Theses of Doctoral (PhD) dissertation

CHEMOMAPPING OF ARTICHOKE (*CYNARA SCOLYMUS* L.) AND FENUGREEK (*TRIGONELLA FOENUM-GRAECUM* L.) EXTRACTS, AND ANALYSIS OF GENERATED BIOLOGICAL EFFECTS USING *IN VITRO* AND *IN VIVO* MODELL SYSTEMS

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1. INTRODUCTION AND OBJECTIVES OF THE DISSERTATION

In the 21st century, despite the huge progress of pharma industry, it becomes an evergrowing trend to turn our attention towards herbal plants and remedies to ease the preasure on the general health condition of humans across the planet. Such medicinal plants and their bioactive constituents were of great inspiration for drug formulations, and recently they are widely used for nutraceuticals manufacturing, yet many times their pharmacological effects and mechanism of actions are not directly proved neither on individual nor on cellular levels. Many herbal remedies not being toxic are licensed for human consumption, but their dose-dependent effect remain largely unknown not talking about the individual consumers' genomes and health statuses determined responses. It has also demonstrated that the bioactive compounds administration in order to treat chronic diseases or their preventive utilization can induce health threatening effects too. Taken together all these considerations, it seems likely that the bioactive compounds preventive and/or therapeutic applications must pay attention to every single detail, and in the postgenomic era, a system biology type of approach would be the right choice to follow to fulfill the mentioned requirements.

Several epidemiological studies are indicating that the advanced consumer lifestyle contributes substantially to death cases seen for patients with chronic diseases. Hungarian people general health condition does not excels as compared to other European contries, while on a world wide scale it is midway positioned (Tompa, 2011; WHO). In Hungarian terms, among middle and elderly individuals, the heart and coronary deseases are represented with a staggering 54%, cancer with 27%, gastro-intestinal diseases with 8% and respiratory system specific diseases share is 4% with respect to the death causes. It has been demonstrated that the heart and coronary diseases therapy and prevention efectiveness increased substantially during the last decades. The efficiency of cancer therapy is growing, though its prevention keep on failing despite the evergrowing efforts to carry out screens on national levels. In Hungary since 2013 the cancer deaths rate looks ruther constant, and the lung with colon cancers are the leading cancer types among both men and women. Breast and prostate cancers are on the third position, and unfortunately their prevention is far more unefficient as compared to lung and colon cancers.

The causes of cancer are ruther complicated, and besides genetic susceptibility, the environmental factors like nutrition, reduced physical activity and stress are also playing influential roles. On the other hand a growing body of evidencies are suggesting that proper environmental interventions can increase the prevention and/or treatments success.

Actually we are not left with other options because the gene therapy is still struggling with identifying the proper intervention method in order to substitute the genetic susceptibility responsibele mutant alleles with their normal counterparts.

Lately the application of bioactive compounds of plant origin has become a major imperative with respect to heart and coronary diseases and cancer treatment and/or prevention (Dietz *et al*., 2016). Identification and characterization of such bioactive compounds attracted much attention from the scientific community (American Cancer Society, 2016). To Youyou's 2015 Nobel Prize for medicine is a great example of one such a plant derived compound. The artemisinin isolated from *Artemisia annua* could be used to efficeniently treat malaria.

My research work is layed on the above mentioned scientific fondations. The artichoke (*Cynara scolymus* L.) and fenugreek (*Trigonella foenum-graecum* L.) based plant extracts production and chemical characterization with UHPLC-ESI-MS was follwed by the in vitro and in vivo analysis of their cytotoxic and genotoxic effects. The aim of my research was to identify and characterize novel plant derived extracts that show anti-tumor effects.

During my work I have been looking for answers in relation with the following questions:

1. What is the bioactive compound content of aqueous and hydro-alcoholic artichoke and fenugreek extracts?
2. What kind of similarities and differencies exist between the bioactive compound profiele of artichoke and fenugreek extracts?
3. What biological effects could be expected from the extracts based on their bioactive compound content?
4. What is the total polyphenol, flavonoid and antocyanin content of extracts, and what *in vitro* antioxidant activity do they feature?
5. How are going to affect the aqueous and hydro-alcoholic artichoke and fenugreek extracts the viability of T-47D and ZR-75-1 human breast cancerous cell lines?
6. Are there going to affect the artichoke and fenugreek extracts the viability *Drosophila melanogaster* (fruitfly), and can it be proved their cytotoxic and/or genotoxic effect?
7. Could it be predicted the human application of artichoke and fenugreek extracts based on their bioactive compound profile and the associated *in vitro* and *in vivo* effects?

2. MATERIALS AND METHODS

2.1. Preparation of plant extracts

The artichoke dried leaves were produced by the TTDR 2000 Ltd., Hungary. 10 g of sample was extracted for 5 min with 200 ml of boiling distilled water. It was allowed to cool to room temperature (RT), filtered and stored in refrigerator. Another 50 g of dried artichoke leaves were extracted twice with 500 ml aqueous 50% ethanol solution by stirring for 4 hrs on a magnetic stirrer. This extract was filtered, evaporated under vacuum and stored in refrigerator

The fenugreek seeds were produced by TRIGONELLA MED. LTD., Mosonmagyaróvár, Hungary. Fenugreek seeds were dried at 40 °C and powdered. 15 g of the powder was extracted with 300 ml of boiling distilled water for 5 min. The mixture was allowed to cool at RT, filtered and stored in refrigerator. Other 15 g of powdered fenugreek seeds were refluxed on a magnetic stirrer with 300 ml aqueous 80% ethanol for 3 hrs. The mixture was filtered and the filtrate was concentrated by rotary evaporator and stored in refrigerator.

2.2. UHPLC-ESI-MS analysis

The UHPLC system (Dionex Ultimate 3000RS equipped with a Thermo Accucore C18 column, 100/2.1 with a particle size of 2.6 μm) was coupled to a Thermo Q Exactive Orbitrap mass spectrometer equipped with an electrospray ionization source (ESI). Eluent A (500 ml of water containing 10 ml of acetonitrile, 0.5 ml of formic acid and 2.5 mM of ammonium formate) and eluent B (500 ml of acetonitrile containing 10 ml of water, 0.5 ml of formic acid and 2.5 mM of ammonium formate) were used in the HPLC separation in positive ionization mode, and eluent A (500 ml of water containing 10 ml of acetonitrile and 2.5 mM of ammonium acetate) and eluent B (500 ml of acetonitrile containing 10 ml of water and 2.5 mM of ammonium acetate) were used in the HPLC separation in negative ionization mode. Flow rate was 200 μl/min. The following gradient elution program was used both positive and negative ionization mode: 0-1 min, 95% A, 1-22 min, 20% A; 22-24 min, 20% A; 24-26 min, 95% A; 26-40 min, 95% A. 5 μl of samples were injected in every run. The Q Exactive hybrid quadrupole-orbitrap mass spectrometer was operated with the following parameters: capillary temperature 320 °C, spray voltage 4.0 kV in positive mode and 3.8 kV in negative mode, the resolution was set to 35000 in the case of MS and to 17500 in the case of MS/MS. The mass range scanned was 100-1000 m/z. Collision energy was 40NCE in the MS/MS scans.

2.3. Spectrophotometric methods on the plant extracts

2.3.1. Determination of total phenolic content

Total phenolic content was determined using the method developed by Singleton and Rossi (1965). Total phenolic content was estimated by the Folin-Ciocalteu colorimetric method, using gallic acid as a standard phenolic compound. 200 µl of extracts were added to 3000 µl of distilled water, 500 µl of Folin-Ciocalteu reagent and 2000 µl of sodium carbonate solution (15 g/100ml), and the mixture was allowed to stand for 20 min at RT. 4300 µl of distilled water was added and the absorbance was measured at 765 nm after 1 hour incubation period with the spectrophotometer (Perkin Elmer Lambda 35). The total flavonoid contents were calculated from a calibration curve with 50, 100, 300, 500, 750 µg/ml points and the results were expressed as mg gallic acid equivalent per g dry weight. Each extract was measured in triplicate.

2.3.2. Quantification of total flavonoids

The total flavonoid content of the extract was determined by the aluminium chloride colorimetric method following the procedure described by Dae-Ok Kim *et al*. (2003). We have made a reagent solution containing 5 ml (10g/ml) of aluminium chloride, 5 ml of KOAc (potassium acetate, 1M), 75 ml of methanol and 140 ml of distilled water (AS). 0.5 ml of the extract was mixed with 4.5 ml of AS, filtered (0.45 µm) and the absorbance was determined at 415 nm in the spectrophotometer (Perkin Elmer Lambda 35). The concentrations were calculated based on the equation obtained from the standard rutin curve (10, 40, 70, 100 µg/ml). Results were expressed as mg rutin equivalents. Each extract was measured in triplicate.

2.3.3. Determination of total anthocyanins

The total anthocyanin (TA) content was determined using an earlier described method (Lee *et al*., 2005). It detects the total monomeric anthocyanin concentration by the pH differential method. The method is a rapid and simple spectrophotometric application based on the anthocyanin structural transformation that occurs upon a change in pH (colored at pH1 and colorless at pH4). The standard solution was prepared by weighing 82.2 mg of cyanidin-3-glucoside chloride, dissolving in distilled water and diluting to a final volume of 1 L in a volumetric flask. 0.3 ml of extract was added to 2 ml of buffer (pH=1 or pH=4) and 1.7 ml of distilled water. Samples were filtered (0.45 µm), and absorbance recorded using a Perkin Elmer Lambda 35 spectrophotometer at wavelengths of 520 and 700 nm, for solutions at pH 1.0 and pH 4.5, respectively. Results

were expressed as cyanidin-3-glucoside (% w/w) equivalents. Total anthocyanin contents were calculated as follows:

$$TA_{(mg/L)} = \left(\frac{A * MW * DF * 10^3}{\varepsilon} \right) \div l$$

where: A = $(A_{520nm} - A_{700nm})_{pH1} - (A_{520nm} - A_{700nm})_{pH4}$; MW (molecular weight) = 449.2 g/mol for cyanidin-3-glucoside; DF = dilution factor; l = path length in cm; ε = 26 900 molar extinction coefficient, in L* mol^{-1} * cm^{-1} , for cyd-3-glu; and 10^3 = factor for conversion from g to mg. Each extract was measured in triplicate.

2.3.4. Determination of antioxidant capacity by the DPPH method

The DPPH (2,2- Diphenyl-1-picrylhydrazyl, Sigma-Aldrich) method was used to determine the antioxidant capacity of artichoke and fenugreek extracts. The method is based on the scavenging of DPPH radical through the action of an antioxidant that decolourizes the DPPH solution. The procedure was described by Molyneux (2004) [5]. 200 µl of extract were added to 300 µl of methanol and 2400 µl DPPH solution (22.6 µg/ml). The mixture was left to stand at room temperature for 30 minutes in the dark and filtered (0.45 µm) before the absorbance was measured at 517 nm. (UV/VIS Spectrometer, Perkin Elmer Lambda 35). All the measurements were performed in triplicate. The results were expressed as ascorbic acid equivalents by means of the dose-response calibration curve. The sample concentration providing 50 % inhibition (IC50) was calculated from the graph of inhibition percentage against sample concentration. Each extract was measured in triplicate.

2.4. *Drosophila melanogaster* based tests

2.4.1. Methods to study viability of *Drosophila melanogaster*

To collect fertilized eggs, 50 females and males were placed in egg collection cages that were put on egg collecting media containing agar, active carbon and yeast paste. Flies were kept at 25°C and left overnight to lay eggs that were collected every morning and batches of 50 embryos were put on normal media containing vials. Such vials were placed in 25°C incubator and supplemented at different time point with different amount of crude and alcoholic artichoke or fenugreek extracts. Later the hatched adults were scored, and the viability expressed as the % of treated embryos/hatched adults.

2.4.2.SMART (Somatic Mutation and Recombination Test) method

Drosophila melanogaster is a suitable organism to study the mutagenic effect of physical, chemical and biological mutagens. Several mutagenic tests were developed to study such events in both somatic and germ line cells. One such a method is called SMART (somatic mutation and recombination test), and is suitable to detect both chromosomal breaks and gene type of mutations induced during larval wing disc development by assessing the adults wings (*Figure 1*).

Initially, at the beginning of larval development the wing disc is composed of 10-12 cells

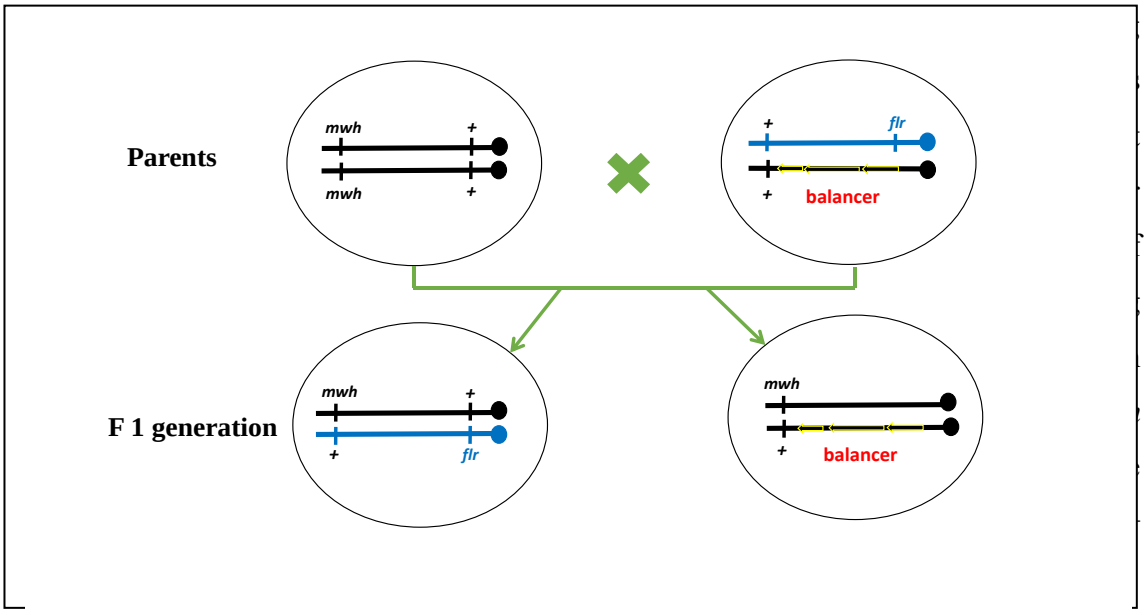

Figure 1. SMART system. Chromosomes depicted in black and blue are representing the 3rd homologous pair, yellow regions along the black balancer chromosome are indicating multiple inversions. The (+) signs correspond to the wild-type allel of *mwh* or *flr* gene, respectively.

Taking in consideration that all mutations arise from a single cell, Szabad et al. (1983) suggested that the frequency (f) of chromosomal (mitotic recombination) mutations can be estimated as f=((n x m))/((N x C)) , where m is the mean size of the clone (cell number), n is the number of clones, N is the number of scored wings, and C is the number of cells in the adult wing (30,000 wing blade cells). In order to determine the frequency (f) of *mwh* gene specific mutations, the above mentioned formula has to be modified as $= \frac{(\boldsymbol{2n\ x\ m})}{(\boldsymbol{N\ x\ C})}$, where **m** is the mean size of the clone (cell number), **2n** is the number of *mwh/mwh* clones, **N** is the number of scored wings, and **C** is the number of cells in the adult wing (30,000 wing blade cells).

Virgin females of *mwh/mwh* were mated to *flr*^{3}*/In (3LR) TM2, Ubx* males. Eggs from such cross were collected at 25 °C and 60%–80% humidity, in the dark, for 4 h in egg cages

placed on egg collecting media supplemented with baker's yeast. Embryos were removed from the egg collecting media and placed on normal media containing vials. Three days later, the larvae (72 ± 4 h) were washed out of the vials with tap water through a meshed stainless steel strainer. These larvae were transferred to vials containing 1.5 g of dry Drosophila Instant Medium (Carolina Biological Supply, Burlington, NC, USA) rehydrated with 5 mL of the test solutions containing different amounts of tested artichoke or fenugreek extracts. The larvae were allowed to feed on these media until pupation. Approximately 10 to 12 days after treatment, the emerging flies were collected, and separated into two phenotypic classes based on the presence or absence of TM2, Ubx balancer chromosome. After phenotypic classification the flies were stored separately in a solution containing 2 part of 70% ethanol and 1 part of glycerol. Wings of flies of both sexes from the two phenotypic classes were mounted on slides using Faure's solution (30 g gum Arabic, 20 mL glycerol, 50 g chloral hydrate, 50 mL water) and searched microscopically for *mwh/mwh* and flr^3/fl^3 single or twin wing spot phenotypes. Because of the weak expression of the flr^3 marker in small clones and its lethality in large clones of mutant cells, only the *mwh/mwh* clones were used to calculate clone formation frequencies as we have already described in previous chapter. The frequency of each type of spot (small single, large single or twin) and the total frequency of spots per wing for each fenugreek extract were compared pair-wise with the frequency of negative concurrent controls (normal media).

2.5. Human cell lines based *in vitro* tests

2.5.1. Cell lines

The two types of human, adherent breast cancer cell lines (T-47D and ZR-75-1) were obtained from ATCC (American Type Culture Colection, USA). At normal culturing conditions the cell cycle lasts for 32 hr in case of T-47D, while the ZR-75-1 cells doubling time is 80 hr.

2.5.2. Cell culturing conditions

These ductal carcinoma cell lines were grown in T25 or T75 flasks (Greiner Bio-One Gmbh) in RPMI-1640 (Lonza) culture medium supplemented with 1% Antibiotic Antimycotic Solution (Invitrogen-Gibco), 10% heat-inactivated FBS (Fetal Bovine Serum; Sigma) and 0,1% 1 mM Na-pyruvate (Biochromag). All cell culture experiments were carried out at 37°C in 5% CO_2 incubator.

2.5.3. MTT viability/proliferation test

Cells were used in this studies when 80% confluence was reached in T75 flasks (Greiner Bio-One GmbH). Cells were washed twice with sterile PBS, and harvested with Trypsin in the incubator. For counting the cells we used Trypan blue dye (Sigma) exclusion method and counted with Bürker chamber. After it the cells were seeded into 96-well plates at a density of 10^4 cells/well and left to attach to the plates. After 24 hours cells were incubated for two and three days with various concentrations (10-5-2.5-1.25-0.62-0.31-0.16-0.08-0.04 v/v%) of extracts. After the exposure time removed the extracts and the cells were incubated with ten microliter of MTT (5 mg/ml) (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) (Sigma) at 37°C for three hours. After dissolving the formazan crystals in MTT solubilization solution, plates were read in a microplate reader (BioTek EL808) at 570 nm. This experiment was performed in sextuplicates and repeated three times (Mosmann, 1983).

2.5.4. Immunostaining

The treatments with both fenugreek extracts were carried out in six-well plates and at the examined concentration range. At the bottom of each well, a coverslip was laid and cells were let to grow on the surface of coverslip. After 2- and 3-day treatments the cells were fixed with formaldehyde and stained as described by Mathe *et al*. (2004) and Lemos *et al*. (2000). The microtubules were detected with YL1/2 rat monoclonal anti-α-tubulin antibody (Sera Lab, Inc.), and POLO like kinase-1 (Plk1) was stained with mouse monoclonal Anti-hPlk1 antibody (P5998 Sigma-Aldrich), while the DNA was counterstained with the DAPI dye (Molecular Probes). Digital images of optical sections were collected with an Olympus Cell R inverted fluorescent microscope system equipped with a Hamamatsu CCD camera controlled by the Olympus XcellenceR imaging software, using a Plapon 60x objective.

2.6. Statistical analysis

Statistical analysis were performed by the SPSS 16.0 software. Statistical differences among treated and untreated cells were determined by one-way ANOVA (Analysis of Variance). To compare several groups was applied the Tukey post-hoc test, and mean differences with $p< 0.05$ were considered statistically significant.

3. MAIN OBSERVATIONS AND RESULTS OF DISSERTATION

3.1. Characterization of bioactive compound profile of different of artichoke and fenugreek extracts by UHPLC-ESI-MS system

Our study was meant to identify all possible bioactive compounds with the applied UHPLC-ESI-MS system, and as a consequence 49 and 51 molecules were found in the aqueous and hydro-alcoholic artichoke extracts, respectively (*Figure 2-5*).

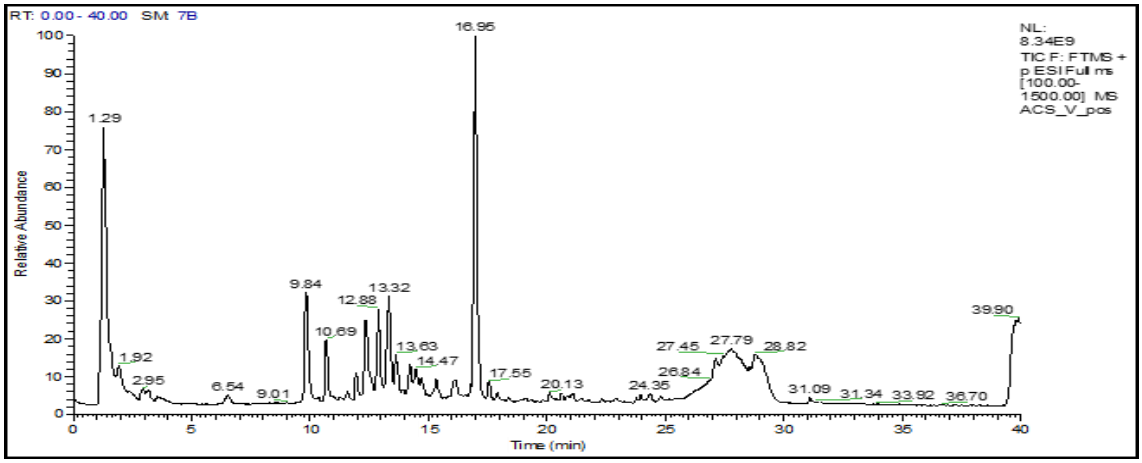

Figure 2. Total ion chromatogram of aqueous extract of Artichoke in positive ionisation mode.

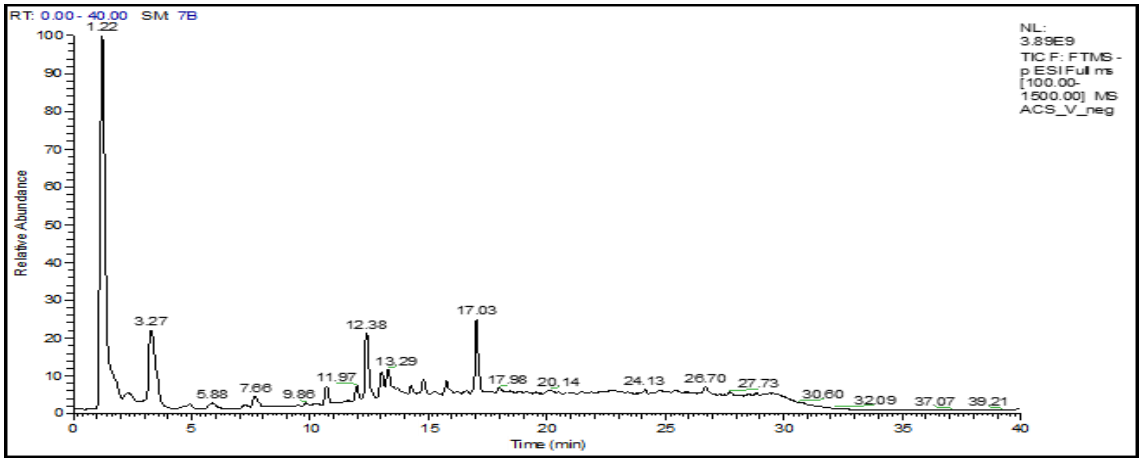

Figure 3. Total ion chromatogram of aqueous extract of artichoke in negative ionisation mode.

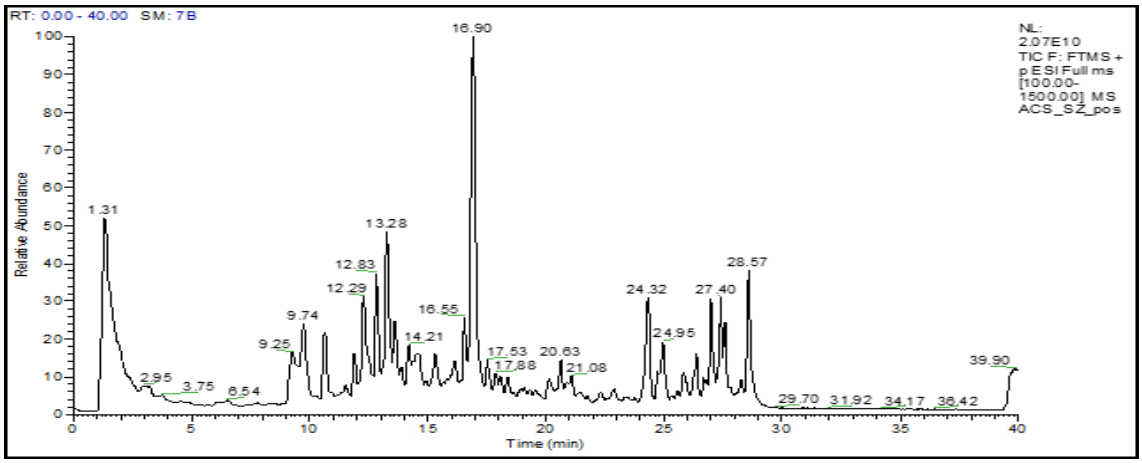

Figure 4. Total ion chromatogram of hydro-alcoholic extract of artichoke in positive ionisation mode.

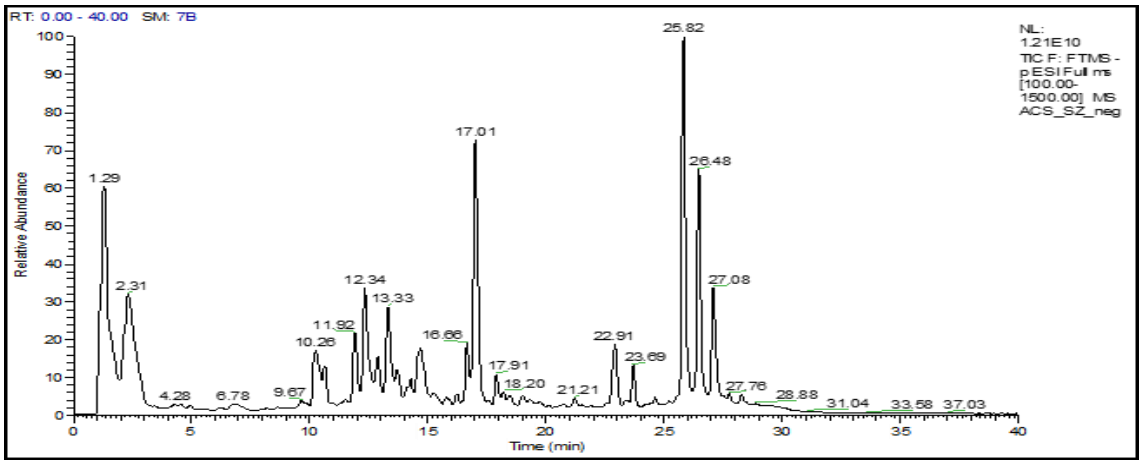

Figure 5. Total ion chromatogram of hydro-alcoholic extract of artichoke in negative ionisation mode.

There have been 54 bioactive compounds identified in the aqueous fenugreek seed extract, while in case of hydro-alcoholic extract 67 molecules were found as shown on coresponding chromatograms (*Figure 6-9*).

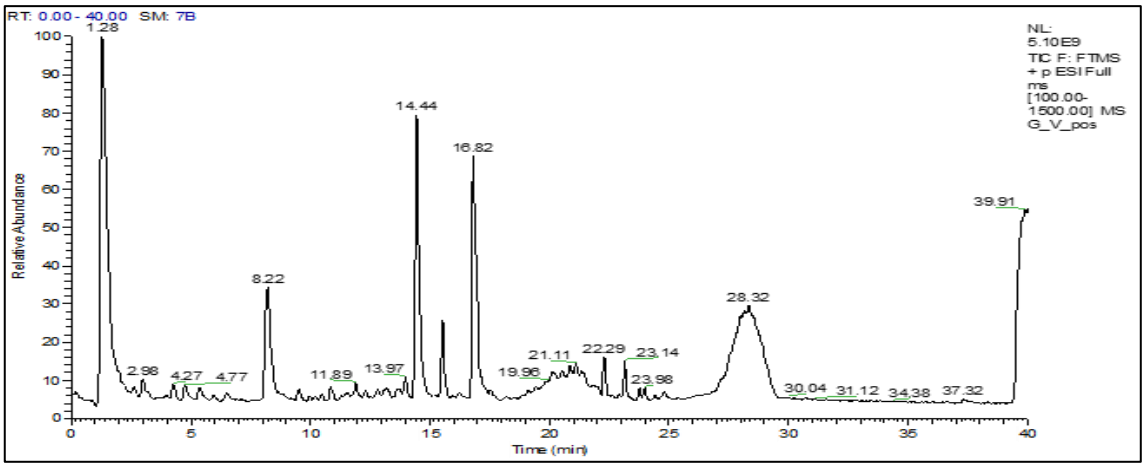

Figure 6. Total ion chromatogram of aqueous extract of fenugreek in positive ionisation mode.

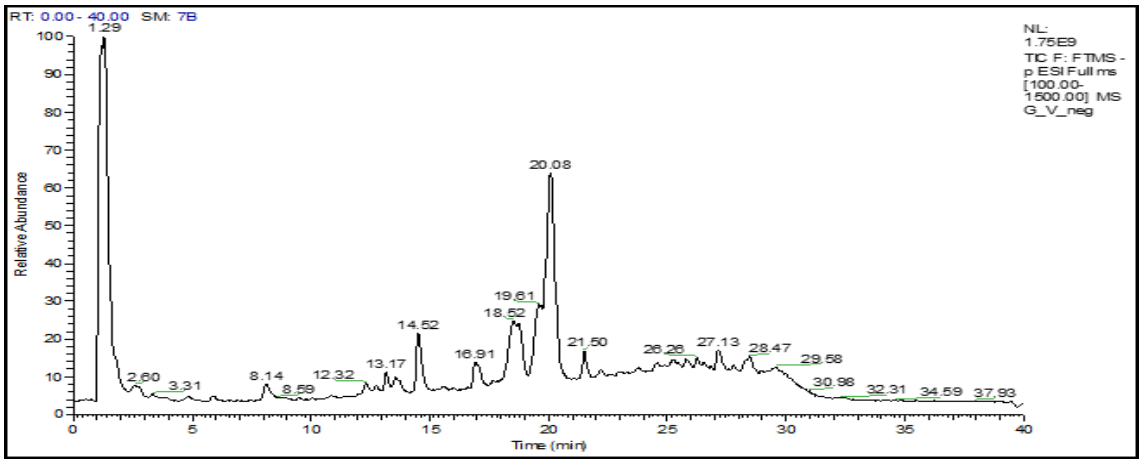

Figure 7. Total ion chromatogram of aqueous extract of fenugreek in negative ionisation mode.

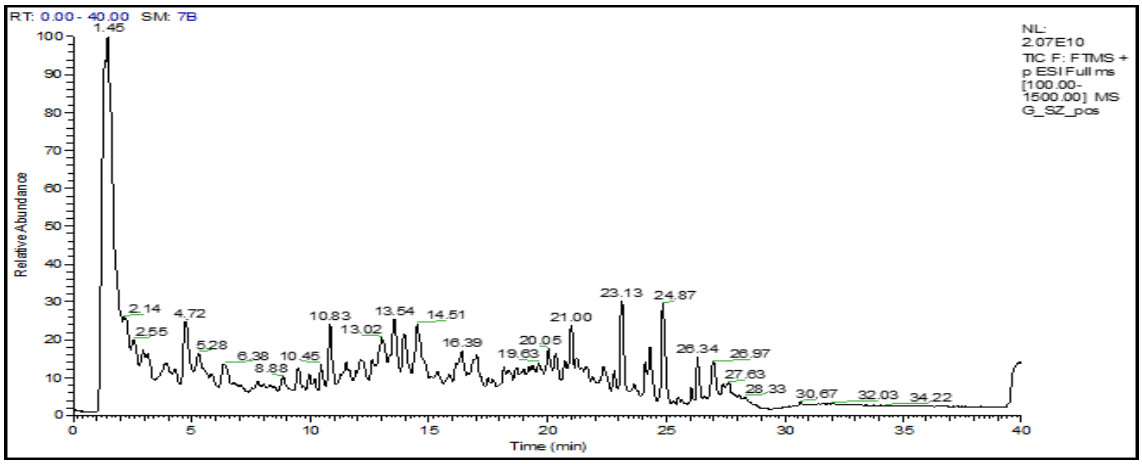

Figure 8. Total ion chromatogram of hydro-alcoholic extract of fenugreek in positive ionisation mode.

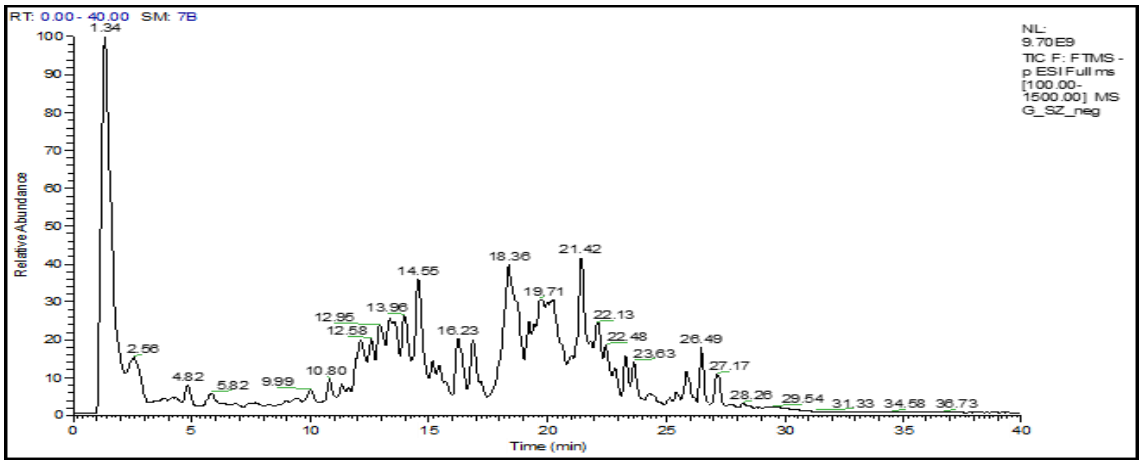

Figure 9. Total ion chromatogram of hydro-alcoholic extract of fenugreek in negative ionisation mode.

The phytoconstituents were defined based on specific retention time, accurate mass, isotopic distribution and fragmentation pattern, and by screening MS databases like Metlin, mzCloud and Massbank. The UHPLC-ESI-MS analysis of artichoke and

fenugreek extracts revealed similarities and differencies regarding their bioactive compound profile. In Table 1. are indicated the numbers of identified compounds specific for the studied bioactive compound cathegory.

Table 1. Number of bioactive compounds identified in the aqueous and alcoholic artichoke and fenugreek extracts

Bioactive compound category	**Aqueous artichoke**	**Hydro-alcoholic artichoke**	**Aqueous fenugreek**	**Hydro-alcoholic fenugreek**
Alkaloids	3	2	4	4
Aminoacids	7	9	12	11
Coumarins	2	3	-	1
Flavonoids	7	13	7	16
Other metabolites	3	4	3	3
Polyphenols	9	3	1	2
Purines and pirimidines	9	6	10	9
Saponins	2	2	14	16
Terpenoids	1	2	-	1
Steroids	-	2	-	-
Vitamines	5	4	3	4
Total	***48***	***50***	***54***	***67***

It is important to notice that the preparation methods for the aqueous and hydro-alcoholic extracts were identical for artichoke and fenugreek. Moreover, the same UHPLC-ESI-MS method was applied for the analysis of the extracts, and therefore the obtained results are directly comparable.

In case of artichoke the described bioactive compound profile strongly supports the liver and gallbladder tonic effect of artichoke by interfering with lipid metabolism. Moreover, some kind of anti-cancerous effect could also be expected based on some phytoconstituents. Based on individual effects of the identified phytoconstituents, multiple mechanisms could be evoked to explain the artichoke health promoting effects like the inhibition of cholesterol synthesis and lipolysis, together with the activation of anti-inflammatory, anti-tumour growth cellular pathways.

The comparative chemomapping of aqueous and hydro-alcoholic fenugreek seed extracts revealed already known and new bioactive compounds that further support the antidiabetic effects of fenugreek seeds. The presence of some bioactive compounds in our extracts is expected to improve blood lipid spectre (4-OH-Ile, diosgenin), and to show

reno-protective (4-OH-Ile, trigonelline), neuroprotective (trigonelline) and antioxidant (diosgenin, trigonelline) properties. Other phytoconstituents identified in our seed extracts plead for a more substantial neuroprotective (kynurenine, genistein, vitexin, isovitexian), anti-inflammatory (trigonelline, scopoletin, ursolic acid, vitamins), hypocholesterolemic (saponins), muscle and/or hepatic insulin resistance reducing (choline) effects.

3.2. Comparative quantitative analysis of some bioactive compounds from artichoke and fenugreek extracts and their associated antioxidant capacity

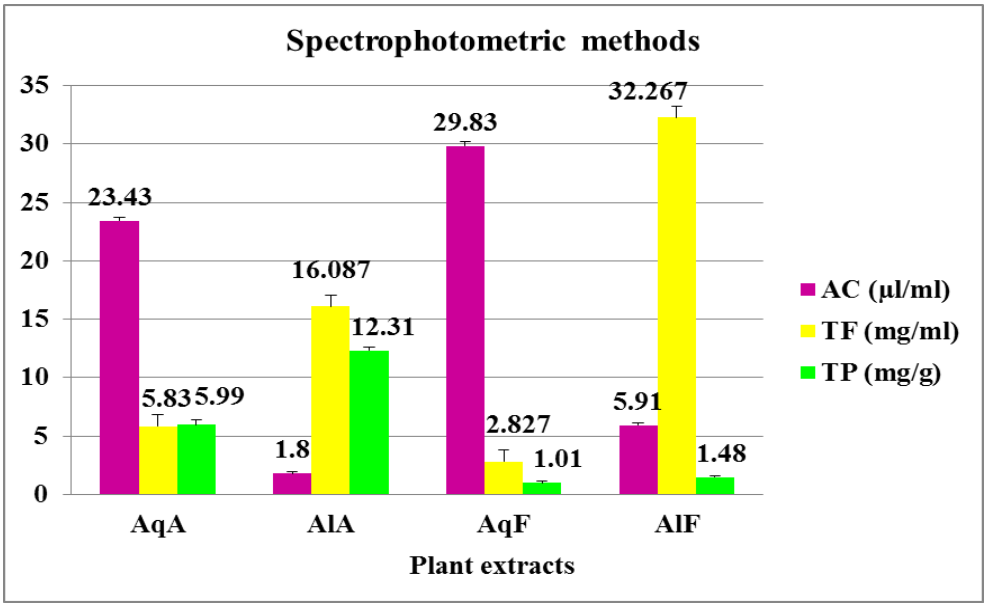

Figure 10. Spectrophotometric methods: Note: (AC) antioxidant capacity, (TF) total flavonoid, (TP) total phenolic, (AqA) aqueous artichoke extract, (AlA) alcoholic artichoke extract, (AqF) aqueous fenugreek extract, (AlF) alcoholic fenugreek extract

From the obtained data we can conclude that the aqueous artichoke and fenugreek extracts antioxidant capacities were relatively high, while the alcoholic artichoke and fenugreek extracts antioxidant capacities were markedly reduced as compared to their corresponding aqueous counterparts. The aqueous extract compared to the hydro-alcoholic extract showed an approximately 16 times greater antioxidant capacity in case of artichoke. In case of fenugreek the aqueous extract showed a 5 time greater antioxidant capacity than the hydro-alcoholic extract (*Figure 10*).

The total flavonoid content of artichoke and fenugreek hydro-alcoholic extracts exceeded substantially the flavonoid content seen in both plants derived aqueous extracts. Accordingly, the artichoke specific hydro-alcoholic extract total flavonoid content appears 3 times greater as compared to the aqueous extract. The fenugreek total flavonoid content of the hydro-alcoholic extracts looks almost 12 times greater than the aqueous

extract related values. Moreover, if the two plants specific hydro-alcoholic extracts are compared, it looks obvious that the fenugreek values are two times greater than the artichoke values for total flavonoid content suggesting that the fenugreek seeds contain twice as much flavonoid than the artichoke. Surprisingly, when the aqueous extracts of artichoke and fenugreek are compared, the artichoke extract specific total flavonoid content was approximately double to that seen in case of fenugreek extract.

The total phenolic content of artichoke hydro-alcoholic extract exceeded more than 2 times the values observed for the artichoke aqueous extract suggesting that the hydro-alcoholic artichoke extract contains twice as many phenolic compounds like the aqueous artichoke extract. However, the aqueous and hydro-alcoholic fenugreek extracts seemed to contain similar amount of phenolic compounds. Surprisingly we were unable to detect anthocyanin in any of our artichoke and fenugreek extracts (*Figure 10*).

3.3. *In vivo* analysis of artichoke and fenugreek extracts effects using *Drosophila melanogaster*

3.3.1. Analysis of artihoke and fenugreek extracts effects on the viability of fruitflies

In order to assess the putative toxic effect of our fenugreek extracts we set to study the way they could affect the viability of treated fruit flies. F1 embryos from two crosses were collected, while their pupariation and adult hatching were monitored on normal media and media containing undiluted or diluted (10^{-1}, 10^{-2}) artichoke or fenugreek aqueous or hydro-alcoholic extracts. The obtained data are summarized in Tables 2-3.

Table 2. Viability assessment of untreated and artichoke extracts treated flies. Control experiments specific data are marked with yellow. Green color refers to aqueous artichoke extract, while blue color represents the hydro-alcoholic artichoke extract experiments. The viability rate refers to the hatched adults as compared to the extract treated embryos. Control-NM indicates the normal media, while Control-DIM refers to the instant media.

Parents crossing	Treatment	Σ F1 treated embryo	F1 larva	Hatched F1 imago	F1 viability rate (%)	Average F1 viability rate (%)
♀w^{m4h} x ♂w^{m4h}	Control-NM	1000	972	956	95.6	
	Control-DIM	1000	988	952	95.2	
	Aqueous artichoke stock solution	1000	968	962	96.2	95.2
	Aqueous artichoke 1:10 dilution	1000	957	952	95.2	
	Aqueous artichoke 1:100 dilution	1000	972	944	94.4	
	Hydro-alcoholic artichoke stock solution	1000	958	949	94.9	93.8
	Hydro-alcoholic artichoke 1:10 dilution	1000	977	932	93.2	
	Hydro-alcoholic artichoke 1:100 dilution	1000	968	936	93.4	
♀*mwh/mwh* x ♂ *flr^3/TM2, Ubx*	Control-NM	1000	984	961	96.1	
	Control-DIM	1000	982	966	96.6	
	Aqueous artichoke stock solution	1000	966	951	95.1	95.9
	Aqueous artichoke 1:10 dilution	1000	982	964	96.4	
	Aqueous artichoke 1:100 dilution	1000	975	963	96.3	
	H.-alcoholic artichoke stock solution	1000	977	943	94.3	95.1
	H.-alcoholic artichoke 1:10 dilution	1000	988	95	96.5	
	H.-alcoholic artichoke 1:100 dilution	1000	968	945	94.5	

Table 3. Viability assessment of untreated and fenugreek extracts treated flies. Control experiments specific data are marked with yellow. Green color refers to aqueous fenugreek extract, while blue color represents the hydro-alcoholic fenugreek extract experiments. The viability rate refers to the hatched adults as compared to the extract treated embryos. Control-NM indicates the normal media, while Control-DIM refers to the instant media.

Parents crossing	Treatment	Σ F1 treated embryo	F1 larva	Hatched F1 imago	F1 viability rate (%)	Average F1 viability rate (%)
♀w^{m4h} x ♂w^{m4h}	Control NM	1000	968	956	95.6	
	Control DIM	1000	972	944	94.4	
	Aqueous fenugreek stock solution	1000	946	938	93.8	94.5
	Aqueous fenugreek 1:10 dilution	1000	953	942	94.2	
	Aqueous fenugreek 1:100 dilution	1000	972	957	95.7	
	Hydro-alcoholic fenugreek stock solution	1000	948	926	92.6	93.3
	Hydro-alcoholic fenugreek 1:10 dilution	1000	966	947	9.7	
	Hydro-alcoholic fenugreek 1:100 dilution	1000	952	928	92.8	
♀*mwh/mwh* x ♂ *flr^3/TM2, Ubx*	Control NM	1000	938	912	91.2	
	Control DIM	1000	942	921	92.1	
	Aquaous fenugreek stock solution	1000	947	922	92.2	92.6
	Aquaous fenugreek 1:10 dilution	1000	966	941	94.1	
	Aqueous fenugreek 1:100 dilution	1000	933	916	91.6	
	H.-alcoholic fenugreek stock solution	1000	955	932	93.2	91.9
	H.-alcoholic fenugreek 1:10 dilution	1000	934	917	91.7	
	H.-alcoholic fenugreek 1:100 dilution	1000	943	921	92.1	

The average F1 hatching rate was above 91% for both crosses, and the hatching rate of aqueous or hydro-alcoholic artichoke and fenugreek extract treated flies were similar to the controls, suggesting that the applied artichoke and fenugreek undiluted and diluted extracts do not exert toxic effects on *Drosophila melanogaster*.

3.3.2. Artihoke and fenugreek extracts assessed for their genotoxic effect using the SMART method

Having seen that neither the aqueous nor the hydro-alcoholic artichoke and fenugreek extracts are affecting the viability of F1 individuals, we treated the F1 larvae from the ♀mwh/mwh x ♂ flr^3/TM2, Ubx cross with undiluted extracts to perform the wing specific SMART assay. Among the F1 progeny, the wings of mwh, +/+, flr^3 transheterozygous flies were analysed for mosaic clones, and the corresponding data are shown in Table 4.

Table 4. Frequency of clone induction in case of mwh, +/+, flr transheterozygous flies wings. Control experiments specific data are marked with yellow. Green colors refer to fenugreek extracts, while brown color indicates the fenugreek extracts based experiments.

Treatment	Wing (N)	Mosaic clone				Frequency of clones (n/N)	Mean cell no./ clone (m)	Frequency of clone induction (f=nm/NC)
		twin	single		Total clone number (n)			
			mwh	*flr*				
Control	**107**	**3**	**55**	**1**	**59**	**0.55**	**1.1±0.2**	**2.0×10^{-5}**
Aqueous artichoke	**98**	**5**	**51**	**-**	**56**	**0.57**	**1.2±0.4**	**2.3×10^{-5}**
Hydro-alcoholic artichoke	**106**	**4**	**53**	**1**	**58**	**0.55**	**1.1±0.4**	**2.0×10^{-5}**
Aqueous fenugreek	**135**	**5**	**53**	**3**	**61**	**0.49**	**1.6±0.4**	**2.4×10^{-5}**
Hydro-alcoholic fenugreek	**142**	**6**	**54**	**5**	**65**	**0.46**	**1.4±0.6**	**2.1×10^{-5}**

Looking at the frequency of clones, mean cell number/clone and the frequency of clone induction there were no obvious differences between the untreated control and crude or alcoholic fenugreek extract treated mwh, +/+, flr transheterozygous individuals. These data are indicating that the crude or alcoholic fenugreek extracts are not inducing chromosomal breaks (mitotic recombination) at a higher extent than those seen in case of control untreated individuals.

Among the F1 progeny, the wings of mwh/ TM2, Ubx transheterozygous flies were analyzed for mosaic clones, and the corresponding data are shown in Table 5.

Table 5. Frequency of clone induction in case of mwh/Balancer flies wings. Control experiments specific data are marked with yellow. Green colors refer to fenugreek extracts, while brown color indicates the fenugreek extracts based experiments.

Treatment	Wing (N)	Total *mwh* clone number (n)	Frequency of clones (n/N)	Mean cell no./clone (m)	Frequency of clone induction (f=2nm/NC)
Control	**106**	**11**	**0.1**	**2.4 ± 1.6**	**1.6×10^{-5}**
Aqueous artichoke	**114**	**12**	**0.1**	**2.2 ± 1.2**	**1.5×10^{-5}**
Hydro-alcoholic artichoke	**102**	**11**	**0.1**	**2.4 ± 1.4**	**1.7×10^{-5}**
Aqueous fenugreek	**112**	**12**	**0.11**	**2.4 ± 0.8**	**1.7×10^{-5}**
Hydro-alcoholic fenugreek	**116**	**14**	**0.12**	**2.2 ± 0.6**	**1.8×10^{-5}**

Looking at the frequency of clones, mean cell number/clone and the frequency of clone induction there were no obvious differences between the untreated control and crude or alcoholic fenugreek extract treated mwh/TM2, Ubx individuals. These data are indicating that the crude or alcoholic fenugreek extracts are not inducing mutations in the mwh locus situated on the TM2 balancer chromosome at a higher extent than those seen in case of control untreated individuals.

The toxicity and mutagenic effect of aqueous and hydro-alcoholic artichoke and fenugreek extracts when tested on Drosophila melanogaster revealed no viability reducing effect or increased mutational (gene and chromosomal breaks) rata, suggesting that both extracts are suitable for studying their mechanisms of action with respect to the contained bioactive compounds.

3.4. Artichoke and fenugreek extracts *in vitro* testing on human breast cancer cell lines

The T-47D and ZR-75-1 human breast cancer cell lines were treated for 2 to 3 days at a wide concentration spectra of different aqueous and hydro-alcoholic artichoke and fenugreek extracts, respectively. For every applied extract concentration using the MTT test, the viability of treated breast cancer cells was determined. In the following I will present only the 3-day treatment specific data (*Figure 11-16*).

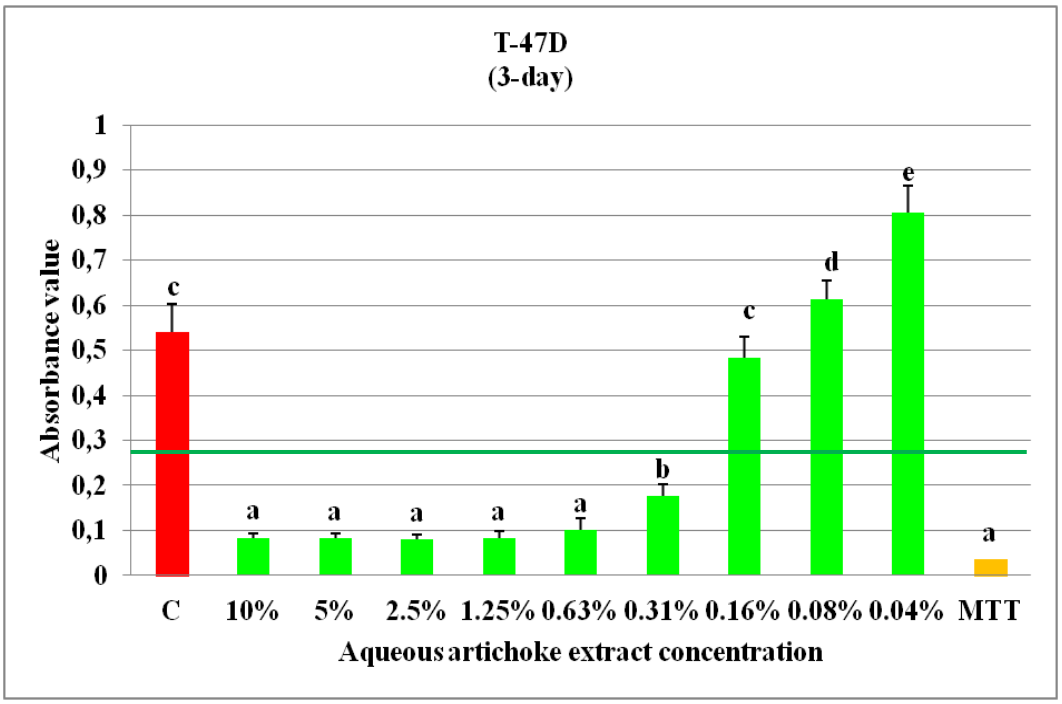

Figure 11. Cell viability after 3-day treatment using aqueous artichoke extract. The T-47D cell viability was measured by MTT assay. C-stands for untreated or positive control cells. MTT- represents the negative control.

Looking at the 3-day treatment experiments of T-47D breast cancer cells, in the 10-0.63% concentration range of aqueous artichoke extract, the viability of treated cancerous cells was 5 times reduced, yet constant, throughout the assessed concentration values as compared to control untreated cells. It seems likely that the 0.31% concentration could be close to the LD50 value in this experiment. Started from the 0.16% concentration point, and ended with the 0.04% concentration of artichoke leaf extract, the viability of treated cells was increasing progressively. At 0.08% and 0.04% extract concentrations, the viability of treated cells surpassed approximately 1.5 times the values of untreated control cells (*Figure 11*).

Looking at the 3-day treatment experiments of ZR-75-1 breast cancer cells, in the 10-0.31% concentration range of aqueous artichoke extract, the viability of treated cancerous cells was markedly reduced, yet constant, throughout the assessed concentrations at about 8.5 times lower viability was detected as compared to control untreated cells. The 0.16% concentration could be considered the LD50 value in this experiment. In the 0.08-0.04% concentration range of artichoke extract, the viability of treated cells was increasing progressively. At 0.04% extract concentration, the viability of treated cells surpassed 1.2 times the untreated control cells specific values (*Figure 12*).

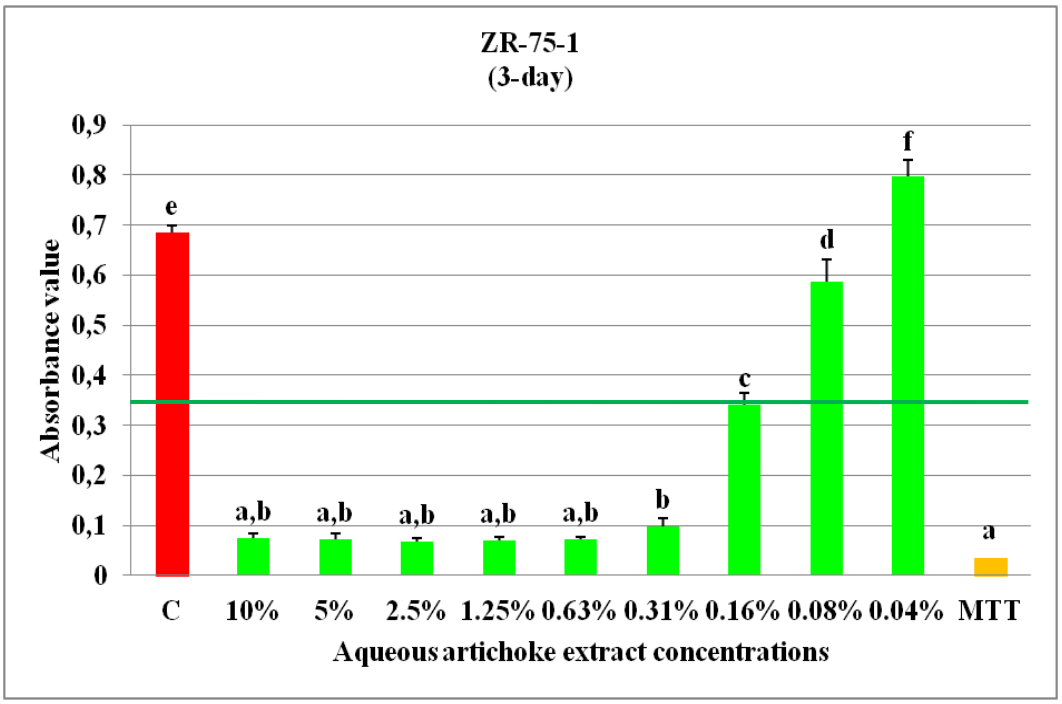

Figure 12. Cell viability after 3-day treatment using aqueous artichoke extract. The ZR-75-1 cell viability was measured by MTT assay. C-stands for untreated or positive control cells. MTT- represents the negative control.

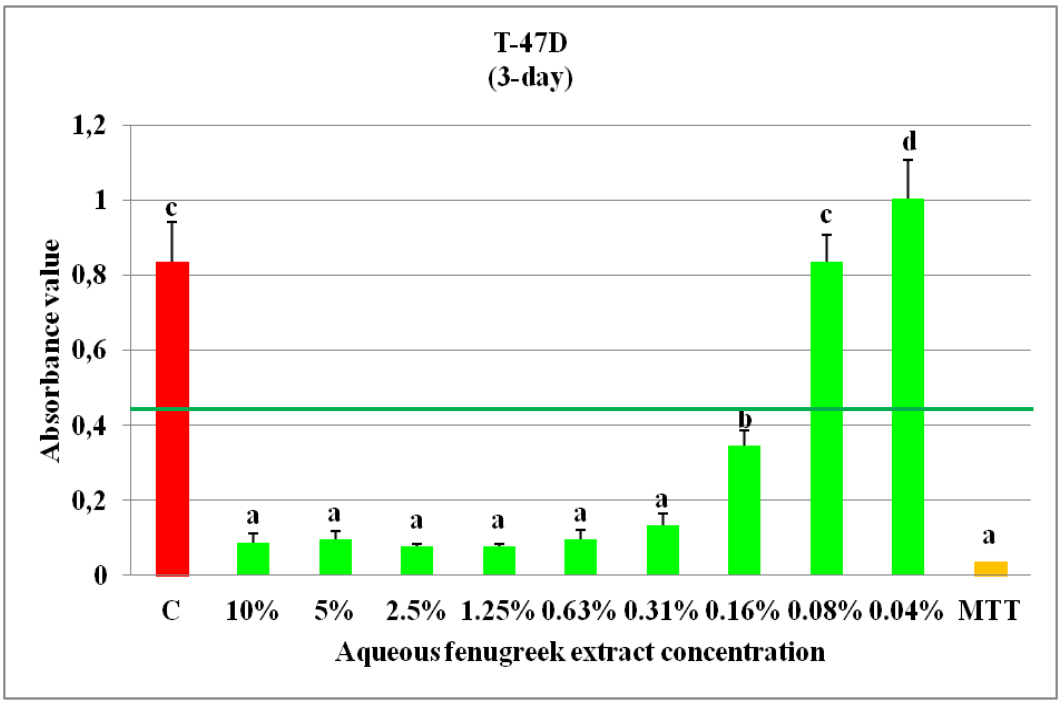

Figure 13. Cell viability after 3-day treatment using aqueous fenugreek extract. The T-47D cell viability was measured by MTT assay. C-stands for untreated or positive control cells. MTT- represents the negative control.

Looking at the 3-day treatments of T-47D breast cancer cells, in the 10-0.31% concentration range of aqueous fenugreek extract, the viability of treated cancerous cells

was 8 times lower, yet constant, throughout the assessed concentration range as compared to control untreated cells. The 0.16% concentration seems to be close to the hypothetical LD50 value in this experiment. Across the 0.16-0.04% concentration range, the viability of treated cells was steadily increasing. At 0.08% extract concentrations, the viability of treated cells equaled those specific to the control cells, while at 0.04% extract concentration the viability of cells surpassed showed a 1.2 fold increase as compared to the values of untreated control cells (*Figure 13*).

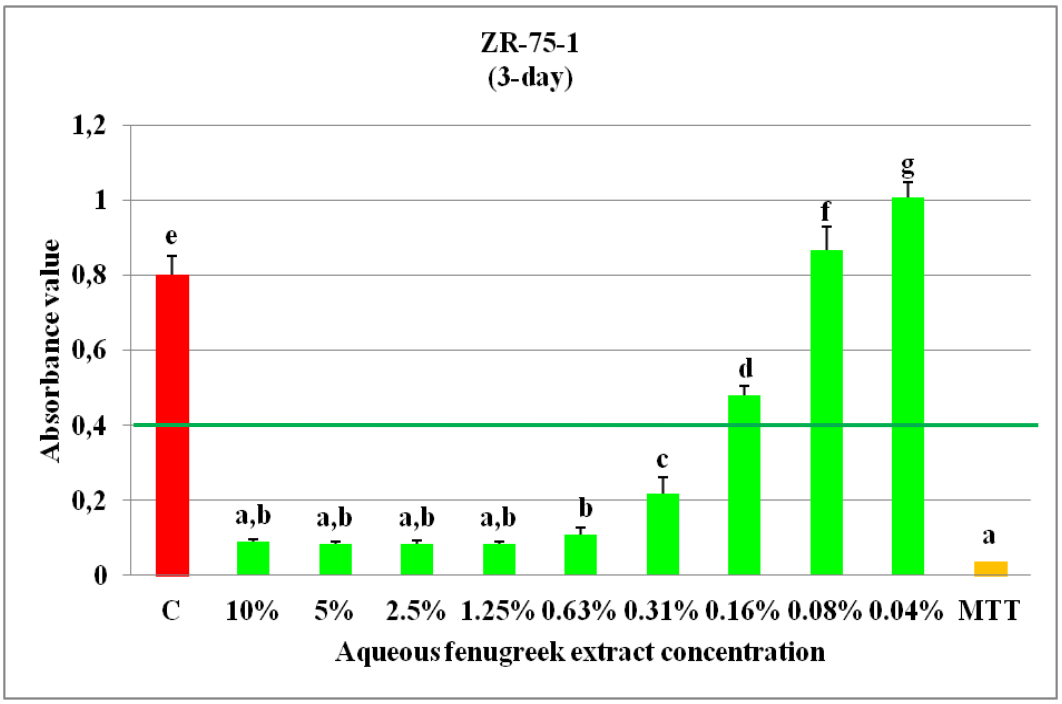

Figure 14. Cell viability after 3-day treatment using aqueous fenugreek extract. The ZR-75-1 cell viability was measured by MTT assay. C-stands for untreated or positive control cells. MTT- represents the negative control.

Looking at the 3-day long treatments of ZR-75-1 breast cancer cells, at the 10-0.63% concentration interval, the viability of treated cancerous cells was markedly reduced, and constant, showing at about 8 times lower values as compared to control untreated cells. The LD50 value is expected to fit in between 0.31-0.16% concentration interval in this experiment. In the 0.31-0.04% concentration range, the viability of treated cells was increasing progressively. At 0.08% extract concentration, the viability of treated cells was similar to the untreated control cells. However, at 0.04% extract concentration, the viability of treated cells surpassed 1.2x the values observed for untreated control cells (*Figure 14*).

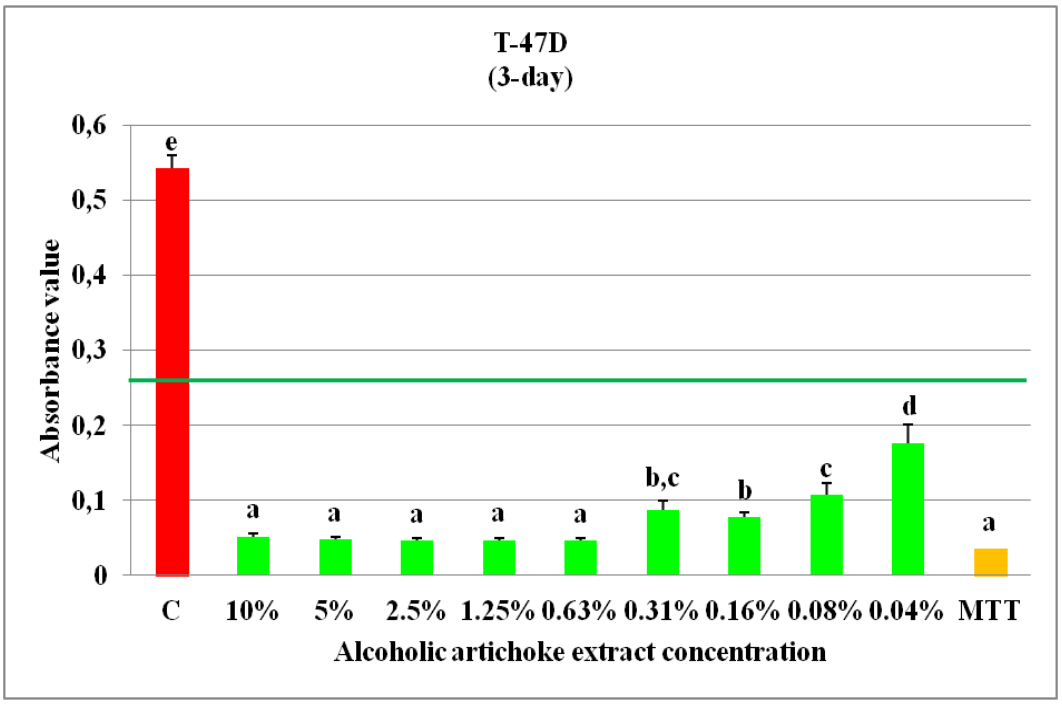

Figure 15. Cell viability after 3-day treatment using hydro-alcoholic artichoke extract. The T-47D cell viability was measured by MTT assay. C-stands for untreated or positive control cells. MTT- represents the negative control.

At the 3-day treatment experiments of T-47D breast cancer cells, I could observe in the 10-0.08% concentration range that the viability was greatly reduced for treated cancerous cells. Throughout the above mentioned concentration range the low viability values show some degree of variation. Accordingly, in the 10-0.63% concentration range the viability of treated cells was approximately 9 times lower as compared to control cells. In the 0.31-0.08% concentration rage, the viability of treated cells increased, yet compared to controls was approximately 5.5 times lower. Nevertheless, in the case of the applied lowest 0.04% concentration, the viability of treated cells increased but, did not reach 50%, suggesting that this working concentration does not corresponds to a LD50 value (*Figure 15*).

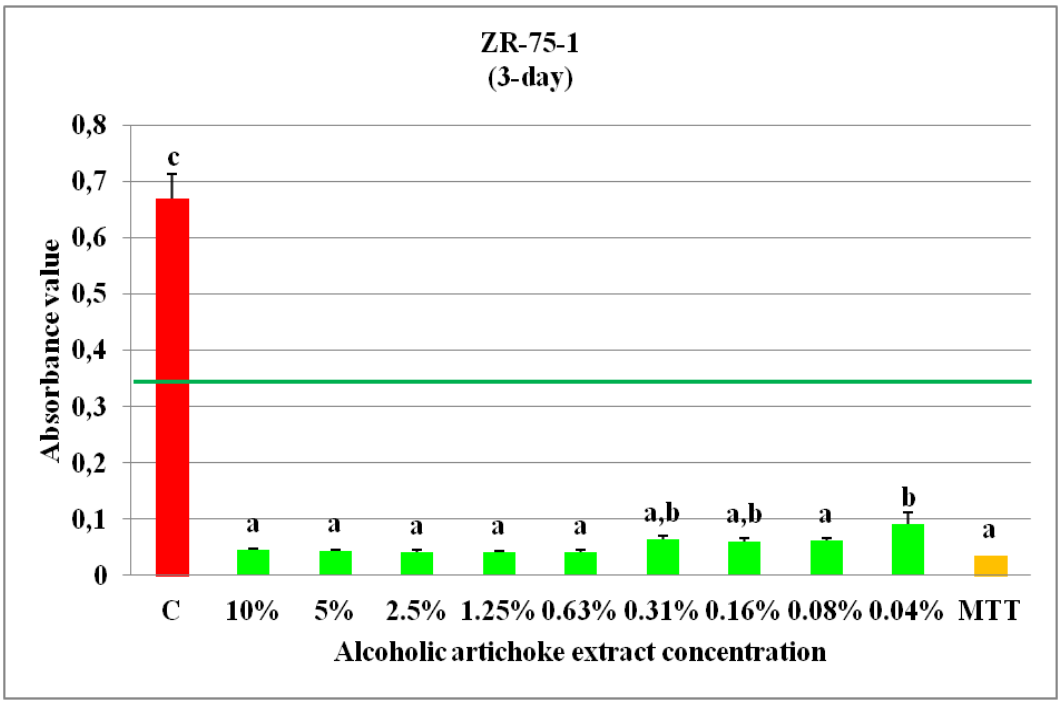

Figure 16. Cell viability after 3-day treatment using hydro-alcoholic artichoke extract. The ZR-75-1 cell viability was measured by MTT assay. C-stands for untreated or positive control cells. MTT- represents the negative control.

At the 3-day treatment experiments of ZR-75-1 breast cancer cells, I could observe throughout the analyzed concentration range that the viability was greatly reduced for treated cancerous cells. The observed viability values showed some minor variation throughout the monitored concentrations but did not differ significantly from each other. The viability of treated cells was approximately 13 times lower as compared to control cells. All the observed viability values were far from reaching the expected LD50 value (*Figure 16*).

Having seen the above described viability reducing effects in the case of our artichoke leaf extracts treated cells we set to analyze the cells phenotype by assessing their chromosomal structure. Among the artichoke leaf extract treated cells we were able to detect some normal interphase and mitotic cells, but there were also polyploidy cells, binuclear cells, and cells showing minispindle and sometime multipolar spindle (*Figure 17*). The polyploidy cells quite often were showing overcondensed and/or fragmented chromosomes features that are specific to cells entering senescence and/or apoptosis.

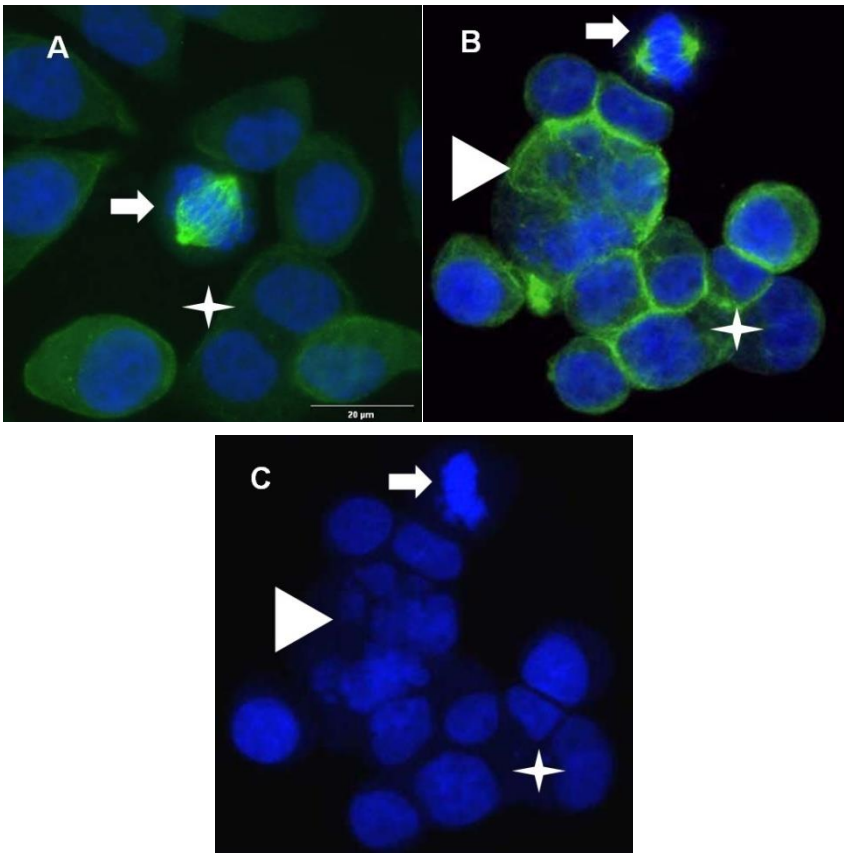

Figure 17. Immunostainigs of artichoke extract treated breast cancer cells.
Note. A. Normal looking cells in interphase, at mitotic metaphase (arrow) and binucleated cell (star); green indicates microtubules, blue stands for DNA. B-C. Polyploidy cell with fragmented chromosomes and/or micronuclei (arrowhead). Minipindle with overcondensed chromosomes (arrow). Binucleated cell (star); green indicates microtubules, blue stands for DNA.

At the 3-day treatment experiments of T-47D breast cancer cells, I could observe that the viability was greatly reduced for treated cancerous cells at all concentration assessed (*Figure 18*). The observed significantly low viability values showed some but minor degree of variation. Accordingly, the viability of treated cells was approximately 8-16 times lower as compared to control cells. It is also evident that the 3-day long applied alcoholic fenugreek extract concentrations are featuring a great level of toxicity.

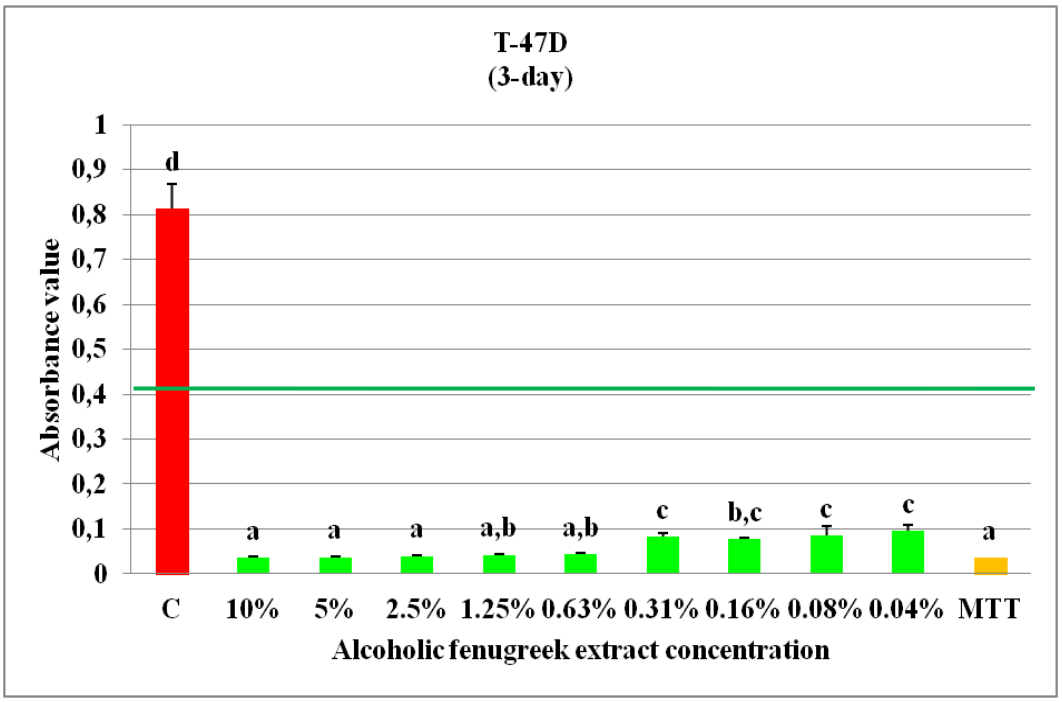

Figure 18. Cell viability after 3-day treatment using alcoholic fenugreek extract. The T-47D cell viability was measured by MTT assay. C-stands for untreated or positive control cells. MTT- represents the negative control.

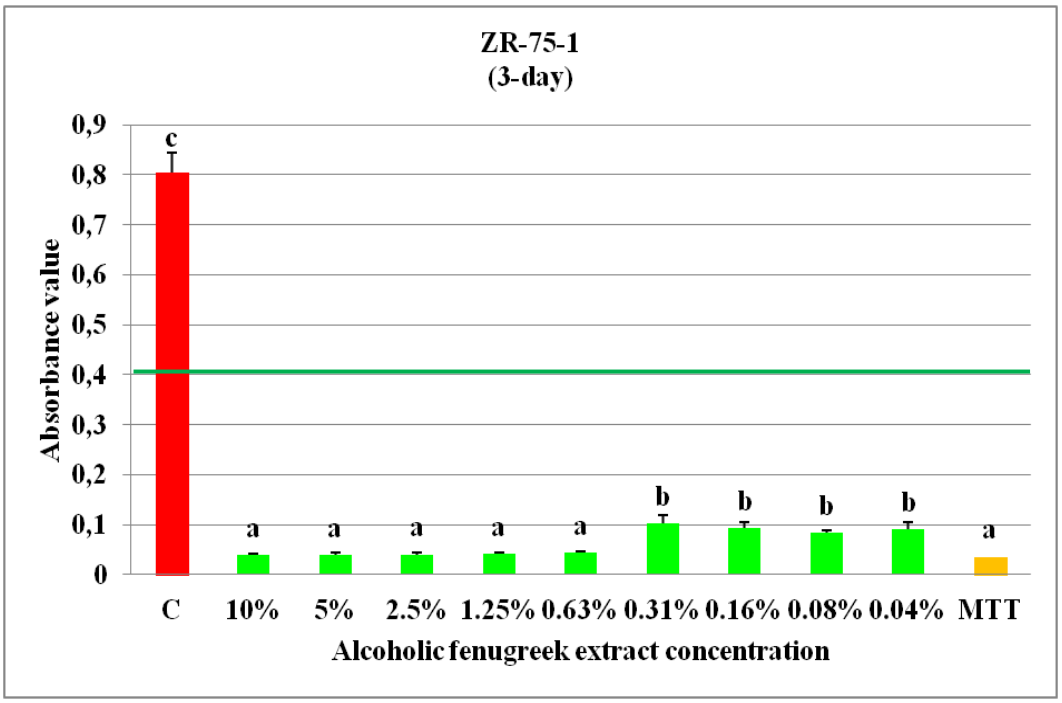

Figure 19. Cell viability after 3-day treatment using alcoholic fenugreek extract. The ZR-75-1 cell viability was measured by MTT assay. C-stands for untreated or positive control cells. MTT- represents the negative control.

At the 3-day treatment experiments of ZR-75-1 breast cancer cells, I could observe throughout the analyzed concentration interval that the viability was even more pronouncedly reduced in case of treated cancerous cells. The viability of treated cells was

approximately 20 times lower as compared to control cells in the concentration interval 10-0.63%. Next, in the concentration interval 0.31-0.04%, the viability of treated cells was about 9 times lower than in case of control untreated cells. All the observed viability values were very far from the expected LD50 value (*Figure 19*).

In order to find out how these extracts are influencing the treated cells we set to analyze by immunostaining their phenotype, hoping that the identified defects would reveal details regarding the affected cellular phenomena. Such an approach would correspond to a phenotypic study, but contrary to a classical genetic analysis where mutant alleles generated phenotypes are described, in our experiments the extracts are responsible for the impeded biological phenomenon. Both fenugreek seed extracts are affecting the viability of the analyzed cancerous cell lines in a dose dependent fashion, and by immunostaining we were able to detect multiple cellular defects. We could identify cell division defects and apoptosis like characteristics. Therefore, we were able to detect normal looking interphase and dividing cells, together with multi-nucleated presumably aneuploid cells, other cells with small or multi-polar spindles, and cells showing nuclear blobbing or fragmented chromosomes, the latter being specific to apoptosis (*Figure 20-21*). We also looked at the structure of mitotic spindles, and by monitoring the Polo like kinase-1 (Plk1) subcellular localization we taught to gain more information about mitotic entry, spindle assembly, chromosome alignment, sister chromatid segregation, metaphase-anaphase transition and cytokinesis. It has been demonstrated that Plk1 regulates almost every aspect of mitotic events, and its overexpression is a marker for poor prognosis in many cancers (Weng *et al*., 2016). In the case of fenugreek extracts treated cells we were able to found that Plk–1 in some instances is associated with centrosomes, while in others shows some accumulation along the central spindle microtubules of the spindle apparatus (*Figure 20*).

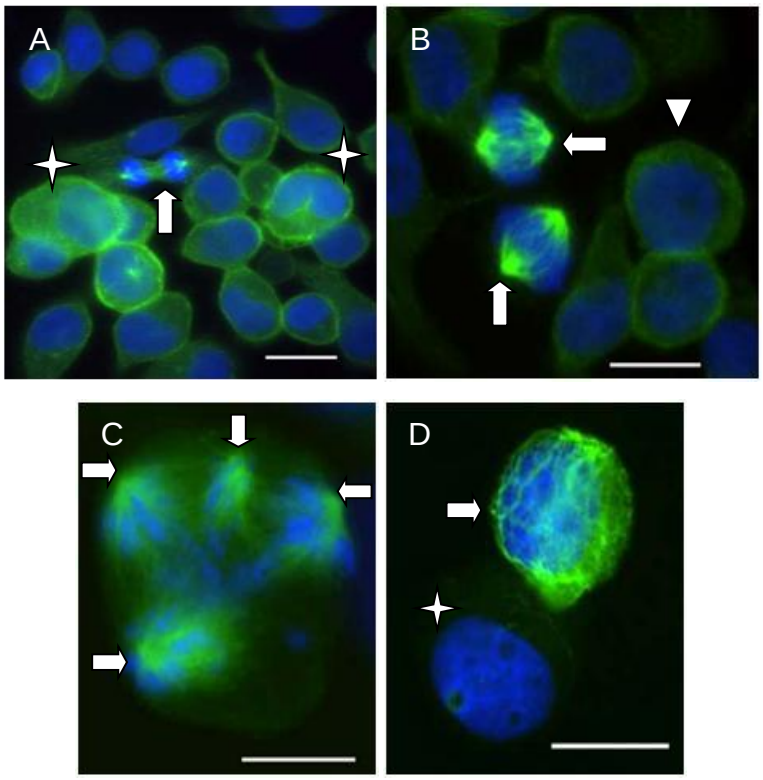

Figure 20. Immunostaining of fenugreek extract treated cancer cells.
Note. A. Untreated cells in interphase together with giant nuclei cells (star) and normal anaphase (arrow). B. Untreated cells at interphase (arrowhead) and mitotic metaphase (arrow). C. Extract treated cell with abnormal multipolar spindle showing accumulation of chromosomes at spindle poles (arrowhead). D. Extract treated cell showing nuclear blobbing and abnormal microtubule network (arrow) as compared to a normal interphase cell (plus).Green indicates microtubules and blue shows the chromosome specific DNA. The scale bar represents 20μm.

Despite the fact that the performed immunostaining revealed certain cellular defects presumably generated by the fenugreek extract based treatments, we did not carry out a quantitative analysis since after 2- and 3-day treatments usually the cell number was greatly reduced. To gain a much clearer picture it will be necessary to carefully assess the phenotypes all treated cells by FACS (Fluorescence-activated cell sorting) analyses.

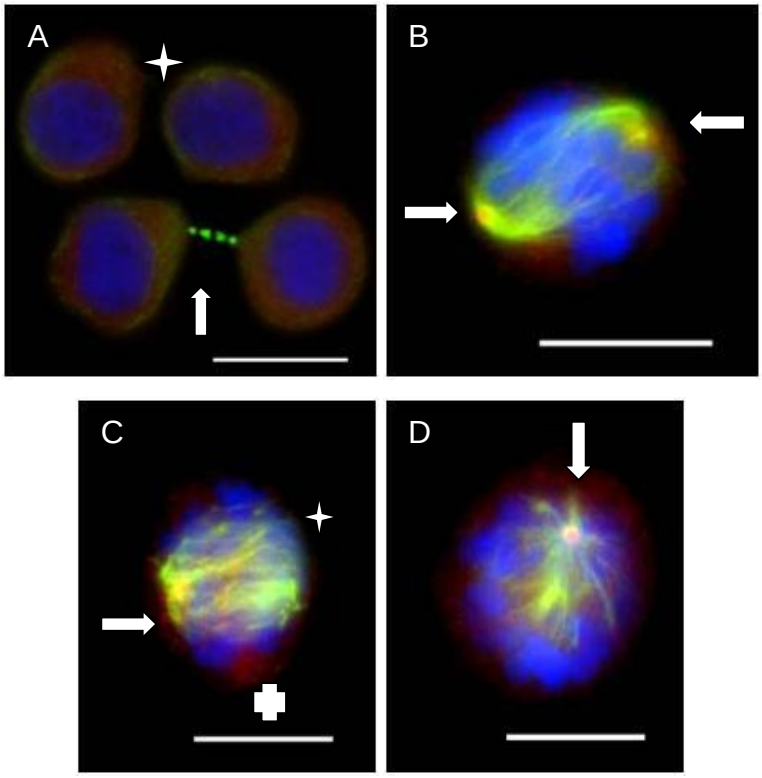

Figure 21. Immunostaining of fenugreek extract treated cancer cells. Note. A. Normal cells in interphase (star) and cytokinesis (arrow). B. cell at mitotic metaphase-anaphase transition with centrosomes at spindle poles and PLK associated with centrosomes but not kinetochores (arrow). C. Extract treated cell with abnormal bipolar spindle showing normal centrosome at one pole with Plk-1 accumulation (arrow), while the opposite pole is much broader with no centrosome (arrowhead) and some PLK accumulation along the spindle microtubules (plus). D. Extract treated cell with abnormal monopolar spindle showing PLK accumulation at the spindle pole associated centrosome (arrow). Green indicates microtubules, red stands for Plk, and blue shows the chromosome specific DNA. The scale bar represents 20μm.

4. NEW SCIENTIFIC RESULTS OF THE DISSERTATION

1. In the dried artichoke leaves made aqueous and hydro-alcoholic extracts I have identified 49 and 51 bioactive compounds, respectively. Among them, 27 bioactive compounds have been for the first time shown to be present in artichoke. These newly identified bioactive compoundsa are the following: kynurenic acid, trigonelline, stachydrine (alkaloids); 4-Guanidinobutyric acid, arginine, tryptophan, N-Acetyl-isoleucine, N-Acetil-leucin (aminoacids); esculin, 5-O-Caffeoylshikimic acid I, 5-O-Caffeoylshikimic acid II, Coumaroylquinic acid I, Coumaroylquinic acid II (polyphenols); diosmetin, salvigenin, naringin-dihydro-chalcone, vicenin-2 (flavonoids); 7-Methoxy-4-methyilcoumarin (coumarin); choline, methyl cinnamate, phenethylamine (other metabolites); ursolic acid (terpenoid); stearidonic acid methyl ester, stearidonic acid ethyl ester (steroids); pantothenic acid (B5), pyridoxal, pyridoxine (B6) (vitamines).
2. Using the Hungarian fenugreek seeds I was able to identify 54 compounds in the aqueous extract, while in the hydro-alcoholic extract 67 molecules were identified. All together in the two extracts 25 new bioactive compounds were found like ecgonine, ecgonine methyl ester (alkaloids); resveratrol (polyphenol); tricin-7-O-glucoside, genistein, isovitexin (apigenin-6-C-glucoside), medicarpin, scoparin, apigenin-6-C-glucoside-8-C-rhamnoside (flavonoids); sotolone, saccharopine (other metabolites); 5'-S-Methyl-5'-thioadenosine, 2'-deoxyadenosine, adenine, adenosine, adenosine 3',5'-cyclic-monophosphate (cAMP), cytidine, flavin mononucleotide (FMN), guanine, guanosine, S-adenosyl-homocysteine, uridine, xanthine (purines and pyrimidines); ursolic acid (terpenoid); nicotinamide (vitamine).
3. The aqueous artichoke and fenugreek extracts tested on T-47D and ZR-75-1 human breast cancerous cell lines are featuring a hormetic type of response with biphasic effect.
4. The hydro-alcoholic artichoke and fenugreek extracts are cytotoxic and induce apoptosis on T-47D and ZR-75-1 human breast cancerous cell lines at a very wide concentration spectra.
5. The *Drosophila melanogaster* based experiments are indicating that any of the tested artichoke or fenugreek extracts do not affect the viability of fruitflies. Moreover, any of the extracts did not increased the gene and chromosomal mutational rate as revealed by the SMART method, so that they are not genotoxic.

5. PRACTICAL UTILIZATION OF THE SCIENTIFIC RESULTS

Based on all my results presented in my dissertation it seems likely that the artichoke and fenugreek aqueous and hydro-alcoholic extracts might be suitable after careful assesments for the development of nutraceuticals or functional foods to prevent breast cancer or to use as complementary treatment for breast cancer patients.

6. REFERENCES

American Cancer Society, Cancer Facts & Figures, Atlanta, 2016.

Dietz B.M. - Hajirahimkhan A. - Dunlap T.L. - Bolton J.L.: 2016. Botanicals and Their Bioactive Phytochemicals for Women's Health. *Pharmacol Rev*. 68(4), 1026-1073.

Kim D.O. - Chun O.K. - Kim Y.J. - Moon H.Y. - Lee C.Y.: 2003. Quantification of polyphenolics and their antioxidant capacity in fresh plums. *Journal of Agricultural and Food Chemistry*. 51(22): 6509-6515.

Lee J. - Durst R.W. - Wrolstad R.E.: 2005. Determination of total monomeric anthocyanin pigment content of fruit juices, beverages, natural colorants, and wines by the pH differential method: collaborative study. *Journal of AOAC International*. 88(5): 1269-1278.

Lemos C.L. - Sampaio P. - Maiato H. - Costa M. - Omel'yanchuk V.L. - Liberal V. - Sunkel C.E.: 2000. Mast, a conserved microtubule-associated protein required for bipolar mitotic spindle organization. *EMBO J*. 19: 3668–3682.

Máthé E. - Kraft C. - Giet R. - Deák P. - Peters J.M. - Glover D.M.: 2004. The E2-C vihar is required for the correct spatiotemporal proteolysis of cyclin B and itself undergoes cyclical degradation. *Curr Biol*. 14(19): 1723-1733.

Molyneux P.: 2004. The use of the stable free radical diphenylpicryl-hydrazil (DPPH) for estimating antioxidant activity. *Journal of Science and Technology*. 26: 211-219.

Mosmann T.: 1983. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *Journal of Immunology Methods*. 65 (1-2): 55-63.

Singleton V.L. - Rossi J.A.: 1965. Colorimetry of total phenolics with phosphomolybdic- phosphotungstic acid reagents. *American Journal of Enology and Viticulture*. 16: 144-158.

Tompa A.: 2011. Daganatos betegségek előfordulása, a hazai és nemzetközi helyzet ismertetése. *Magyar Tudomány*. 11: 1333-1345.

Weng Ng W.T. - Shin J.S. - Roberts T.L. - Wang B. - Lee C.S.: 2016. Molecular interactions of polo-like kinase 1 in human cancers. *J Clin Pathol*. 69(7): 557-562.

7. LIST OF PUBLICATIONS RELATED TO THE DISSERTATION

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Registry number: DEENK/184/2017.PL
Subject: PhD Publikációs Lista

Candidate: Szabolcs Vigh
Neptun ID: SZABOLCS.VIGH
Doctoral School: Hankóczy Jenő Doctoral School of Crop Production, Horticulture and Food Sciences
MTMT ID: 10035790

List of publications related to the dissertation

Hungarian book chapters (1)

1. **Vigh, S.**, Zsvér-Vadas, Z., Máthé, E.: Articsóka és görögszéna növényi kivonatok citogenetikai hatásvizsgálata hagyma gyökércsúcs merisztéma sejteken.
   In: Őshonos- és tájfajták - Ökotermékek - Egészséges Táplálkozás - Vidékfejlesztés: A XXI. század mezőgazdasági stratégiái Konferencia. Szerk.: Irinyiné Oláh Katalin, Kosztyuné Krajnyák Edit, Tóth Csilla, Lajtos István, Nyíregyházi Egyetem, Nyíregyháza, 305-309, 2016. ISBN: 9786155545696

Foreign language scientific articles in international journals (7)

2. **Vigh, S.**, Cziáky, Z., Sinka, L. T., Pribac, C., Moş, L., Turcuş, V., Gálné Remenyik, J., Máthé, E.: Analysis of phytoconstituent profile of fenugreek - Trigonella foenum-graecum L. - seed extracts.
   Stud. Univ. Babes-Bolyai Chem. "Accepted by Publisher", 1-20, 2017. ISSN: 1224-7154. IF: 0.244 (2016)
3. **Vigh, S.**, Cziáky, Z., Sinka, L. T., Pribac, C., Moş, L., Turcuş, V., Máthé, E.: Antioxidant activity, total flavonoid, total phenolic and anthocyanin contents of Cynara scolymus L. leaves and Trigonella foenum-graecum L. seeds.
   Annals of the Academy of Romanian Scientists Series on Science and Technology of Information. 6 (1), 174-179, 2017. ISSN: 2069-1149.
4. **Vigh, S.**, Cziáky, Z., Sinka, L. T., Pribac, C., Moş, L., Turcuş, V., Gálné Remenyik, J., Máthé, E.: Comparative chemomapping of phytoconstituents from different extracts of globe artichoke - Cynara scolymus L.
   Stud. Univ. Babes-Bolyai Chem. "Accepted by Publisher", 1-17, 2017. ISSN: 1224-7154. IF: 0.244 (2016)

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5. **Vigh, S.**, Zsvér-Vadas, Z., Pribac, C., Moş, L., Cziáky, Z., Czapár, M., Mihali, C., Turcuş, V., Máthé, E.: Artichoke (Cynara scolymus L.) extracts are showing concentration-dependent hormetic and cytotoxic effects on breast cancer cell lines.
Stud. Univ. "Vasile Goldiş" Arad, Ser. Ştiinţ. vieţii. 26 (4), 423-433, 2016. ISSN: 1584-2363.

6. **Vigh, S.**, Zsvér-Vadas, Z., Pribac, C., Moş, L., Cziáky, Z., Czapár, M., Mihali, C., Turcuş, V., Gálné Remenyik, J., Máthé, E.: Fenugreek (Trigonella foenum-graecum L.) extracts are inducing dose-dependent hormetic response and cytotoxic effects in case of human breast cancer cell lines.
Stud. Univ. "Vasile Goldiş" Arad, Ser. Ştiinţ. vieţii. 26 (4), 435-448, 2016. ISSN: 1584-2363.

7. Máthé, E., Moş, L., Szőke-Kovács, Z., Szőke-Kovács, R., **Vigh, S.**, Pribac, C., Czapár, M., Oláh, C.: Plant extracts affect dose-dependently the hatching rate of Drosophila melanogaster but do not induce chromosome loss and/or aneuploidy.
Anal. Univ. Oradea Fac. Ecotoxicologie, Zootehnie şi Tehnologii de Industrie Alimentară. 11B, 399-404, 2012. ISSN: 1583-4301.

8. Czapár, M., Domina, R., **Vigh, S.**, Máthé, E., Pribac, G., Moş, L., Damian, S., Turcuş, V., Covaci, A., Cotoraci, C., Paiusan, L.: Effects of some plant extracts on various tumor cell lines.
Stud. Univ. "Vasile Goldiş" Arad, Ser. Ştiinţ. vieţii. 20 (1), 29-35, 2010. ISSN: 1584-2363.

List of other publications

Hungarian book chapters (1)

9. **Vigh, S.**, Czapár, M., Tarek, M., Máthé, E., Dinya, Z.: Elfelejtett táplálékunk a tejsavó: A tejsavó kémiai összetételének és humán rákos sejtvonalak proliferációjára gyakorolt hatásának vizsgálata.
In: A IV. nyíregyházi doktorandusz (PhD/DLA) konferencia kiadványa. Szerk.: Semsei Imre, Mikóné Márton Julianna, Debreceni Egyetemi Kiadó, Debrecen, 225-229, 2010. ISBN: 9789633181744

Foreign language scientific articles in international journals (1)

10. Pribac, G., Czapár, M., **Vigh, S.**, Dinya, Z., Nagyné Uhrin, H., Szathmáry, M., Steiber, Z., Máthé, E., Ardelean, A., Moş, L., Bodó, E.: Apoptosis on colorectal HT-29 cancer cells may be induced by bioactive principles of Dioscorea Sp. extracts.
Annals Romanian Soc Cell Biol. 14 (2), 33-37, 2009. ISSN: 1583-6258.

Hungarian abstracts (2)

11. **Vigh, S.**: Növényi hatóanyagok tesztelése humán emlőtumoros sejtvonalakon.
In: Aktualitások a táplálkozástudományi kutatásokban című V. PhD konferencia Összefoglalói. Szerk.: Gelencsér Éva, Horváth Zoltánné, Magyar Táplálkozástudományi Társaság, Budapest, 10, 2015. ISBN: 9789638810885

12. Czapár, M., **Vigh, S.**, Dinya, Z., Nagyné Uhrin, H., Szathmáry, M., Steiber, Z., Máthé, E., Bodó, E.: Természetes növényi hatóanyagok tumorellenes hatásainak vizsgálata kolorektális eredetű HT-29 sejtvonalon.
In: VIII. Magyar Genetikai Kongresszus, XV. Sejt- és Fejlődésbiológiai Napok : program, összefoglalók. Szerk.: Maróy Péter, Sass Miklós, Putnoky Péter, Magyar Genetikusok Egyesülete, Szeged, 143-144, 2009.

Foreign language abstracts (1)

13. Czapár, M., Pribac, G., Domina, R., **Vigh, S.**, Máthé, E., Ardelean, A., Turcuş, V., Damian, S., Moş, L., Cotoraci, C.: Effects of some plant extracts on HT-29 colorectal adenocarcinoma cell line.
In: 20th Edition of the International Conference Arad Academic Days : Book of Abdstracts. Coord.: Liana Mos, George Pribac, Vasile Goldis University Press, Arad, 20, 2010. ISBN: 9789736643972

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