Research Article Open Access

Lucia Pirvu*, Amalia Stefaniu, Georgeta Neagu, Bujor Albu, Lucia Pintilie

In Vitro Cytotoxic and Antiproliferative Activity of Cydonia oblonga flower petals, leaf and fruit pellet ethanolic extracts. Docking simulation of the active flavonoids on anti-apoptotic protein Bcl-2

https://doi.org/10.1515/chem-2018-0062
received February 12, 2018; accepted April 14, 2018.

Abstract: This study aimed to compare *in vitro* cell cytotoxicity and antiproliferative potency of three standardized ethanolic extracts (5mg GAE/mL sample) from quince flower petals, leaves and fruit pellet on four cell lines (L-929, and HepG2, Caco-2 and BT-20 respectively). Comparative analytical qualitative studies (HPTLC) indicated that if quince leaf extracts (Col40) mainly contain quercetin and kaempferol derivates, the flower petal extracts (Cof40) contain caffeoylquinic acid derivates, while the fruit pellet extracts (Cop40) are comprised of quercetin and caffeoylquinic acid derivates. Pharmacological studies demonstrated the lack of toxicity of test extracts; the most important antiproliferative effects were observed on the hepatic cancer cell line HepG2 (up to 75%, 53% and 70% inhibition in the case of Col40, Cof40 and Cop40 test extracts), followed by the colon cancer cell line Caco-2 (up to 69%, 77% and 40% inhibition) and breast cancer cell line BT-20 (up to 54%, 61% and 19% inhibition). The docking simulations on hyperoside, isoquercitrin, astragalin, and quercetin and kaempferol compared to the synthetic co-crystallized LI0 A1000 ligand (a strong inhibitor of anti-apoptotic protein Bcl-2) indicated astragalin as the most feasible protein inhibitor, but quercetin and kaempferol respected all the parameters involved in the *Lipinski* rule, making them the most promising antiproliferative candidates.

Keywords: Quince extracts; polyphenols profile; antiproliferative effect; docking analysis on quercetin and kaempferol derivates.

1 Introduction

Quince fruit (*Cydonia oblonga* Miller) is recognized since ancient times for its high nutritional value owing to its high content of carbohydrates, mucilage and pectin, proteins and amino acids, lipids and numerous vitamins (thiamine, riboflavin, niacin, ascorbic acid, carotene and retinol), but also of minerals (Na, K, Ca, Mg, Fe, Cu, Zn and Mn) and essential oils (a mixture of aromatic aldehydes, fatty acids, oxygenated monoterpenes and sesquiterpenes) [1, 2].

Medicinal properties of *Cydonia oblonga* derived products are mainly based on polyphenols content, namely tannins, flavonols (kaempferol and quercetin derivates) and caffeoylquinic acid derivates, but also on polyphenols cooperation with polysaccharides fractions. Therefore, *Cydonia oblonga* plant parts (eg., fruit, pulp, peel, seed and also leaf) represent an important source of functional foods and natural medicines; based on the essential oils contained, *Cydonia oblonga* plant parts are also an important source for flavor products in the food and cosmetic industries [3].

Regarding the potential phyto-medicinal and functional food uses of products derived from *Cydonia oblonga* plant parts, the tannins fraction obtained from the fruit part (using hot/water extraction, or hydro-alcoholic and hydro-acetone extraction) was shown

***Corresponding author: Lucia Pirvu,** National Institute of Chemical Pharmaceutical Research and Development (ICCF), 112 Vitan Road, Sector 3, Bucharest, Romania; Department of Pharmaceutical Biotechnologies, E-mail: lucia.pirvu@yahoo.com
Amalia Stefaniu, Georgeta Neagu, Bujor Albu, Lucia Pintilie: National Institute of Chemical Pharmaceutical Research and Development (ICCF), 112 Vitan Road, Sector 3, Bucharest, Romania; Department of Pharmaceutical Biotechnologies
Georgeta Neagu: Department of Pharmacology
Bujor Albu: Department of Physical Chemical Analysis and Quality Control
Lucia Pintilie: Department of Synthesis and Fine Chemicals

to have antibacterial, anti-fungal and anti-influenza properties; the best results were given by alcoholic extracts from quince peel and quince seed products [4]. Polyphenol extracts from different *Cydonia oblonga* plant parts were shown with augmented antioxidant properties; proving these, after treatment with polyphenol extracts from quince the leaf and quince fruit plant parts, certain benefits were found in diabetic rats [5], as well as in ischemia, cardiovascular and other endothelial diseases [6] that develop with high oxidative stress respectively. Polyphenol extracts from quince leaves were also effective in managing cholesterol levels, suggesting further benefits for liver [7], kidney [8] and reproductive apparatus [9]. Studies on the extracts enriched in caffeoylquinic acid derivates, and quercetin and kaempferol glycosides from both the quince fruit and the quince leaf have been shown to have lipid lowering effects comparable with *statins* products. It was concluded that their real potency was found in its reduction of progressive atherosclerosis diseases [10]. Anti-allergic [11, 12] and skin healing effects [13] of quince products are attributed to the presence of polyphenols and their antioxidant properties, but also their cooperation with the mucilage fraction; for example, the (hot) water extracts and the separate polysaccharides fraction from different quince plant parts were both shown to be effective in atopic dermatitis [14]. Similarly, the beneficial effects in gastrointestinal injuries, such as acute and chronic inflammatory bowel lesions and chronic gastric ulcerations, were attributed to the presence of polyphenols (mainly tannins presence) and their interaction with polysaccharides fraction [15].

Antiproliferative potency of the polyphenolic extracts from quince products have also been proved [16]; studies of cancer cell lines A-498 and 796-P (renal), and Caco-2 (colon) indicated that while the leaf extracts presented a concentration dependent growth inhibitory activity on the colon cancer cells and no effect on the renal cancer cells, the seed extracts (500 mg/Kg) inhibited the proliferation of the renal cancer cells, some of the most resistant cancer cells in current chemotherapeutic treatment. Similarly, quince peel polyphenol fraction indicated anti-proliferative properties on murine melanoma cells B16-F1 [17], also known for their chemotherapeutic resistance.

In relation to their antioxidant potency, the extracts from quince peel, pulp, seeds and leaves were shown to have anti-hemolythic activity. While the extracts from the seeds were less effective (EC_{50}=12.2 mg/mL), the extracts from the leaf pulp and peels presented strong antioxidant activities (2,2-diphenyl-1-picrylhydrazyl/DPPH method), EC_{50}=0.6 mg/mL and EC_{50}=0.8 mg/mL respectively; as expected, the intensity of anti-hemolythic activity matched that of the antioxidant activity (2,2'-Azobis-2-amidinopropane-dihydrochloride/AAPH test) [18]. Quince leaf extracts showed a much higher antioxidant potency, EC_{50}= 21.6 µg/mL (DPPH method), and much more augmented anti-hemolythic activity (AAPH test). In comparison, the green tea extract has been evaluated at EC_{50}=12.7 µg/mL [19]; it must be noted that the evidence for a stabilizing effect upon the erythrocyte membranes, i.e. an anti-hemolythic activity, suggests a stabilizing effect on the lysosomal membranes, a hepatoprotective activity respectively.

The main polyphenol compounds found in quince products are 3-O-, 4-O- and 5-O-caffeoylquinic acids, 3,5-O-dicaffeoylquinic acid along with quercetin-3-O-galactoside, quercetin-3-O-rutinoside, and kaempferol-3-O-glucoside and kaempferol-3-O-rutinoside [20]. Data indicate 5-*O*-caffeoylquinic acid to be the main phenolic compound found in fruit (measuring from 29% to 57%), pulp and peels. The total phenols content was appraised at 0.4 g (seed), 6.3 g (peel) and 2.5 g (pulp) *per* 1 Kg dry material; the leaf had a content of total phenols compounds from 4.9 to 16.5 g *per* 1 Kg dry material [21, 22]. Studies also reported high contents of procyanidins, procyanidin B2 (1.4 mg), oligomeric procyanidin (46.6 mg) and polymeric procyanidin (200 mg) respectively, and 3-caffeoylquinic acid (14.1 mg), 5-caffeoylquinic acid (12.3 mg) and 4-caffeoylquinic acid (1.5 mg) *per* 100 g quince fruit [23]. Polysaccharides fraction, the mucilage fraction isolated from seeds respectively, indicated the presence of a partially O-acetylated(4-O-methyl-D-glucurono)-D-xylan structure with a high percentage of glycuronic acid residues; the molar ratio between D-xyl : 4-O-Me-D-GlcA : D-GlcA : O-Ac unities was valued at 2 : 0.8 : 0.2 : 1 [24].

Considering the huge therapeutic potential of the polyphenolic extracts from quince products (fruit, peel, pulp, seed and leaf), and the lack of correlation between chemical qualitative data and pharmacological results, the present study aimed to study polyphenols profile (HPTLC method) and *in vitro* cytotoxic and antiproliferative potency on three human cancer cell lines (breast cancer cell line BT-20, hepatic cell line HepG2 and colon cancer cell line Caco-2) of three standardized ethanolic extracts with exactly 5 mg total phenols content (expressed as gallic acid [GAE] equivalents *per* 1 mL sample) isolated from quince flower petals, quince leaves and quince fruit pellets (peel and seed residue); it must be noted that studies on the quince flower petals are not well described in scientific literature. A docking analysis of the anti-apoptotic protein Bcl-2 was also carried out to predict the binding modes, the binding affinities and the orientation of five active flavonoids compounds (hyperoside,

isoquercitrin/isoquercetin, astragalin and their aglycones quercetin and kaempferol), available in quince derived products.

2 Experimental Procedure

2.1 Materials

2.1.1 Plant material description

The *Cydonia oblonga* tree was authenticated by the biologist team from the National Institute of Chemical-Pharmaceutical R&D, ICCF, Bucharest, Romania. The three plant parts studied (flower petals, leaves and fruits) were collected from a single tree in 2016, within the South Romania, Ilfov region respectively; the flower petals were collected in April, the leaves were collected in August, while the fruits were harvested at the end of October. Voucher specimens (Cof-AP16, Col-AP16, Cop-AP-16) are deposited in ICCF *Plant Material Storing Room*.

2.1.2 Vegetal extracts preparation

The flower petals and the leaves were washed (separately) with distilled water, dried at 45-50°C, and milled into a fine plant powder. The fruits were washed, boiled for 30 minutes, and the resulting mashed material passed through a sieve (3 mm pore diameter); the residue (material left on the sieve) consisting of peel and seeds (i.e. the fruit pellet) was used in the extraction process.

Ten (10) grams of powdered quince leaves, 10 g of powdered quince flower petals and 100 g of quince fruit pellet were extracted (separately) with a 100 mL, 100 mL and 1000 mL 70% (v/v) ethanol solution, for one hour, at boiling temperature. The three resulting extracts were filtered (quince leaves and flower petals) or centrifuged (fruit pellet, 5000 rpm), and the extracts obtained were analyzed for total phenol content (*Folin Ciocalteau* method). After that, the three ethanolic extracts were concentrated (using the rotavapory device) at a *spiss* residue. The three *spiss* residues were passed into a 40% (v/v) ethanol solution so as to achieve the final concentration of 5 mg of total phenols (expressed as gallic acid equivalents [GAE]) *per* 1 mL sample. Three standardized (5mg [GAE]/1 mL sample) 40% ethanolic extracts were obtained: Cof40 - flower petals extract, Col40 - leaf extract and Cop40 - fruit pellet extract, further used for HPTLC analytical (polyphenols profile) and *in vitro* pharmacological (citotoxic and antiproliferative potency) studies.

2.1.3 Chemicals, reagents and references

Chemicals, reagents, solvents and reference products (polyphenols) were purchased from *Fluka and Sigma-Aldrich* Co Bucharest, Romania (see bellow); reference compounds were prepared as 10^{-3}M in (70%, v/v) ethanol then mixed at precise ratio and used for HPTLC studies.

Product name	*Puriss.*	Product name	*Puriss.*
Sodium carbonate	*99.999%*	Rutin	*min. 95%*
Sodium acetate	*>99.0%*	Quercetin	*95%*
Aluminium chloride	*99.999%*	Isoquercitrin	*90%*
Folin Ciocalteu's phenol reagent	*for the determination of phenols*	Hyperoside	*97%*
Natural Product (2-Aminoethyl diphenylborinate)	*>97.0%*	Apigenin	*97%*
PEG (Polyethylene glycol 4000)	*20%*	Kaempferol	*95%*
Ethanol	*analytical standard*	Cynarin	*analytical standard*
Ethyl acetate	*99.8%*	Chlorogenic acid	*95%*
Formic acid	*98 - 100%*	Caffeic acid	*99%*
Glacial acetic acid	*>99.7%*	Gallic acid	*95%*
		Ferulic acid	*98%*

2.2 Experimental Design

2.2.1 Qualitative (HP)TLC analysis

Qualitative HPTLC studies were performed according to *Plant Drug Analysis* [25] and *High-Performance Thin-Layer Chromatography for the Analysis of Medicinal Plant* [26], as described in our previous work [27]. Studies intended polyphenols profile' assessment of the three standardized 40% (v/v) ethanolic extracts: Cof40 - flower petals extract, Col40 - leaf extract, and Cop40 - fruit pellet extract.

2.2.2 Estimation of Total Phenolics Content

Studies were performed according to standard *Folin Ciocalteau* method [28], also detailed in previous work [27].

2.2.3 Pharmacological studies *in vitro*

2.2.3.1 Cell cultures

The tumor cell lines used for the evaluation of the antiproliferative effect were: human breast carcinoma BT-20 line (ATCC® HTB19™), human colorectal cancer Caco-2 line (ATCC® HTB-37™) and human liver cancer HepG2 line (ATCC®HB-8065™). The normal cells selected for cytotoxicity studies were murine fibroblasts, L-929 cell line (ATCC®CCL-1™) and it was used as a comparative control, to check for selective specificity towards cancer. All four types of cells were obtained from ATCC (LGC Standards, Germany) and were cultured in EMEM (Eagle's Minimum Essential Medium) supplemented with 10% (BT-20 and HepG2 lines) or 20% (Caco-2 cells) of fetal bovine serum (FBS), while L-929 line required10% of horse serum. All cell culture types were kept in similar growth conditions (at 37ºC in 5% CO_2 humid atmosphere).

2.2.3.2 MTS test

The viability test was performed according to the Technical Bulletin of Promega Corporation, CellTiter 96 AQueous One solution Cell Proliferation Assay [29]. *General principle*. The MTS tetrazolium [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxy-phenyl)-2-(4-sulfophenyl)-2H-tetrazolium] is bioreduced by cells into a colored formazan product that is soluble in a tissue culture medium (this conversion is presumably accomplished by NADPH or NADH produced by dehydrogenase enzymes in metabolically active cells). Assay is performed by adding a small amount of the CellTiter 96 AQueous One Solution Reagent directly to culture wells, incubating for 1–4 hours then recording the absorbance at 490 nm with a 96-well plate reader. The quantity of the formazan product, as measured by an absorbance of 490 nm, is directly proportional to the number of living cells in the culture. Each treatment (cell series treated with test extract at different doses – dilution series) is carried out in quadruplicate and the cell survival is calculated with respect to the untreated controls. *Materials*. The Cell Proliferation Kit, CellTiter 96 Aqueous One solution Cell Proliferation Assay (MTS) was purchased from Promega Corporation, while EMEM, Foetal Bovine Serum (FBS) were purchased from ATCC (LGC, Germany).

2.2.3.3 Cytotoxicity and antiproliferative studies

Briefly, after reaching confluency, the effects of test-extracts on both types of experiments (cytotoxicity and antiproliferative activity) were evaluated using MTS [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium] reduction, which is a colorimetric test based on the selective ability of viable cells to reduce the tetrazolium component of MTS into purple colored formazan crystals, as described earlier. Depending on how the test is conducted, it is possible to evaluate the cytotoxic effect or cellular proliferation. In the proliferation assay, the application of the modulating factor (test extract) for proliferation is done on "sub-confluent" cell cultures, and the determination of the cell population is performed after at least one division cycle (population doubling interval). Regarding the cytotoxicity assay, the cells are exposed to "semiconfluent" cultures (about 70%) and the activity is measured for a shorter time period rather than a doubling time (usually 6-12 hours). After reaching confluence, the cells were detached from the flask with Trypsin-EDTA. The cell suspension was centrifuged at 2000 rpm for 5 min and then resuspended in the growth medium for further study. For all the experiments, cells were seeded in 96-well plates at a density of 4000 cells per well in 200 µl of the culture medium. L-929 cells were allowed to attach and achieve approximately 80% confluence prior to the start of the experiments for determining the cytotoxicity of the three samples, while the tumor cell lines, had the culture medium from the wells - removed the next day (before the cells started the duplication process) and replaced with 100 µl new culture media containing test vegetal extracts (namely Cof40, Col40 and Cop40) at dilution series (5, 10, 25, 50 and 100 µg EAG/mL); each test vegetal sample, dilution point respectively, was prepared as triplicate and compared to a control sample with identical concentration of test vegetal sample solvent, 40% (v/v) ethanol solution respectively. After 22 hours (L-929 cells) or 44 hours (the three tumoral cell lines) of exposure, the culture medium was removed. After two hours of incubation with MTS solution, viability of the adherent cells was determined using the *CellTiter 96 AQueous One Solution Cell Proliferation Assay* (*Promega, USA*).

The absorbance of the test/treated and control samples were measured at 490 nm with a Microplate Reader (Chameleon V Plate Reader, LKB Instruments) and the recorded values used for cell viability estimation (*see formula*).

2.2.3.4 Statistical analysis

Results were calculated as mean ± SD, n=3.

2.2.4 Procedure for molecular docking simulations

Molecular Docking studies carried out using *CLC Drug Discovery Workbench* Software on a well known apoptosis regulator, Bcl-2 protein, downloaded from Protein Data Bank (PDB ID:2O2F) [30], which also included the co-crystalized ligand coded LI0, a benzamide derivative, proved to be a potent inhibitor of Bcl-2 protein. The Molecular Docking procedure consisted of the following steps: test vegetal ligands (hyperoside, isoquercitrin, astragalin, quercetin and kaempferol) preparation, import form Chemspider as *.mol, by structure optimization; calculate structural properties according *Lipinski*'s rule of five* [31]; explore protein-ligand interactions using docking: setup the binding site (the existing active site of the protein-receptor- the binding site of the co-crystallized ligand) in a Molecule Project, dock ligands imported to a Molecule Table, inspect the docking results; validate the docking method: to ensure that the ligand orientations and position obtained from the molecular docking simulations are valid and reasonable potential binding modes of ligands, the docking methods and parameters used have been validated by redocking; analyze and compare the final docking results (binding mode, hydrogen bond interactions, docking score and RMSD).

***Note:** According to Lipinski's rule of five, when evaluating drug-likeness, in order to find an efficient oral drug candidate, the analyzed compounds should meet the following criteria: i). maximum five hydrogen bond donors (HBD), as total number of nitrogen-hydrogen and oxygen-hydrogen bonds; ii). maximum 10 hydrogen bond acceptors (HBA), as total number of nitrogen and oxygen atoms; iii). maximum molecular (M) weight of 500 Da; iv). the octanol-water partition coefficient (log P) value less than 5. All value limitations of parameters that should be observed according the rule **are multiples of five** (HBD < 5 × 1; HBA < 5 × 2; M < 5 × 10; log P < 5 × 1).

Ethical approval: The conducted research is not related to either human or animals use.

3 Results and Discussion

3.1 Analytical aspects of *Cydonia oblonga* ethanolic extracts

Figure 1, chromatograms A and B, presents polyphenols profile of the three standardized (5 mg GAE / 1 mL) *Cydonia oblonga* derived products: 40% (v/v) ethanolic extracts (chromatograms A1 and B1) and corresponding hydrolyzed samples (chromatograms A2 and B2) from flower petals (T2 and T2H tracks), leaves (T3 and T3H tracks) and fruit pellet (T4 and T4H tracks), comparatively to several polyphenols compounds, reference products respectively (T1, T5, T7, T8, T9, T10, T11, T12, T13, T14, T15, T16, T17, T18 tracks.).

As shown HPTLC fingerprints, flower petal ethanolic extract Cof40 (chromatograms A1 and B1, T2 tracks) indicated the occurrence of numerous caffeoylquinic acid derivates (blue fl. spots s2/chlorogenic acid, s3/neochlorogenic acid and several isochlorogenic acids, s6, s7 and s9 spots respectively, plus cynarin) and less augmented quantities of quercetin derivates (orange fl. spots s1/rutin, s4/hyperoside, s5/isoquercitrin and s8/quercitrin). Cof40 hydrolyzed sample (chromatograms A2 and B2, T2H tracks) confirmed the abundance of caffeic acid and its derivates, and the presence of cynarin (chromatograms A2 and B2, blue fl. spot at *Rf* ~0.28).

Quince leaf ethanolic extract Col40 (chromatogram B1, T3 track) pointed out a much higher increase in the quantities of quercetin derivates and the presence of kaempferol derivates (blue-green fl. spots in the areas of s2 and s6), kaempferol-3-O-rutinoside/nicotiflorin (kaempferol 1) and kaempferol-3-O-glucoside/astragalin (kaempferol2) respectively. Col40 hydrolyzed sample (chromatograms A2 and B2, T3H tracks) confirmed both, high quantities of quercetin aglycone (chromatogram A2 and B2, orange fl. spot at *Rf* ~0.70) and the abundance of kaempferol aglycone (chromatogram A2 and B2, intense green fl. spot at *Rf* ~0.86/0.89); cynarin has not been evidenced either.

Fruit pellet ethanolic extract Cop40 (chromatograms A1, T4 tracks) showed fewer augmented quantities of caffeoylquinic acid derivates, but more increased quantities of quercetin derivates, plus quantities of p-coumaric acid (dark blue fl. spot s10). Cop40 hydrolyzed sample (chromatograms A2 and B2, T4H tracks) confirmed the predominance of caffeic acid and its derivates and did not reveal the presence of cynarin.

All three test extracts (Cof40, Col40 and Cop40) were prepared with an identical total phenol content, 5 mg [GAE] *per* 1 mL sample respectively.

Accordingly, **Figure 2** depicts a polyphenol distribution (quantitative and qualitative aspects) between the three quince derived products, 40% ethanolic extracts from petal flowers(**a**), leaves(**b**) and fruit pellet(**c**) respectively.

In summary, analytical studies have shown that if quince leaves abound in flavonoids compounds (quercetin and kaempferol glycosides) and less augmented quantities caffeoylquinic acid derivates, flower petals mainly contain

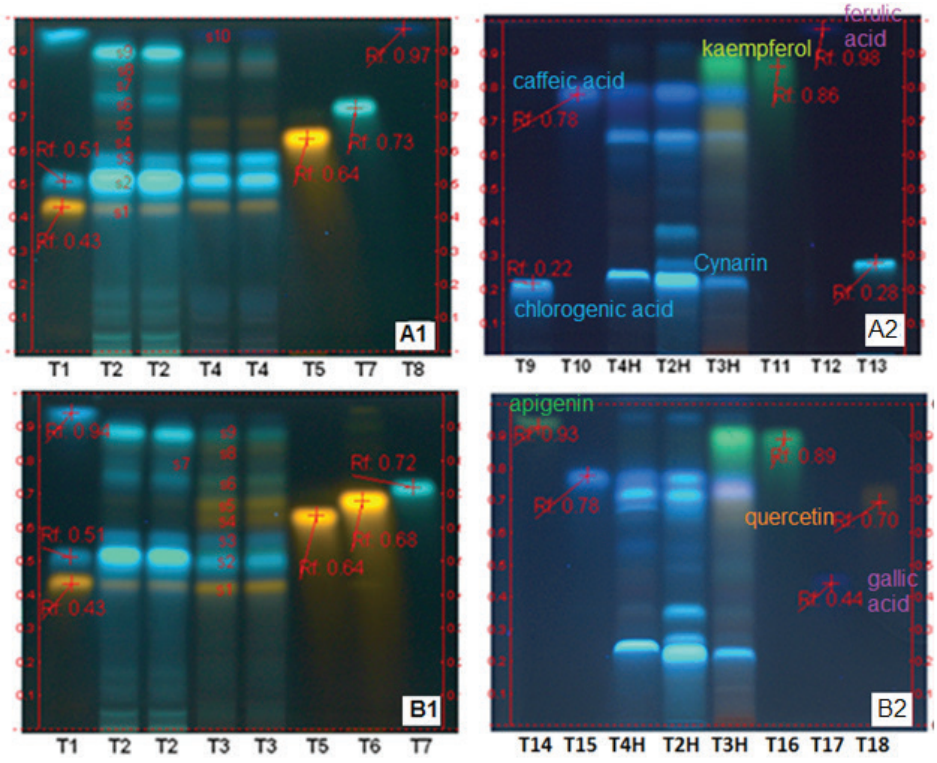


Figure 1: Polyphenols profile of *Cydonia oblonga* 40% ethanolic extracts and corresponding hydrolyzed samples from quince flower petals (T2 and T2H tracks), quince leaves (T3 and T3H tracks) and quince fruit pellet (T4 and T4H track) face to the references (ref.). **Where: Chromatogram A1:** T1 track – rutin, chlorogenic acid, caffeic acid (ref.); T2 tracks – Cof40 ethanolic extract (duplicate sample); T4 tracks – Cop40 ethanolic extract (duplicate sample); T5 track - Hyperoside (ref.); T6 - Cynarin (ref.); T7 track – Ferulic acid (ref.). **Chromatogram A2:** T9 track – Chlorogenic acid (ref.); T10 track - Caffeic acid (ref.); T4H track – Cop40 hydrolyzed sample; T2H track – Cof40 hydrolyzed sample; T3H track – Col40 hydrolyzed sample; T11 track - Kaempferol (ref.); T12 track - Ferulic acid (ref.); T13 track - Cynarin (ref.); **Chromatogram B1:** T1 track – rutin, chlorogenic acid, caffeic acid (ref.); T2 tracks – Cof40 ethanolic extract (duplicate sample); T3 tracks – Col40 ethanolic extract (duplicate sample); T5 track - Hyperoside (ref.); T6 - Isoquercitrin (ref.); T7 track – Cynarin (ref.). **Chromatogram B2:** T14 track – Apigenin (ref.); T15 track - Caffeic acid (ref.); T4H track – Cop40 hydrolyzed sample; T2H track – Cof40 hydrolyzed sample; T3H track – Col40 hydrolyzed sample; T16 track - Kaempferol (ref.); T17 track - Gallic acid (ref.); T18 track - Quercetin (ref.).

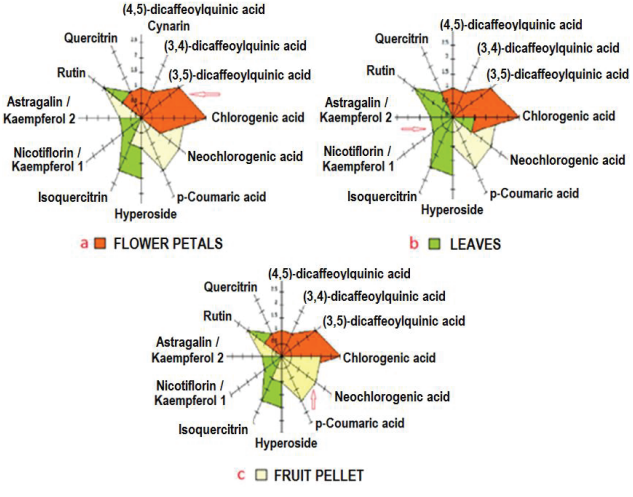


Figure 2: Polyphenols distribution (qualitative and quantitative aspects) between *Cydonia oblonga* 40% (v/v) ethanolic extracts; **a)** Cof40 - flower petals extract, **b)** Col40 - leaves extract, **c)** Cop40 - fruit pellet extract.

caffeoylquinic acid derivates and only small amounts of quercetin derivates; the fruit pellet (peel and seeds residue) plant material also is characterized by small amounts of quercetin derivates and (neo)chlorogenic and isochlorogenic caffeoylquinic acid derivates, plus p-coumaric acid.

3.2 *In vitro* pharmacological results

3.2.1 Cytotoxicity results on *Cydonia oblonga* ethanolic extracts

The first part of the pharmacological study aimed to evaluate potential toxic effects of the three tested vegetal extracts (Col40, Cop40 and Cof40) on L-929 cells.

The protocol presented (see Section 2.2.3) essentials consisting in a colorimetric test based on the selective

ability of the viable cells to reduce the tetrazolium component of MTS into a purple colored formazan crystal. The quantity of the formazan product, as measured by absorbance at 490nm, is directly proportional to the number of living cells in the culture, so that the effect of *the sample* on the proliferation of L-929 fibroblasts can be calculated as a cell viability percentage (%).

Figure 3 summarizes the results on the three tested vegetal extracts (Col40, Cop40 and Cof40) *versus* control cells.

The results indicate that the L-929 fibroblasts were viable in all studied concentrations for all quince extracts; the most important stimulatory effects, compared to the negative control cells, were done by Col40 (SD<0.78%), followed by Cof40 (SD<1.01%) and Cop40 (SD<0.71%). It was also determined that the smallest concentration (5 µg GAE / mL sample) did not affect the cell's viability, but with an increasing extract concentration (the interval 10 - 100 µg GAE / mL), the cell's viability percentage increased in a concentration dependent manner; **Table 1S** presents the general view of the effects of *Cydonia oblonga* extracts on the L-929 cell line, the percentage of cell proliferation face to control sample, relative ratio ± SD(%), and the deviation from the linearity (R^2) respectively. Therefore, the viability test indicated the safety of usual doses (10 - 100 µg GAE / mL) of the quince derived products, leaves, fruit pellets and flower petals 40% ethanolic extracts; the highest doses of 100 µg GAE/mL were not practical as they are impossible to achieve *in vivo*.

3.2.2 Antiproliferative potency of *Cydonia oblonga* ethanolic extracts

Tests were done on several human cancer cell lines demonstrated to be sensitive of quercetin and kaempferol derivates [32 - 37]. The three cell lines were as follows: a. breast cancer cell line BT-20, b. hepatocellular carcinoma (hepatic cancer) line HepG2 and c. colorectal adenocarcinoma (colon cancer) cell line Caco-2.

Figure 4 shows antiproliferative efficacy, cell viability inhibition (%) compared to positive control cells respectively, of the three tested vegetal extracts (Col40, Cop40, Cof40) on the three human cancer cell lines in the interval 5 -100 µg GAE/mL sample.

The viability test of the three cancer cell lines indicated a general linear tendency ((see **Table 1S,** the general view of the effects of *Cydonia oblonga* extracts on the three cancer cell lines, percent of cell proliferation face to control sample, relative ratio ± SD(%), and the deviation from the linearity (R^2) respectively)) in the interval 10 - 100 µg GAE / mL sample, the antiproliferative efficacy increased with polyphenol content in extracts respectively.

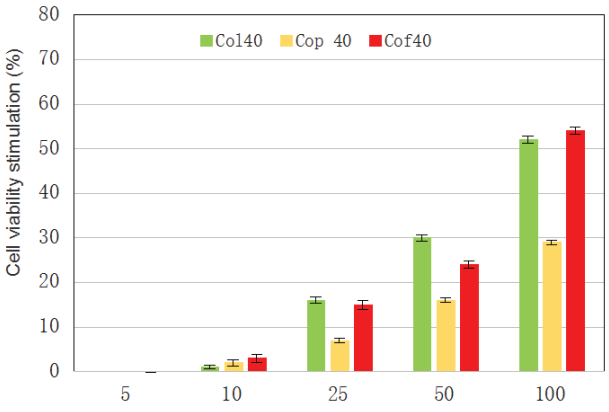


Figure 3: Cell viability stimulation (%) of L-929 fibroblasts exposed to different concentrations of the three test vegetal extracts (Col40, Cop40 and Cof40) compared to control negative cells; n=3, ±SD(%)

In this context, the quince leaf ethanolic extract (Col40) indicated the best ability to inhibit cancer cell viability. The best results were obtained on hepatic cancer cell line HepG2 (up to 75% of inhibition compared to the positive control sample, SD<1.70%) followed by colon cancer cell line Caco-2 (up to 69% inhibition, SD<1.00%) and breast cancer cell line BT-20 (up to 54% inhibition, SD<2.37%). Quince flower petals ethanolic extract (Cof40) indicated high activity on colon cancer cell line Caco-2 (up to 77% inhibition, SD<1.44%), followed by breast BT-20 (up to 61%, SD<1.39%) and hepatic HepG2 (up to 53% inhibition, SD<1.11%) cancer cell lines respectively. The fruit pellet ethanolic extract (Cop40) indicated the best results for the hepatic cancer cell line HepG2 (up to 70% inhibition, SD<1.67%), followed by colon Caco-2 (up to 40% inhibition, SD<1.13%) and breast BT-20 (up to 19% inhibition, SD<1.35%) cancer cell lines respectively.

Table 1 shows the IC_{50} values of the studied vegetal extracts on the three human cancer cell lines, BT-20, HepG2 and Caco-2 respectively.

The results clearly confirmed the high antiproliferative potency of quince leaf ethanolic extracts (Col40), mainly on human hepatic cancer cell line HepG2 (IC_{50}=26 µg GAE / mL test sample), but also on human colon cancer cell line Caco-2 (IC_{50}=62 µg GAE / mL test sample); flower petals ethanolic extract also indicated good results on human colon cancer cell line Caco-2 (IC_{50}=65 µg GAE / mL test sample) and potential benefits as natural anti-tumor agent.

Table 1: The antiproliferative activity expressed as IC_{50} ± SD (µg GAE / mL test sample); n=3, ±SD(%).

Test sample	IC_{50} (µg GAE / mL test sample)			
	BT-20 cell line (ATCC® HTB19™) breast carcinoma	HepG2 cell line (ATCC®HB-8065™) hepatic cancer	Caco2 cell line (ATCC®HTB-37™) colon cancer	L-929 cell line (ATCC®CCL-1™) fibroblasts
Cof40	92 ± 10.11	96 ± 11.57	**65** ± 05.44	93 ± 06.43
Col40	90 ± 09.38	**26** ± 02.41	**62** ± 05.15	93 ± 11.33
Cop40	257 ± 28.23	64 ± 05.23	121 ± 12.76	166 ± 15.87

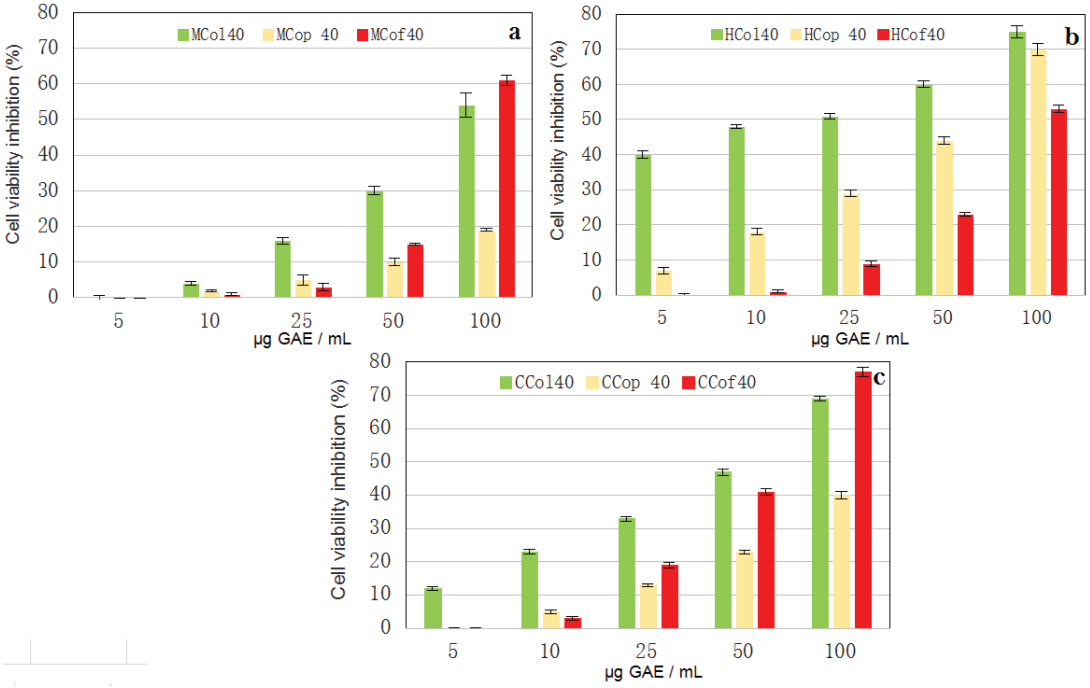


Figure 4: Antiproliferative potency of *Cydonia oblonga* ethanolic extracts (Col40, Cop40, Cof40) on **a)** human breast cancer cell line (BT-20), **b)** human hepatic cancer cell line (HepG2) and **c)** human colon cancer cell line (Caco-2); n=3, ±SD(%)

3.3 Docking results

The starting point of the docking analysis was the *in vitro* pharmacological results, antiproliferative potency of the quince leaf extracts (Col40) respectively. It was also noted that the quince leaf ethanolic extracts indicated several very active flavonoid compounds (namely quercetin-3-O-galactoside/hyperoside, quercetin-3-O-glucoside/isoquercitrin/isoquercetin and kaempferol-3-O-glucoside/astragalin). These were either absent or found in small quantities in the quince fruit pellet or quince flower petal extracts. In accordance, five active flavonoid compounds with high biodisponibility [38, 39] in humans (namely hyperoside, isoquercitrin and astragalin plus their aglycones, quercetin and kaempferol), available in *Cydonia oblonga* derived products, mainly in leaf extracts, were assumed to have anti-tumor properties and were subjected to the docking analysis (CLC Drug Discovery Workbench Software).

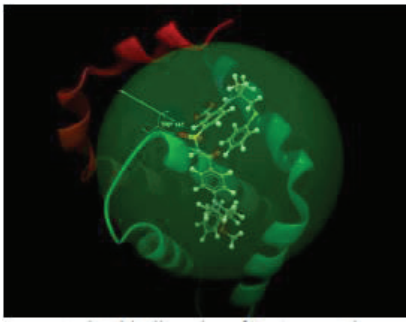
The analysis predicted the binding modes, the binding affinities and the orientation of the selected ligands on the anti-apoptotic protein Bcl-2 [40].

The results were compared with that of the co-crystallized LI0, a –sulfonamide based ligand 4-(4-Benzyl-4-Methoxypiperidin-1-Yl)-N-[(4-{[1,1-Dimethyl-2-(Phenylthio)ethyl]amino}-3-Nitrophenyl)sulfonyl] benzamide [30], which was shown to have inhibitory activity on the anti-apoptotic proteins Bcl-2 and Bcl-xL [41]; the Bcl-2 family of regulator proteins was involved in both, cell death and cell growth and replication and, due

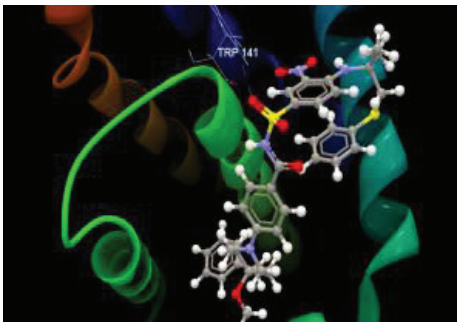
to the pro/anti-apoptotic effects, Bcl-2 protein also acted as a tumor promoter and allows cancer progressing [42].

Therefore, the docking simulations were conducted to analyze and measure the interactions of *the selected vegetal ligands* with the surrounding amino acid interaction group of BCL-2 (PDB ID:2O2F) protein chain A, to compare the docking score and the root mean square deviation (RMSD) parameters which could suggest the ligands contribution and potency as natural anti-tumor agents; the docking scores were given by the contributions of the hydrogen bond score, metal score interaction, steric interaction score and ligand conformation penalty contributions.

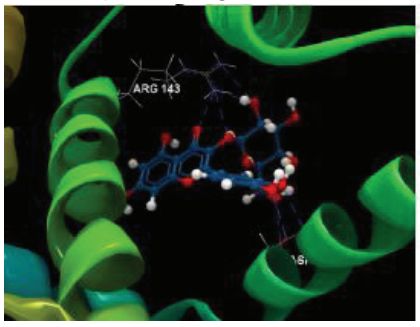
Figure 5 illustrates: the active binding site of 2O2F Bcl-2 protein (a); interaction between the co-crystallized LI0 and TRP141 residue (b); hydrogen bonds between hyperoside and ASP108, ARG143:A residues (c); interacting group of hyperoside and hydrogen bonds with amino acids residues from chain A protein (d); hydrogen bonds between isoquercitrin and ASP108 and ARG143:A residues (e); interacting group of isoquercitrin and hydrogen bonds with amino acids residues from chain



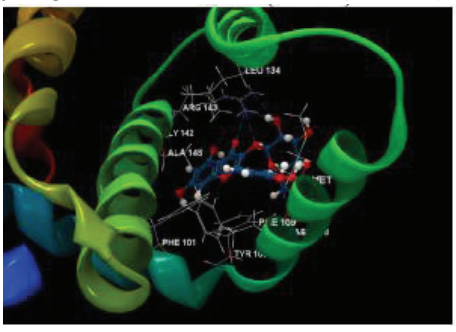
a. Active binding site of 2O2F protein fragment



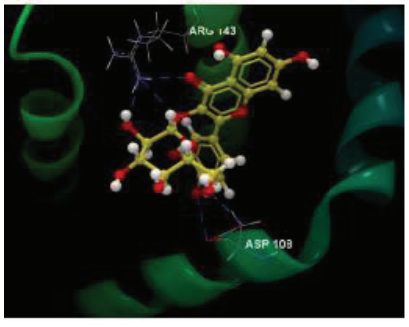
b. Co-crystallized LI0 A 1000 and hydrogen bond with TRP141: A (3.181 Å)



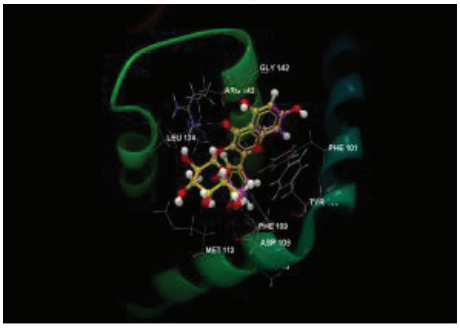
c. Hydrogen bonds between Hyperoside and ASP108, ARG143: A residues



d. Interacting group of Hyperoside and Hydrogen bonds with aminoacids residues from chain A protein

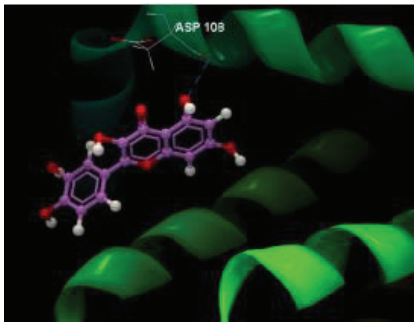


e. Hydrogen bonds between Isoquercitrin and ASP108 and ARG143: A residues

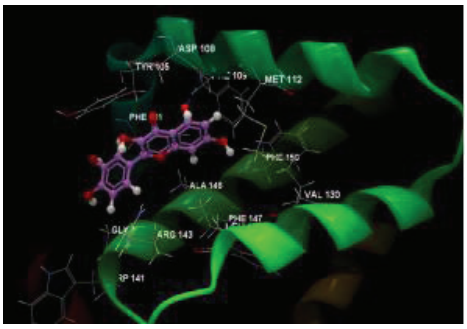


f. Interacting group of Isoquercitrin and Hydrogen bonds with aminoacids residues from chain A protein

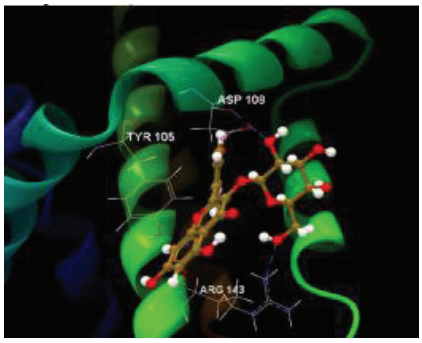
Figure 5: Docking simulations results for the five vegetal flavonoids ligands (hyperoside, isoquercitrin, quercetin, astragalin and kaempferol) and the synthetic compound (L10 A 1000) on the amino acid groups of BCL-2 (PDB ID:2O2F) protein chain A.



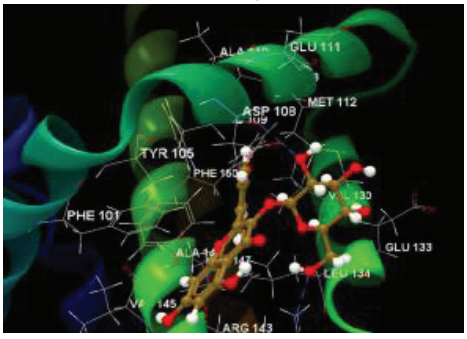
g. Hydrogen bonds between aglycone, Quercetin, and ASP108: A residue



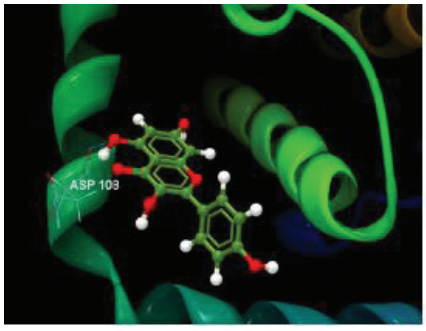
h. Interacting group of Quercetin and Hydrogen bond with aminoacids residues from chain A protein



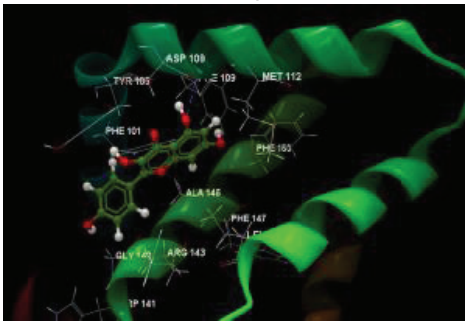
i. Hydrogen bonds between Astragalin and ARG143, TYR105 and ASP108: A



j. Interacting group of Astragalin and Hydrogen bonds with aminoacids residues from chain A protein



k. Hydrogen bonds between aglycone, Kaempferol, and ASP108: A residues



l. Interacting group of Kaempferol and Hydrogen bond with residues from chain A protein

Continued **Figure 5:** Docking simulations results for the five vegetal flavonoids ligands (hyperoside, isoquercitrin, quercetin, astragalin and kaempferol) and the synthetic compound (L10 A 1000) on the amino acid groups of BCL-2 (PDB ID:2O2F) protein chain A.

A protein (f); hydrogen bonds between quercetin and ASP108:A residue (g); interacting group of quercetin and hydrogen bonds with amino acids residues from chain A protein (h); hydrogen bonds between astragalin and ARG143, TYR105 and ASP108:A residues (i); interacting group of astragalin and hydrogen bonds with amino acids residues from chain A protein (j); hydrogen bonds between kaempferol and ASP108:A residues (k) and interacting group of kaempferol and hydrogen bonds with amino acids residues from chain A protein (l).

The docking score, RMSD, interaction group amino-acids and established hydrogen bonds type and the size of the bond length (Å) results from docking simulations on the six studied ligands (five natural flavonoids and one synthetic compound) and A protein chain of apoptosis regulator BCL-2 (PDB ID:2O2F) were also computed (Table 2).

Table 2: The list of intermolecular interactions between the ligands docked with 2O2F using *CLC Drug Discovery Workbench* Software.

Compound	Score/ RMSD	Interacting group: A	Hydrogen bond	Bond Length (Å)
Co-crystallized (LI0 A 1000)	-82.97 / 2.02	GLU133, MET112, ASP108, PHE109, TYR105, ARG104, PHE101, ASP100, GLN96, TYR199, PHE195, ALA97, GLY98, TRP141, VAL145, GLU149, GLY142, PHE150, ALA146, ARG143, ASN140, VAL131, LEU134	O sp^2 – N sp^2 TRP141	3.281
Hyperoside	-46.94 / 0.48	PHE101, TYR105, PHE109, ASP108, MET112, ALA146, GLY142, ARG143, LEU134	O sp^2 – N sp^2 ARG143 O sp^3 – N sp^2 ARG143 O sp^3 – N sp^2 ARG143 O sp^3 – N sp^2 ARG143 O sp^3 – O sp^2 ASP108 O sp^3 – O sp^2 ASP108 O sp^3 – O sp^2 ASP108	3.231 2.931 2.753 3.169 2.713 2.888 2.970
Isoquercitrin	-46.75 / 0.54	MET112, ASP108, PHE109, ALA110, TYR105, PHE101, GLY142, ARG143, LEU134, ALA146	O sp^3 – N sp^2 ARG143 O sp^3 – N sp^2 ARG143 O sp^2 – N sp^2 ARG143 O sp^3 – O sp^2 ASP108 O sp^3 – O sp^2 ASP108	2.769 3.084 2.783 3.075 2.979
Quercetin	-44.20 / 0.02	TRP141, GLY142, ARG143, ALA146, PHE147, LEU134, VAL130, PHE150, MET112, PHE109, ASP108, TYR105, PHE101	O sp^3 – O sp^2 ASP108	2.917
Astragalin	-55.37 / 0.06	GLY142, ARG143, LEU134, GLU133, VAL130, ALA146, VAL145, PHE147, MET112, ASP108, GLU111, ALA110, PHE109, TYR105, PHE150, PHE101	O sp^3 – O sp^3 TYR105 O sp^3 – N sp^2 ARG143 O sp^3 – O sp^2 ASP108 O sp^3 – O sp^2 ASP108	3.025 2.654 2.926 2.986
Kaempferol	-44.96 / 0.02	TRP141, GLY142, ARG143, PHE147, LEU134, ALA146, PHE150, MET112, ASP108, PHE109, PHE101, TYR105	O sp^2– O sp^2 ASP108	2.893

Therefore, compared to the synthetic co-crystallized LI0 A 1000 ligand (Docking score = -82.97/ RMSD = 2.02), the best docking score of the investigated five natural vegetal ligands (flavonoids) was obtained for astragalin (-55.37/0.06), which established four hydrogen bonds with ASP108, TYR105 and ARG143 amino acids residues.

As expected, due to the identical aglycone in their structure, hyperoside and isoquercitrin formed hydrogen bonds with the same amino acid residues (ASP108 and ARG143), they were surrounded by the same interacting amino acids group, resulting in a similar docking score (-46.94 / 0.48 *vs*. -46.75 / 0.54).

Quercetin and kaempferol, both established a single hydrogen bond with ASP108 (-44.20 / 0.02 *vs*. -44.96 / 0.02); this fact could explain a similar, lower docking score respectively.

Table 3 shows the comparative results regarding several molecular properties such as the number of atoms, molecular weight, number of flexible bonds, number of hydrogen donors (the total number of nitrogen-hydrogen and oxygen-hydrogen bonds) and number of hydrogen acceptors (the total number of nitrogen and oxygen atoms), as well as corresponding *Lipinski* violation number and *LopP* values of the studied ligands (hyperoside, isoquercitrin, quercetin, astragalin and kaempferol face to the synthetic compound L10), computed with *CLC Drug Discovery Workbench* Software. One must be reminded of the *Lipinski's* rule of five meanings: i). maximum five hydrogen bond donors (HBD), as total number of nitrogen-hydrogen and oxygen-hydrogen bonds; ii). maximum 10 hydrogen bond acceptors (HBA), as the total number of nitrogen and oxygen atoms; iii). maximum molecular (M) weight of 500 Da; iv). the octanol-water partition coefficient (log P) value less than 5. All value limitations of parameters that should be observed according the rule, **are multiples of five** (HBD < 5 × 1; HBA < 5 × 2; M < 5 × 10; log P < 5 × 1).

According to the data presented in Table 3, three of the studied flavonoid compounds (hyperoside, isoquercitrin and astragalin) failed to respect two parameters (number of hydrogen bond donors greater than 5 and number of hydrogen bond acceptors greater than 10) of the *Lipinsky*

Table 3: Ligands properties, computed with CLC Drug Discovery Workbench Software.

Compound	Atoms	Weight [Daltons]	Flexible bonds	*Lipinski* violations	Hydrogen donors	Hydrogen acceptors	LogP
Co-crystallized (LI0)	88	688.86	13	2	2	10	7.46
Hyperoside	53	464.38	4	2	8	12	0.66
Isoquercitrin	53	464.38	4	2	8	12	1.22
Quercetin	32	302.24	-	0	5	7	3.02
Astragalin	52	448.38	4	2	7	11	1.57
Kaempferol	31	286.24	-	0	4	6	3.38

rule (*Lipinski* violation are 2); a lower number of *Lipinski* violations indicate a most feasible drug candidate.

Therefore, only two ligands, quercetin and kaempferol aglycons respectively, presented a zero violation of all the parameters involved in *Lipinski*'s rule of five, these being the most feasible antitumor candidates in the author's study.

4 Conclusions

Studies in recent years pointed out that parts of the quince plant such as fruits and fruit peels, seeds, and leaves represented a valuable source of functional foods and herbal medicines mainly due to polyphenols content. The present study revealed that quince fruit pellets, quince leaves and quince flower petals 40% (v/v) ethanolic extracts were very different in their polyphenol profile, qualitative and quantitative aspects respectively; it was revealed that if the quince leaf ethanolic extracts (Col40) abound in quercetin and kaempferol glycosides, quince flower petals ethanolic extracts (Cof40) mainly contained caffeoylquinic acid derivates, while the quince fruit pellet ethanolic extracts (Cop40) were abound in (neo)chlorogenic and isochlorogenic caffeoylquinic acid derivates, and p-coumaric acid.

The differences between chemical qualitative content, in the context of an identical chemical quantitative content (5mg GAE/1 mL quince extract) resulted in differences related to the pharmacological properties. Proving this, the MTS test made on BT-20, HepG2 and Caco-2 human cancer cell lines revealed that the treatment with the three quince extracts significantly reduced cell proliferation, especially of HepG2 and Caco-2 cells, and in a dose-dependent manner, quince leaf ethanolic extracts (Col40) being the most active vegetal product; also, the MTS test made on L-929 normal cells proved a low toxicity of the three tested quince extracts (Col40, Cop40 and Cof40). A possible explanation may be a difference in human breast and human colon cancer cells, human hepatic cells having a special enzymatic equipment to use and transform flavonoids derivates, the most feasible antitumor compounds as the literature data have proven [43, 44]. For example, it is well known that the capacity of flavonoids compounds to modulate the activity of cytocrome P450 isoenzymes, which in turn controls the activation/ detoxication balance in hepatic cells, and hence plays a key role in the prevention and progression of hepatic cancer [45]; similarly, the inhibition of fatty acid synthase activity has been statistically related to the inhibition of breast cancer cells growth [46]. In this way, the differences in antiproliferative efficacy of vegetal extracts may be explained by the capacity of specific flavonoids compounds to modulate both the key metabolic pathways of the cells and different tumor targets, mediators of tumor initiation and progression processes respectively.

Furthermore, the docking simulations on five flavonoid compounds with high biodisponibility in humans [38, 39] (ie., quercetin-3-O-galactoside/hyperoside, quercetin-3-O-glucoside/isoquercitrin, kaempferol-3-O-glucoside/astragalin, and corresponding aglycones, quercetin and kaempferol) indicated good antiproliferative potency of the three flavonoid monoglycosides, mainly of the astragalin compound (see the docking score), on the anti-apoptotic protein Bcl-2. Studies regarding the most important molecular properties, including the *Lipinski* violation number and *LopP* values, indicated that only quercetin and kaempferol aglycones presented zero violation of all the parameters involved in Lipinski's rule, so that in a molecular docking approach of the five flavonoids studied, these two flavonoid aglycones were the most feasible antiproliferative drug candidates; the results could be compared with those found by Narumol P. et al, 2010 [47] who studied 28 active polyphenol compounds

on seven antitumor target molecules, including Bcl-2 protein fragment. Therefore, the docking results suggested specific quercetin and kaempferol derivates ability to inhibit the anti-apoptotic protein Bcl-2, a well known tumor promoter and cancer progressing mediator. Regarding the most probable tumor targets of the most active flavonoid compounds studied, some recent studies have revealed kaempferol aglycone capacity to induce authophagic cell death of hepatocellular carcinoma cells *via* activating AMP-activated protein kinase/AMPK signaling (studies were done on HepG2, Huh-7, BEL7402, and SMMC cell lines) [48], and quercetin aglycone capacity to prevent tumor initiation by preventing oxidative stress at the level of hepatic cells (studies were made on rats with hepatocellular carcinoma induced by N-Nitrosodiethylamine/NDEA, a strong tumor inducer and cancer promoter at the level of hepatic tissue) [49], confirming the high antitumor potency of these two flavonoid compounds.

In summary, pharmacological and docking studies, both suggest that quince derived products, mainly the leaf plant part, should be considered a feasible source of natural bioactive compounds against cancer.

Acknowledgments: This research work has been financed by the Romanian National Authority for Scientific Research ANCS, Competitiveness Operational Programme COP-A1-A1.2.3-G-2015, Project title "Innovative technologies for new, natural health products", ID P_40_406, SMIS 105542.

Conflict of interest: The authors declare no competing financial interest.

References

[1] Khoubnasabjafari M., Jouyban A., Review A review of phytochemistry and bioactivity of quince (*Cydonia oblonga* Mill.), Journal of Medicinal Plants Research, 2011, 5, 3577-3594.
[2] Al-Snafi A.E., The medical importance of *Cydonia oblonga* - A review, IOSR Journal of Pharmacy, 2016, 6, 87-99.
[3] Muhammad U.A., Gulzar M., Muhammad A.H., Syed N.A.B., *Cydonia oblonga* M., A Medicinal Plant Rich in Phytonutrients for Pharmaceuticals, Front Pharmacol., 2016, 7, 163.
[4] Fattouch S., Caboni P., Coroneo V., Tuberoso C.I., Angioni A., Dessi S., Marzouki N., Cabras P, Antimicrobial activity of Tunisian quince (*Cydonia oblonga* Miller) pulp and peel polyphenolic extracts, J. Agric. Food Chem., 2007, 55, 963-969
[5] Aslan M., Orhan N., Orhan D.D., Ergun F., Hypoglycemic activity and antioxidant potential of some medicinal plants traditionally used in Turkey for diabetes, J Ethnopharmacol., 2010, 128, 384-389.
[6] Haleh V., Potential of *Cydonia oblonga* leaves in cardiovascular disease, Hypothesis, 2014, 12, 356.
[7] Abliz A., Aji Q., Abdusalam E., Sun X., Abdurahman A., Zhou W., Moore N., Umar A., Effect of *Cydonia oblonga* Mill. leaf extract on serum lipids and liver function in a rat model of hyperlipidaemia, J. Ethnopharmacol, 2014, 151, 970-4.
[8] Attia D.M., Zhenmin N.N., Boer P., Attia M.A., Goldschmeding R., Koomans H.A., Vaziri N.D., Joles J.A., Proteinuria is preceded by decreased nitric oxide synthesis and prevented by a NO donor in cholesterol-fed rats, Kidney Int., 2002, 61, 1776-1787.
[9] Aslam M., Sial A.A., Effect of Hydroalcoholic Extract of *Cydonia oblonga* Miller (Quince) on Sexual Behaviour of Wistar Rats, Advances in Pharmacological Sciences Article. 2014, ID 282698, http://dx.doi.org/10.1155/2014/282698.
[10] Khademi F., Danesh B.,Nejad D.M., Rad J.S., The Comparative Effects of Atorvastatin and Quince Leaf Extract on Atherosclerosis, Iran Red. Crescent Med., 2013, 15, 639-643.
[11] Kawahara T., Iizuka T., Inhibitory effect of hot water extract of quince (*Cydonia oblonga*) on immunoglobulin E-dependent late-phase immune reactions of mast cells, Cytotechnol., 2011, 63, 143-152.
[12] Pieroni A., Quave C.L., Villanelli M.L., Mangino P., Sabbatini G., Santini L., Boccetti T., Profili M., Cicciol T., Rampa L.G., Antonini G., Girolamini C., Cecchi M., Tomasi M., Ethnopharmacognostic survey on the natural ingredients used in folk cosmetics, cosmeceuticals and remedies for healing skin diseases in the inland Marches, Central - Eastern Italy, J. Ethnopharmacol., 2004, 91, 331-344.
[13] Hemmati A.A., Kalantari H., Jalali A., Rezai S., Zadeh H.H., Healing effect of quince seed mucilage on T-2 toxin-induced dermal toxicity in rabbit, Exp. Toxicol. Pathol., 2012, 64, 181-186.
[14] Shinomiya F., Hamauzu Y., Kawahara T., Anti-allergic effect of a hot-water extract of quince (*Cydonia oblonga*), Biosci. Biotechnol. Biochem., 2009, 73, 1773-8.
[15] Minaiyan M., Ghannadi A., Etemad M., Mahzouni P., A study of the effects of *Cydonia oblonga* Miller (Quince) on TNBS induced ulcerative colitis in rats, Res. Pharm. Sci., 2012, 7, 103-110.
[16] Carvalho M., Silva B.M., Silva R., Valentão P., Andrade P.B., Bastos M.L., First report on *Cydonia oblonga* Miller anticancer potential: differential antiproliferative effect against human kidney and colon cancer cells, J. Agric. Food Chem., 2010, 58, 3366-3370.
[17] Alesiani D., Canini A., D'Abrosca B., DellaGreca M., Fiorentino A., Mastellone C., Monaco P., Pacifico S., Antioxidant and antiproliferative activities of phytochemicals from quince (*Cydonia vulgaris*) peels, Food Chem., 2010, 118, 199-207.
[18] Magalhaes A.S., Silva B.M., Pereira J.A., Andrade P.B., Valentao P., Carvalho M., Protective effect of quince (*Cydonia oblonga* Miller) fruit against oxidative hemolysis of human erythrocytes, Food Chem. Toxicol., 2009, 47, 1372-1377.
[19] Costa R.M., Magalhães A.S., Pereira J.A., Andrade P.B., Valentão P., Carvalho M., Silva B.M., Evaluation of free radical-scavenging and antihemolytic activities of quince (*Cydonia oblonga*) leaf: A comparative study with green tea (*Camellia sinensis*), Food Chem. Toxicol., 2009, 47, 860-865.
[20] Oliveira A.P., Pereira J.A., Andrade P.B., Valentão P., Seabra R.M., Silva B.M., Phenolic profile of *Cydonia oblonga* Miller leaves, J. Agric. Food Chem., 2007, 55, 7926-7930.

[21] Silva B.M., Andrade P.B., Ferreres F., Domingues A.L., Seabra R.M., Ferreira M.A., Phenolic profile of quince fruit (*Cydonia oblonga* Miller) (pulp and peel), J. Agric. Food Chem., 2002, 50, 4615-4618.
[22] Silva B.M., Andrade P.B., Ferreres F., Seabra R.M., Oliveira M.B., Ferreira M.A., Composition of quince (*Cydonia oblonga* Miller) seeds: phenolics, organic acids and free amino acids, Nat. Prod. Res., 2005, 19, 275-281.
[23] Hamauzu Y., Yasui H., Inno T., Kume C., Omanyuda M., Phenolic profile antioxidant property, and antiinfluenza viral activity of Chinese quince (*Pseudocydonia sinesis* Schneid.), quince (*Cydonia oblonga* Mill.), and apple (*Malus domestica* Mill.) fruits, J. Agric. Food Chem., 2005, 53, 928-934.
[24] Bengt L., An unusual (4-O-methyl-D-glucurono)-D-xylan isolated from the mucilage of seeds of the quince tree (*Cydonia oblonga*), Carbohydrate Research., 1990, 207, 307-310.
[25] Reikh E., Schibli A., HPTLC for the Analysis of Medicinal Plants, Thieme, N.Y. Stuttgart, 2008.
[26] Wagner H., Bladt S., Plant Drug Analysis, Second Ed., Springer, Berlin, 1996.
[27] Pirvu L., Panteli M., Bubueanu C., Coprean D., *Centaurea cyanus* L Polysaccharides and Polyphenols Cooperation in Achieving Strong Rat Gastric Ulcer Protection, Central European Journal of Chemistry, 2015, 13, 910-921.
[28] *Romanian Pharmacopoeia*, Xth ed., Ed. Medicala, Bucharest, 1993.
[29] https://www.promega.ro.pdf
[30] Bruncko M., Oost T.K., Belli B.A., Ding H., Joseph M.K., Kunzer A., Martineau D., McClellan W.J., Mitten M., Ng S.C., Nimmer P.M., Oltersdorf T., Park C.M., Petros A.M., Shoemaker A.R., Song X., Wang X., Wendt M.D., Zhang H., Fesik S.W., Rosenberg S.H., Elmore S.W., Solution structure of the anti-apoptotic protein Bcl-2 in complex with an acyl-sulfonamide-based ligand, Deposited on: 2006-11-29.
[31] Lipinski C.A., Lombardo F., Dominy BW., Feeney P.J., Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings, Advanced Drug Delivery Reviews, 2001, 46, 3-26.
[32] Kandaswami C., Lee L.T., Lee P.P., Hwang J.J., Ke F.C., Huang Y.T., Lee M.T., The antitumor activities of flavonoids, In Vivo, 2005, 19, 895-909.
[33] Shikha S., Ranganatha R.S., Mahesh H., Mayilaadumveettil N., Satish K.T., Mrinal S., Bibha C., Sathees C.R., Quercetin, a Natural Flavonoid Interacts with DNA, Arrests Cell Cycle and Causes Tumor Regression by Activating Mitochondrial Pathway of Apoptosis, Sci. Rep., 2016, 6, 24049.
[34] Allen Y.C., Yi C.C.N., A review of the dietary flavonoid, kaempferol on human health and cancer chemoprevention, Food Chem., 2013, 138, 2099-2107.
[35] Magne C.B., Zingue S., Winter E., Creczynski-Pasa T.B., Michel T., Fernandez X., Njamen D., Clyne C., Flavonoids, Breast Cancer Chemopreventive and/or Chemotherapeutic Agents, Curr. Med. Chem., 2015, 22, 3434-3446.
[36] Xiang-An Z., Shuangxi Z., Qing Y., Jing Z., Quercetin induces human colon cancer cells apoptosis by inhibiting the nuclear factor-kappa B Pathway, Pharmacogn Mag., 2015, 11, 404–409.
[37] Han J.C., Jung H.Y.P., Kaempferol Induces Cell Cycle Arrest in HT-29 Human Colon Cancer Cells, J. Cancer Prev., 2013, 18, 257–263.
[38] Manach C., Scalbert A., Moarand C., Remesy C., Jimenez L., Polyphenols: food sources and bioavailability, American Society for Clinical Nutrition, 2004, 79, 727-747.
[39] Chen L., Teng H., Xie Z., Cao H., Cheang W.S., Skalicka-Woniak K., Georgiev MI., Xiao J., Modifications of dietary flavonoids towards improved bioactivity: An update on structure-activity relationship, Crit. Rev. Food Sci. Nutr., 2018, 58, 513-527.
[40] Bhat V., Olenick M.B., Schuchardt B.J., Mikles D.C., McDonalds C.B., Farooq A., Biophysical Basis of the Promiscuous Binding of Bcl2 Apoptotic Repressor to BH3 Ligands, J. Mol. Recognit., 2013, 26, 501-513.
[41] Bruncko M., Oost T.K., Belli B.A., Ding H., Joseph M.K., Kunzer A., Martineau D., McClellan W.J., Mitten M., Ng S.C., Nimmer P.M., Oltersdorf T., Paek C.M., Petros A.M., Shoemaker A.R., Song X., Wang X., Wendt M.D., Zhang H., Fesik S.W., Rosenberg S.H., Elmore S.W., Studies leading to potent, dual inhibitors of Bcl-2 and Bcl-xL, J. Med. Chem., 2007, 50, 641-662.
[42] Akl H., Vervloessem T., Kiviluoto S., Bittremieux M., Parys J.B., De Smedt H., Bultynck G., A dual role for the anti-apoptotic Bcl-2 protein in cancer: mitochondria versus endoplasmic reticulum, Biochim Biophys Acta, 2014, 1842, 2240-2252.
[43] Panche A.N., Diwan A.D., Chandra S.R., Flavonoids: an overview, J. Nutr. Sci., 2016, 5, e47.
[44] Ren W., Qiao Z., Wang H., Zhu L., Zhang L., Flavonoids: promising anticancer agents. Med. Res. Rev., 2003, 23, 519-34.
[45] Androutsopoulos V.P., Tsatsakisand A.M., Spandidos D.A., Cytochrome P450 CYP1A1: wider roles in cancer progression and prevention, BMC Cancer, 2009, 9, 187.
[46] Brusselmans K., Vrolix R., Swinnen JV., Induction of cancer cell apoptosis by flavonoids is associated with their ability to inhibit fatty acid synthase activity. J. Biol. Chem., 2005, 280, 5636-45.
[47] Narumol P., Jiraporn U., Molecular docking study on anticancer activity of plant derived natural products, Med. Chem. Res., 2010, 19, 817-835.
[48] Bing H., Yi-Qun Y., Qi-Lian Y., Chun-Ying S., Xiao-Juan W., Kaempferol induces autophagic cell death of hepatocellular carcinoma cells via activating AMPK signaling, Oncotarget., 2017, 8, 86227–86239.
[49] AlaaEddeen M.S., Safinz S.I., Tarek K.E., Elsayed E.H., Preventive effect of the flavonoid, quercetin, on hepatic cancer in rats via oxidant/antioxidant activity: molecular and histological evidences, J. Exp. Clin. Cancer Res., 2009, 28, 80.