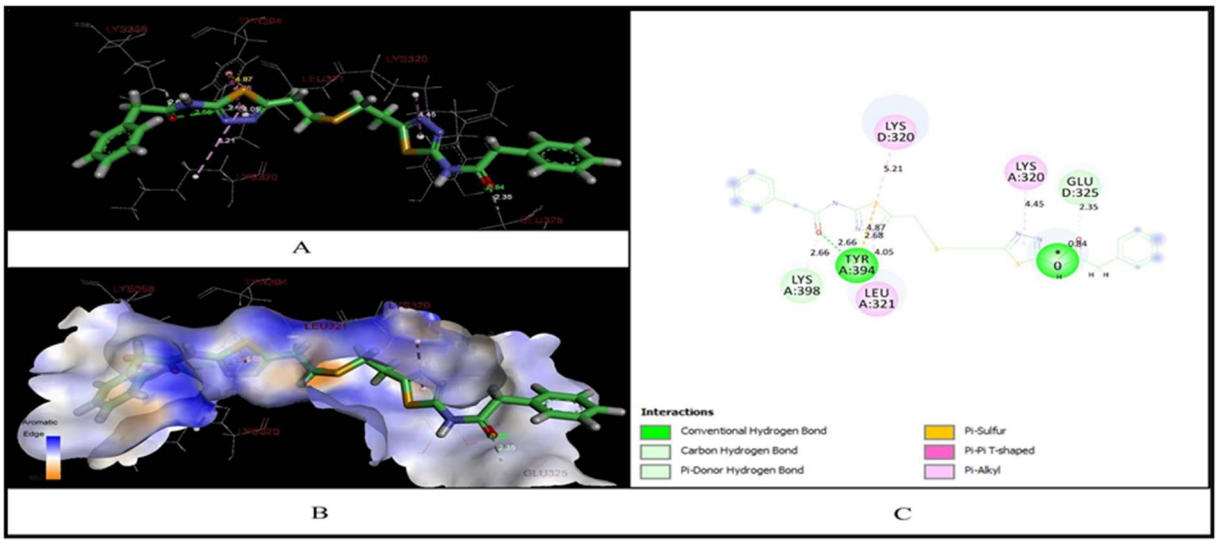

Figure 2: A. The binding mode of the candidate compound (Hesperidin). B. Mapping surface showing (Hesperidin) occupying the active pocket of human kidney-type l- glutaminase
C. (hesperidin) docked in human kidney-type glutaminase, hydrogen bonds (green) and the pi interactions are represented in Purple lines.

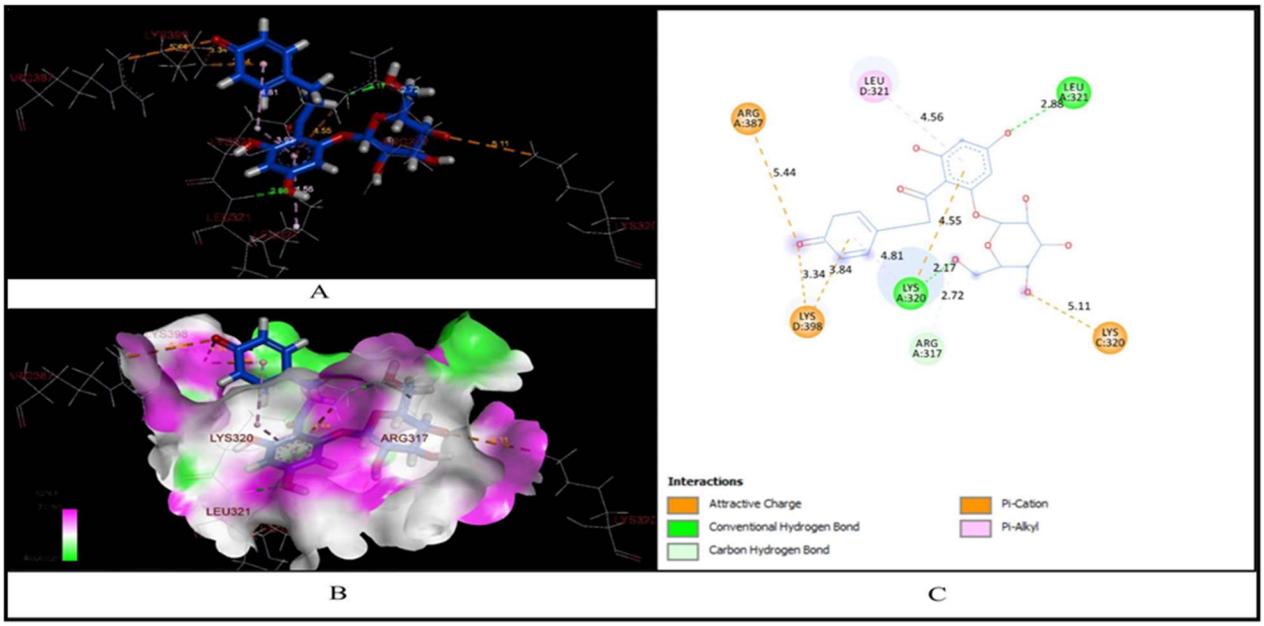

Figure 3: A.The proposed compound's binding mode (Q-3-O-B-glucopyranoside). B. Surface mapping of (Q-3-O-B-glucopyranoside) in the active pocket of human kidney-type l-glutaminase. C. (Q-3-O-B-glucopyranoside) docked in human kidney-type glutaminase, with hydrogen bonds (green) and pi interactions (purple lines) depicted.

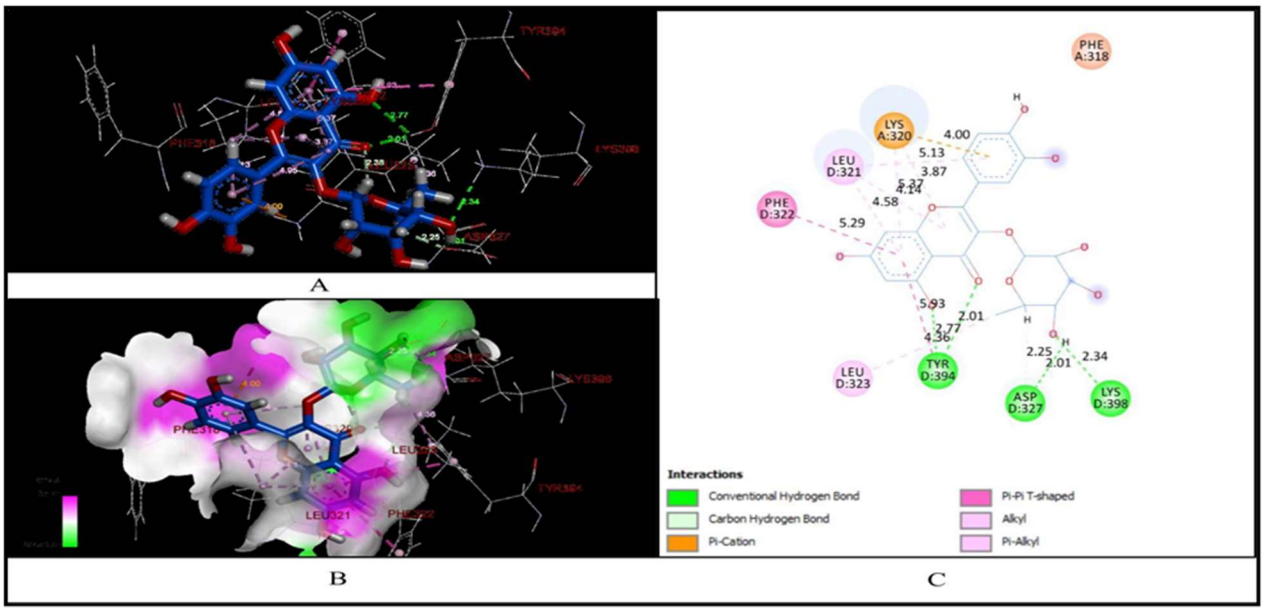

Figure 4: A. The potential compound's binding mode (Quercetin-3- rhamnoside). B. Surface mapping demonstrating (Quercetin-3-rhamnoside) occupies the active pocket of human kidney-type l-glutaminase Flexible. C. (Quercetin-3-rhamnoside) docked in human kidney-type glutaminase, with hydrogen bonds (green) and pi interactions (purple lines).

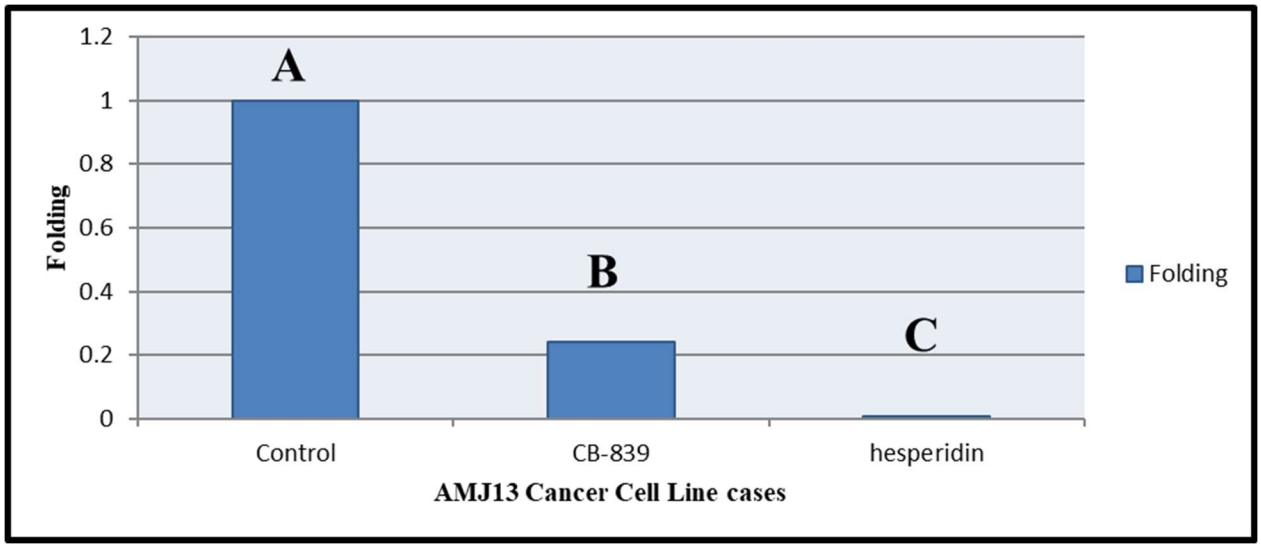

Figure 5: Quantitive analysis of kidney type l- glutaminase in AMJ13 cell line treated with hespridin and CB-839.
A/ AMJ 13 cell line as control.
B/ AMJ13 cell line treated with CB-839.
C/ AMJ13 cell line treated with hesperidin.

Conclusion

In this study, the highly and DPPH radical scavenging and reductive ability activity (after L. nobilis) was demonstrated by C. limon compared with other selected medical plants. Different

active compounds (Hesperidin, Q-3-O-B-glucopyranoside and Quercetin-3-rhamnoside) were subjected to molecular docking utilizing lower estimated binding energies, which are thought to have stronger binding affinity to an enzyme. Hesperidin had the highest binding affinity to kidney type l- glutaminase, compared with those Q-3-O-B-glucopyranoside, and Quercetin-3-rhamnoside which cause downregulation in gene expression of its enzyme. As a result, more research is needed to fully understand the effect of hesperidin and to confirm its effectiveness as a cancer therapeutic and its ability to target cancer cells with specificity.

Conflict of Interest

The authors declare that there are no conflicts of interest regarding the publication of this manuscript.

References

1. Pavlova, N. N. & Thompson, C. B. 2016. The emerging hallmarks of cancer metabolism. Cell metabolism, 23, 27-47.
2. DeBerardinis RJ, Cheng TQ. Q's next: the diverse functions of glutamine in metabolism, cell biology and cancer. Oncogene. 2010;29:313–324
3. Zhang J, Pavlova NN, Thompson CB. Cancer cell metabolism: the essential role of the nonessential amino acid, glutamine. EMBO J. 2017 May 15;36(10):1302-1315.
4. Cluntun, A. A., Lukey, M. J., Cerione, R. A. & Locasale, J. W. 2017. Glutamine metabolism in cancer: understanding the heterogeneity. Trends in cancer, 3, 169-180.
5. Wu C, Zheng M, Gao S, Luan S, Cheng L, Wang L, Li J, Chen L, Li H. A natural inhibitor of kidney-type glutaminase: a withanolide from Physalis pubescens with potent anti-tumor activity. Oncotarget. 2017 Dec 8;8(69):113516-113530.
6. Yun, J., Johnson, J. L., Hanigan, C. L. & Locasale, J. W. 2012. Interactions between epigenetics and metabolism in cancers. Frontiers in oncology, 2, 163.
7. Yuan, H.; Ma, Q.; Ye, L.; Piao, G. The Traditional Medicine and Modern Medicine from Natural Products. Molecules 2016, 21, 559.
8. Al-Dulimi, A. G., Al-Saffar, A. Z., Sulaiman, G. M., Khalil, K. A., Khashan, K. S., Al-Shmgani, H. S. & Ahmed, E. M. 2020. Immobilization of l-asparaginase on gold nanoparticles for novel drug delivery approach as anti-cancer agent against human breast carcinoma cells. Journal of Materials Research and Technology, 9, 15394-15411.
9. Conforti F, Statti GA, Tundis R, Menichini F, Houghton P. Antioxidant activity of methanolic extract of Hypericum triquetrifolium Turra aerial part. Fitoterapia. 2002 Oct;73(6):479-83.
10. Fu, W., Chen, J., Cai, Y., Lei, Y., Chen, L., Pei, L., Zhou, D., Liang, X. & Ruan, J. 2010. Antioxidant, free radical scavenging, anti-inflammatory and hepatoprotective potential of the extract from Parathelypteris nipponica (Franch. et Sav.) Ching. Journal of ethnopharmacology, 130, 521-528.

11. Vilela, J., Martins, D., Monteiro-Silva, F., González-Aguilar, G., de Almeida, J. M., & Saraiva, C. (2016). Antimicrobial effect of essential oils of Laurus nobilis L. and Rosmarinus officinallis L. on shelf-life of minced ―Maronesa‖ beef stored under different packaging conditions. Food Packaging and Shelf Life, 8, 71-80.
12. Darband SG, Kaviani M, Yousefi B, Sadighparvar S, Pakdel FG, Attari JA, Mohebbi I, Naderi S, Majidinia M. Quercetin: a functional dietary flavonoid with potential chemo-preventive properties in colorectal cancer. J Cell Physiol. 2018;233(9):6544–60.
13. Wudtiwai B, Makeudom A, Krisanaprakornkit S, Pothacharoen P, Kongtawelert P. Anticancer Activities of Hesperidin via Suppression of Up-Regulated Programmed Death-Ligand 1 Expression in Oral Cancer Cells. Molecules. 2021 Sep 2;26(17):5345.
14. Barreca D, Gattuso G, Bellocco E, Calderaro A, Trombetta D, Smeriglio A, Lagana G, Daglia M. Meneghini S, Nabavi SM. Flavanones: citrus phytochemical with health-promoting properties. Biofactors 2017; 43:495–506
15. Hughes J.P., Rees S., Kalindjian S.B., Philpott K.L. Principles of early drug discovery. Br. J. Pharmacol. 2011;162:1239–1249.
16. Rafiq, S., Kaul, R., Sofi, S., Bashir, N., Nazir, F. & Nayik, G. A. 2018. Citrus peel as a source of functional ingredient: a review. Journal of the Saudi Society of Agricultural Sciences, 17, 351-358.
17. Senguttuvan J, Paulsamy S, Karthika K. Phytochemical analysis and evaluation of leaf and root parts of the medicinal herb, Hypochaeris radicata L. for in vitro antioxidant activities. Asian Pac J Trop Biomed. 2014 May;4(Suppl 1):S359-67.
18. Jabbar, Abeer M., Ruqaya M. Ibrahim, and Asmaa A. Hussein. "Antitumor Activity of Hesperidin Extracted from Citrus Limon." HIV Nursing 22.2 (2022): 327-330.
19. Ibraheem, R. M., A. A. Mhawesh, and K. W. Abood. "Estimation of the whole flavonoid, antioxidant, anti bacterial challenge concerning viola odorata (banafsha) methanolic extract." The Iraqi Journal of Agricultural Science 49.4 (2018): 655.
20. Adham, A. N. 2015. Qualitative and Quantitative Estimation of Hesperidin in Peel and Juice of Citrus Fruits by RP-HPLC Method Growing in Kurdistan Region/Iraq. Int. J. Pharm. Sci. Rev. Res, 33, 220-224.
21. Livak, K. J., & Schmittgen, T. D. (2001). Analysis of relative gene expression data using real-time quantitative PCR and the 2− ΔΔCT method. methods, 25(4), 402-408.
22. Dias, M., Barros, L. & Duenas, M. 2014. Alves rc, oliveira mb, santos-buelga c., ferreira ic. Nutritional and antioxidant contributions of laurus nobilis l. Leaves: would be more suitable a wild or a cultivated sample. Food chemistry, 156, 339-346.
23. Wolfe, K., Wu, X. & Liu, R. H. 2003. Antioxidant activity of apple peels. Journal of agricultural and food chemistry, 51, 609-614.
24. Schovánková, J. & Opatová, H. 2011. Changes in phenols composition and activity of phenylalanine-ammonia lyase in apples after fungal infections. Horticultural Science, 38, 1-10.

25. Dimitrova, D.Z., Nedialkov, P. and Kitanov, G. (2010). Radical scavenging and antioxidant activities of methanolic extracts from Hypericum species growing in Bulgaria. Pharmacogn Mag., 6: 74-78.
26. Morgan, Abubaker MA, et al. "Anti-osteoporotic and antioxidant activities of chemical constituents of the aerial parts of Ducrosia ismaelis." Bioorganic & Medicinal Chemistry Letters 24.15 (2014): 3434-3439. phenolic components and biological activity of some Senecio species in Turkey. J Pharm Pharmacol.
27. Albayrak, S., Aksoy, A., Yurtseven, L. and Yaşar, A. (2014). A comparative study on phenolic components and biological activity of some Senecio species in Turkey. J Pharm Pharmacol
28. Berber, A., Zengin, G., Aktumsek, A., Sanda, M. A. & Uysal, T. 2014. Antioxidant capacity and fatty acid composition of different parts of Adenocarpus complicatus (Fabaceae) from Turkey. Revista de biologia tropical, 62, 349-358.
29. Mahato, N., Sinha, M., Sharma, K., Koteswararao, R. & Cho, M. H. 2019. Modern extraction and purification techniques for obtaining high purity food-grade bioactive compounds and value-added co-products from citrus wastes. Foods, 8, 523.
30. Dimitrova, D.Z., Nedialkov, P. and Kitanov, G. (2010). Radical scavenging and antioxidant activities of methanolic extracts from Hypericum species growing in Bulgaria. Pharmacogn Mag., 6: 74-78.
31. Patocka, J., Bhardwaj, K., Klimova, B., Nepovimova, E., Wu, Q., Landi, M., Kuca, K., Valis, M. & Wu, W. 2020. Malus domestica: A Review on Nutritional Features, Chemical Composition, Traditional and Medicinal Value. Plants, 9, 1408.
32. Harbeck, N. (2020). Breast cancer is a systemic disease optimally treated by a multidisciplinary team. Nature Reviews Disease Primers, 6(1), 1-2. c 8;8(69):113516-113530.