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THE IMPACT OF *BRASSICA OLERACEA VAR ITALICA* AND *CAPSELLA BURSA PASTORIS* EXTRACTS ON WISH CANCER CELLS

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Objective: *to investigate the impact of aqueous extracts of Brassica oleracea var italica and Capsella bursa pastoris on the WISH (monolayer) cancer cell line. Chemical composition and pharmacological properties of Brassica oleracea var italica and Capsella bursa pastoris are considered.* ***Methods:*** *this article considers microscopic method based on inhibition and percentage determination of cancer cell lines.* ***Results****: experiment shows that Brassica oleracea var italica and Capsella bursa pastoris can be used against cervix cancer cells – human WISH (monolayer) cell line.*

Key words: extraction; *Brassica oleracea var italica; Capsella bursa pastoris*; WISH (monolayer) cell line; inhibition of cancer cell line.

Introduction. Cancer is a fatal illness that, unfortunately, is not treated with one pill. The cancer distribution trend is characteristic not only for Ukraine (the 2nd place in terms of mortality in Europe), but also for the world as a whole. Oncology covers 22 % of the able-bodied population for a given period of time. According to the Cancer Institute, in 2009, 961,183 persons were registered on the account of oncology institutions, including 338,635 men and 622,548 women. But by 2030, the number of cancer patients will increase by 70 % according to the Ministry of Health. Also, after 15 years, scientists predict that cancer will be diagnosed by every other person.

Widespread cancer incidence contributes to several key factors - bad habits, unhealthy diet, loss of control over body weight, stress, adverse environmental

conditions. Also in this series - the problem of untimely diagnosis of the disease due to the lack of opportunities for quality post-colonization.

Common methods of cancer control are chemotherapy, antibiotics and surgical intervention. This method of treatment have influence on healthy cells of the human body.

In connection with above, it is proposed to solve the problem of preventing cancer by the influence of medicinal plant extracts. The family of *Cruciferae* or *Brassicaceae* contains many biologically active substances that have antioxidant and anti-carcinogenic properties, namely indole, sulphoraphane, routine and others [1-8].

Intensive distribution of the disease and increased mortality led to the search for alternative methods of prevention and treatment of cancer. This problem remains the most urgent to date.

Materials and methods. The objects of investigation were samples of raw materials − dried herbs of *Capsella bursa pastoris* and fresh broccoli *Brassica oleracea var italia*. Samples were obtain from local pharmacies and supermarkets in Kiev.

In this work, for the checking antitumor activity we used WISH ATCC-№ CCL-25 (monolayer) cancer cell line. This cell line was supplied by European Collection of Authenticated Cell Cultures (ECACC). General characteristic of WISH cell line is represented in Table 1.

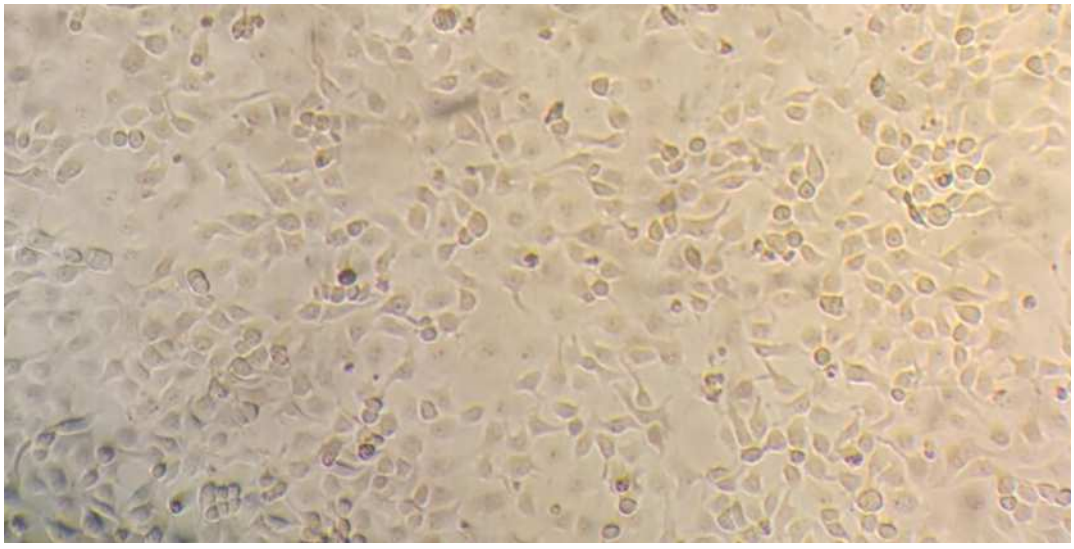

Fig. 1. WISH (monolayer) cell line under microscope (x100)

Table 1

General characteristics of WISH ATCC-№ CCL-25 (monolayer) cell line

Organism	Human
Tissue	Cervix
Cell type	Epithelial cells
Growth properties	Monolayer
Description	Originally derived from human amnion tissue. Have been used in virus studies; susceptible to VSV (Indiana), adenovirus 3 and poliovirus. Useful in differentiating virulent and avirulent measles virus. HeLa marker chromosomes and type A G6PD have been detected. This cell line was found tobe indistinguishable from HeLa by STR PCR DNA profiling. Therefore, the cell line must be considered as derivative from HeLa. Ethnicity: black.
Cancer type	Human cervix carcinoma

Extraction of biologically active compounds from broccoli and herbs was carried out using the 3 different methods. The first (alcohol-water extraction) – in glass bottle with a capacity of 100 ml, 5 g of crushed raw material of *Capsella bursa pastoris* (the degree of crushing 2–3 mm) with 10 ml of distilled water and 10 ml of ethanol 96 % (Figure 2) were added, and kept in a water bath (Figure 3) for 60 minutes at the temperature of 90 °C. After cooling the samples to room temperature (approximately 20 °C), the extract was filtered (Figure 4) into a plastic tubes of 15 ml capacity.

The second (water extraction) – in glass bottle with a capacity of 100 ml, 10 g of crushed raw material of *Capsella bursa pastoris* (the degree of crushing 2–3 mm) with 40 ml of distilled water (Figure 2) were added, and kept in a water bath (Figure 3) for 60 minutes at the temperature of 90 °C. After cooling the samples to room temperature (approximately 20 °C), the extracts was filtered (Figure 4) into a plastic tubes of 15 ml capacity.

The third (mechanical) – broccoli juice is squeezed mechanically. Then this juice was filtered (Figure 4) into a plastic tubes of 15 ml capacity.

Fig. 2. Extracts of *Capsella bursa pastoris*

Fig. 3. Keep of extracts in a water bath (water bath-thermostat with stirring WB-4MS)

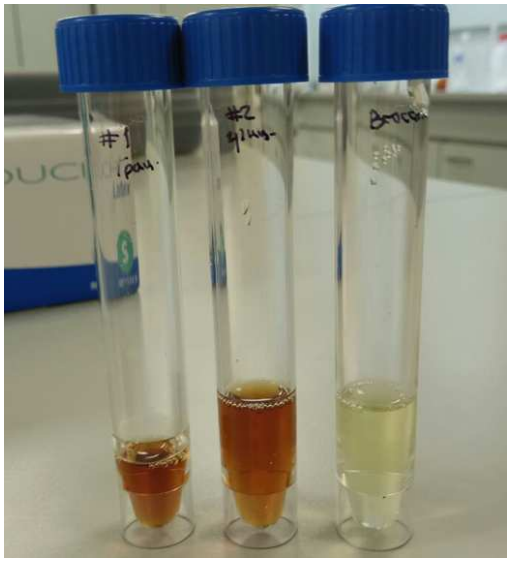

Fig. 4. Filtered extracts

In result, we got 3 different extracts, namely: dried herb with distilled water, dried herb with distilled water and ethanol, fresh broccoli juice. Also, extracts were filtered through a bacteriological filter “Epikriz” with a pore diameter of 0,22 nm before introduction to the cancer cells.

By mixing with a nutrient medium, the concentration of the cells in the suspension was adjusted to 3×105 cells/mL, poured by 100 µL into all wells of 96-well plates and incubated in the atmosphere of 5 % CO_2 at 37 °C and humidity of 95–97 % (standard conditions for cell culture) for 6 hours for cells attachment.

WISH cell line was exposed to extracts of *Capsella bursa pastoris* and *Brassica oleracea var italia* in different concentrations for 72 hours. After 72 hour we check our plates under microscope and compare it with starting point of our experiment (Figure 5).

We have different concentration of extracts, namely 1/1, 1/2, 1/4, 1/8, 1/16, 1/32 and K+. As the start concentration of extracts was 1/4 we can calculate the amount of substance in 1 ml of solution (Table 2).

Table 2

Concentration of extracts

Dillution	Concentation g/ml		
	Capsella bursa pastoris (alc.+water)	*Capsella bursa pastoris (water)*	*Brassica oleracea var italia*
1/1	0,125	0,125	0,125
1/2	0,063	0,063	0,063
1/4	0,031	0,031	0,031
1/8	0,016	0,016	0,016
1/16	0,008	0,008	0,008
1/32	0,004	0,004	0,004

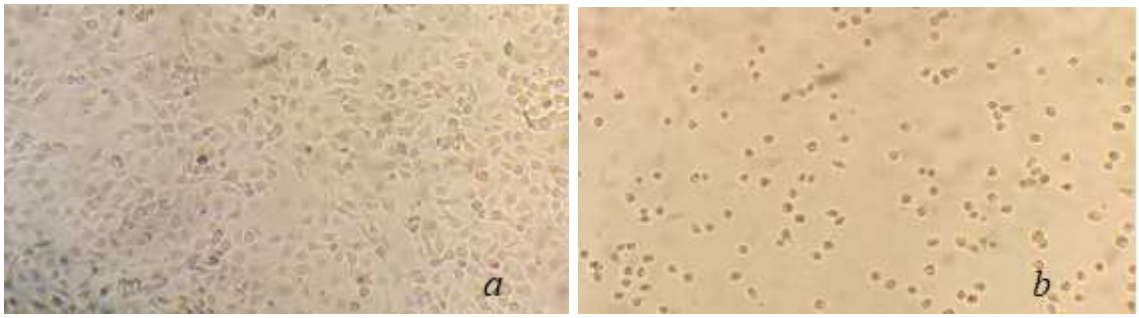

Fig. 5. Comparing of *a)* live cells of WISH with *b)* dead cells of WISH

After fluorescence spectrophotometry with tablet fluorescent spectrophotometer (Cytation3 Viatek, or similar) in Ex560 nm, 590 nm we got data with RFU (relative units fluorescences).

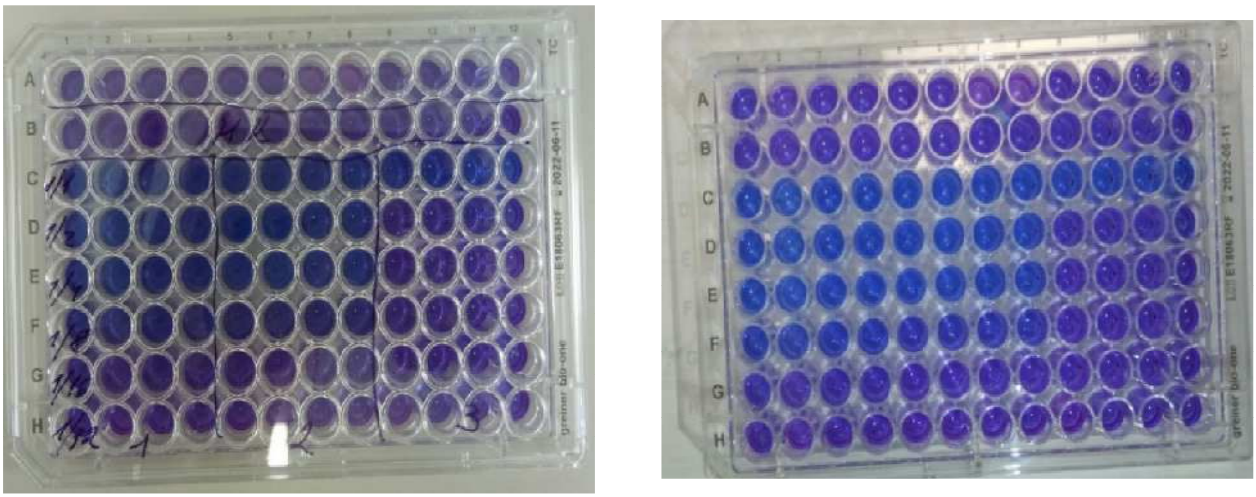

Fig. 6. 96-well plate of cell lines with extracts

Table 3

Relative units fluorescences (RFU)

	1	2	3	4	5	6	7	8	9	10	11	12
B	3801	4195	3961	4136	4031	3882	4136	4255	4175	4017	4287	3955
C	290	231	284	295	290	279	298	296	479	579	577	766
D	280	234	283	288	288	290	293	286	3930	3972	3862	2892
E	292	246	294	294	292	300	297	298	4204	4365	4374	3933
F	1262	1364	1376	1382	2212	2039	2115	2183	4479	4676	4713	4445
G	3239	4064	3868	3737	4408	4254	4221	4246	4783	4753	4740	4446
H	4303	4893	4638	4727	4879	4911	4990	4930	6103	4618	4560	4483

At first, we must calculate average mean of control row. We get 4108 and then we can define the percentage of living cells. Columns 1 and 12 are not taken into account as the effect of evaporation in measuring fluorescence is present.

1 sample				2 sample					3 sample			
	231	284	295		290	279	298	296		479	579	577
	234	283	288		288	290	293	286		3930	3972	3862
	246	294	294		292	300	297	298		4204	4365	4374
	1364	1376	1382		2212	2039	2115	2183		4479	4676	4713
	4064	3868	3737		4408	4254	4221	4246		4783	4753	4740
	4893	4638	4727		4879	4911	4990	4930		6103	4618	4560

Fig. 7. RFU of 1, 2 and 3 samples

Table 4

Determination of effect from *Capsella bursa pastoris* (alc.+water) on cancer cells

Average mean, RFU	Living cells, %	Concentration, g/ml
270	7	0,125
268	7	0,063
278	7	0,031
1374	33	0,016
3890	95	0,008
4753	116	0,004

Table 5

Determination of effect from *Capsella bursa pastoris* (water) on cancer cells

Average mean, RFU	Living cells, %	Concentration, g/ml
291	7	0,125
289	7	0,063
297	7	0,031
2137	52	0,016
4282	104	0,008
4928	120	0,004

Table 6

Determination of effect from *Brassica oleracea var italia* on cancer cells

Average mean, RFU	Living cells, %	Concentration, g/ml
545	13	0,125
3921	95	0,063
4314	105	0,031
4623	113	0,016
4759	116	0,008
5094	124	0,004

Finally, after calculation in Excel we built graph of dependence.

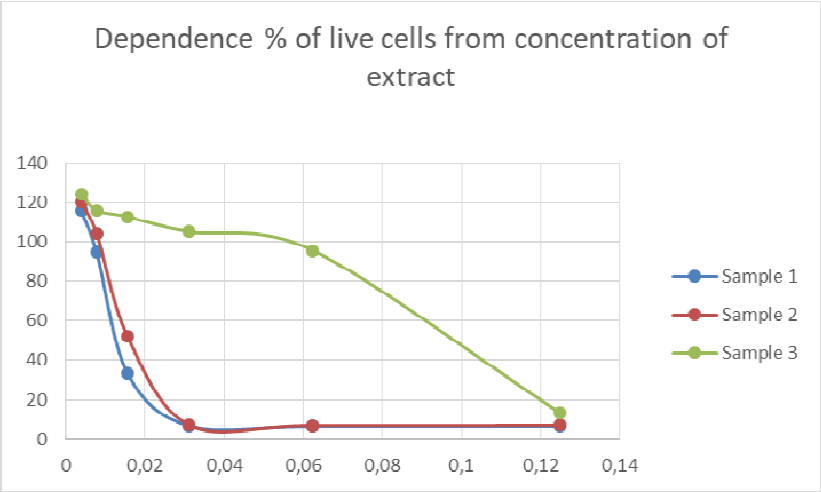

Fig. 8. Graph of dependence: 1 sample – *Capsella bursa pastoris* (alc.+water), 2 sample – *Capsella bursa pastoris* (water), 3 sample – *Brassica oleracea var italia* juice

So, we can observe that *Capsella bursa pastoris* acts on cancer line WISH (monolayer) in minimum concentration of 0,016 g/ml, where the inhibition of cancer cells is 67 %. At the concentration 0,031–0,125 g/ml and more the survival of cell line is 7 %. In contrast, broccoli *Brassica oleracea var italia* have no bright result in our experiment, only at 0,125 g/ml survival of cell line is 13 %.

CONCLUSIONS

The article gives a general description of the chemical composition and pharmacological properties of *Capsella bursa pastoris* and *Brassica oleracea var italia*. The analysis of scientific research devoted to methods of determination of *Capsella bursa pastoris* and *Brassica oleracea var italia* anticancer activity was carried out.

Extraction of biologically active compounds from broccoli and herbs was carried out using 3 different methods: alcohol-water extraction, water extraction and mechanical. Water extraction under 90 °C during 60 minutes with proportions of 1:4 showed the best results.

Capsella bursa pastoris acts on cancer of the WISH line (monolayer) at a minimum concentration of 0.016 g/ml, where the inhibition of cancer cells is 67 %. At a concentration of 0.031–0.125 g/ml or more, the survival rate of cells is 7 %.

Broccoli Brassica oleracea var italia do not have a bright result in our experiment, only at 0,125 g/ml the survival of the cell line is 13 %.

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ВПЛИВ ЕКСТРАКТІВ *BRASSICA OLERACEA* VAR *ITALICA* ТА *CAPSELLA BURSA PASTORIS* НА КЛІТИННУ ЛІНІЮ WISH

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Мета: *дослідити вплив водних екстрактів Brassica oleracea var italica та Capsella bursa pastoris на лінію ракових клітин WISH (моношар). Розглянуто хімічний склад та фармакологічні властивості Brassica oleracea var italica та Capsella bursa pastoris.* ***Методи****: у цій статті розглядається мікроскопічний метод, заснований на інгібуванні та відсотковому визначенні ракових клітинних ліній.* ***Результати****: експеримент показує, що Brassica oleracea var italica і Capsella bursa pastoris можна використовувати проти ракових клітин шийки матки – людської лінії WISH (моношар).*

Ключові слова*: екстракція; Brassica oleracea var italica та Capsella bursa pastoris; WISH (моношар) клітинної лінії; інгібування ракової клітинної лінії.*

ВЛИЯНИЕ ЭКСТРАКТОВ *BRASSICA OLERACEA* VAR *ITALICA* И *CAPSELLA BURSA PASTORIS* НА РАКОВУЮ КЛЕТОЧНУЮ ЛИНИЮ WISH

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Цель*: изучить влияние водных экстрактов Brassica oleracea var italica и Capsella bursa pastoris на линию раковых клеток WISH (монослой). Рассмотрен*

химический состав и фармакологические свойства Brassica oleracea var italica и Capsella bursa pastoris. ***Методы:*** *в данной статье рассматривается микроскопический метод, основанный на ингибировании и определении процентного содержания раковых клеточных линий.* ***Результаты****: эксперимент показывает, что Brassica oleracea var italica и Capsella bursa pastoris могут быть использованы против клеток рака шейки матки - клеточной линии человека WISH (монослой).*

Ключевые слова*: эктракция; Brassica oleracea var italica; Capsella bursa pastoris; WISH (монослойная) клеточная линия; ингибирование линии раковых клеток.*