Evaluation of toxicity and determination IC_{50} of tea leaf extracts on Hela cervical cancer cell line and MCF7 breast cancer cell line in vitro

Nguyen Thi Van Anh[1], Nguyen Duc Thanh[2*]

[1]*Vietnam University of Traditional Medicine, Hanoi*

[2]*Vietnam Military Medical University, Hanoi*

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Abstract

The tea leaves were extracted in methanol 80% (acidified with HCl) and the extracts were purified through two following phases to obtain K powder: the first stage used the silica gel column with gradient elution, the second stage involved in isolating on Sephadex LH-20 column with isometric elution mode. The toxicity tests of the K powder on two MCF37 breast cancer cell lines and Hela cervical cancer line revealed that IC_{50} of K powder with MCF37 cell line was 31.62 µg/mL and with Hela cell line was 50.1 µg/mL. When being compared with the Taxol positive control, the products showed that the efficacy of K powder was lower. However, this is a new direction in the use of drugs derived from plants, in general, and tea leaves, in particular.

Key word: *Tea leaf, uterine cancer, breast cancer, Hela, MCF7, in vitro.*

1. INTRODUCTION

Camellia sinensis O. Ktze Tea belongs to the *Theaceae* family, a wild tree can be 20 m tall. In Vietnam, tea is grown in many places: Thai Nguyen, Son La, Hoa Binh, Phu Tho, Lam Dong, Quang Nam, etc. The used parts are leaves and flower buds. Tea leaves contain tannins, alkaloids, flavonoids, etc. Tea is used to make drinking beverages, stimulate the central nervous system, diuretic, hold mild diarrhea, dysentery [1]. Recently, Japanese scientists have reported about tea against radiation Sr^{90}. Many studies worldwide show that drinking tea, especially green tea, can bring health benefits, preventive support against some dangerous diseases such as blood pressure, obesity, diabetes, some types of cancer, and is also used in treatment therapy combination with chemotherapy [2-4]. Besides, green tea also helps control Alzheimer's and Parkinson's disease, reducing blood clot formation.

Vietnam is the fourth largest tea producer and exporter in the world. The annual production of tea exceeds 1 million tons per year. Therefore, the investment in research and development of tea is needed to ensure product quality and to create new value-added products. Recently, research results around the world show that some of the chemical components of tea, especially EGCG, have remarkable biological activities. As a result, many new health care products have been developed from tea [5].

In this study, we conducted the experiments to extract flavonoids from tea leaves and perform initially *in vitro* tests about the effects of obtained extracts with two cancer cell lines: uterine and breast cancer.

2. MATERIALS AND METHODS

2.1. Materials

The green tea leaves were collected in Hoa Binh province in the spring. Two cell lines of

* *Corresponding author: Tel 0987396956 Email: nguyenducthanh@vmmu.edu.vn*

cancer include Hela cervical cancer and MCF7 breast cancer. Paclitaxel (Taxol) was used as the positive control.

2.2. Methods

2.2.1. Extraction protocol

Flavonoids were isolated from tea leaves with various solvents [6-8], in this study, tea leaves were extracted with methanol, then the extract was isolated through two stages: 1) phase one: Using silicagel 60 column was stuffed in the laboratory, with gradient elution mode; 2) the second phase was isolated on Sephadex LH-20 column (Sigma) with isocratic elution mode. The specific process is as follows:

Five hundred grams of tea leaves were weighed and soaked twice with 80% methanol. After that, the extracts were combined, and the solvent was evaporated under low pressure with a vacuum rotary evaporator to obtain the residue. The residue was hydrolyzed by 10 mL concentrated HCl, continuing to treat hydrolysate with 20 mL $CHCl_3$, adjusted the pH of the extract to about 3-4. After that, the extract was treated with 20 mL ethyl acetate, and dried with anhydrous Na_2SO_4, then evaporated under low pressure to obtain the total flavonoid residue. Firstly, total flavonoid residue was purified by a silica gel column with various solvents, including chloroform, acetone, ethyl acetate, and methanol. Secondly, total flavonoid residue was continued to pass through the Sephadex column with methanol as the eluting solvent. Water was drained off from the eluate to obtain K powder. The effect of K powder on two cancer cell lines was examined.

2.2.2. Activation and propagation of research cell lines

Hela cervical cancer cell line and MCF7 breast cancer cell line were provided by the Department of Biology, University of Science - Vietnam National University, these cell lines were thawed in a 37ºC incubator. Cell pellets were dispersed into culture medium: DMEM + 10% FBS + 1% penicillin. The resulting cell suspension was stirred and then transferred into a Petri dish or bottle, and incubated in a 37ºC incubator with 5% CO_2. When the cell grew tightly to 75 - 90% of the bottle/ petri dish surface, the cells were harvested or transferred into a new petri dish containing the abovementioned culture medium.

Cells were dissociated by adding 5 mL trypsin solution, and collected as well. The percentage of dead cells was tested by staining with Blue trypan. For culture bottles/plates with less than 10% cell death rate were used to conduct toxicity tests *in vitro*.

2.3. Evaluation of toxicity and determination of IC_{50} value by MTS method

To evaluate qualitatively the toxicity of a substrate to cultured cells, we used microscopy to observe cell morphology and MTS method to quantify cell level based on absorbance values (D); Subsequently, the proliferation rate (A%) was calculated and the IC_{50} value was determined for each cell line.

- Transferring cells: proceed to transfer cells in the culture plate 96 wells so that each well has 5×10^3 cells in 180 mL of culture medium.

- Preparing the test concentration series after 24h when the cells in the wells had been stable. Each well was added 20 µL of K powder solution (according to the concentration ranges shown in Table 1) and incubated for 48 hours at 37° C, 5% CO_2. K powder was dissolved in DMSO solvent and then diluted in the culture medium until the nominal concentration of DMSO was 0.5% or below.

- The IC_{50} value is the concentration of the reagent at which K powder inhibits 50% of

cell proliferation compared to the positive control.

- The proliferation rate A% was calculated as follows

$$A\% = V/Vh \times 100\%$$

In which:

V: D value was measured in the testing well

Vh: D value measured in solvent control well

The positive control drug was Taxol.

2.4. Preparation of cell bottles/plates

K powder concentration were prepared (shown in Table 1) and transferred into plate to test toxicity. Taxol positive control was used in this study.

Table 1. *Concentration range of K powder Test*

Cell line	*Code and testing concentration (µg/mL)*									
	C1	**C2**	**C3**	**C4**	**C5**	**C6**	**C7**	**C8**	**C9**	**C10**
Hela	1.95	3.91	7.81	15.62	31.25	62.5	125	250	500	1000
MCF7	1.95	3.91	7.81	15.62	31.25	62.5	125	250	500	1000
[C_{Taxol}]	0.06	0.12	0.234	0.47	0.94	1.87	3.75	7.5	15	30

3. RESULS AND DISCUSSION

3.1. Influence of the solvent on cell growth

After 48 hours of incubation, cell images in positive control wells, incubating with 0.5% DMSO without K powder, are illustrated in Figures 1 and 2. Figure 1A and 2A show cell culture wells without DMSO, while Figure 1B and 2B shows a well with 0.5% DMSO.

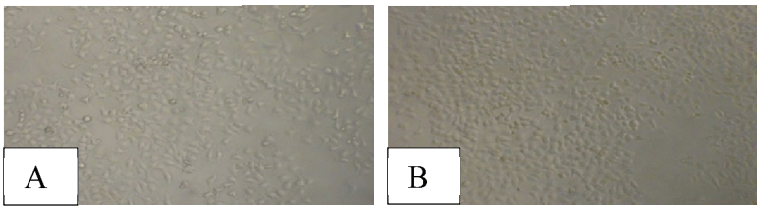

Figure 1. *Image of Hela cells in DCSH well (A) (100 × 4,6) and DCDM (B) (100 × 4,6)*

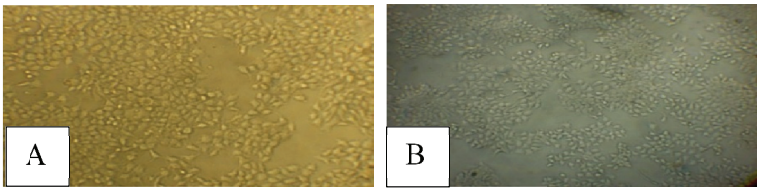

Figure 2. *Image of MCF7 cells in DCSH well (A) (100 × 4,6) and DCDM (B) (100 × 4,6)*

Our findings showed that Hela cell lines and MCF7 cells line were firmly adhered to the bottom of the culture plates with a moderate density, and accounted for about 60 - 70% of the culture bottle surface area; they did not differ between the DCSH well (A) and DCDM well (B).

These results demonstrate that the concentration of 0.5% DMSO in the culture medium does not significantly affected on cell morphology. Based on the cell image in the positive control well, the obtained results are the basis for comparing with the morphology of the cells in the testing wells.

3.2. Testing toxicity and determining the IC_{50} value of powder K in Hela and MCF7 cell lines

The result of testing K powder and Taxol positive control are shown in Figures 3, 4, 5, 6. Figures C1 to C10 are wells with K powder solution, respectively, with the concentration shown in Table 1. After incubating with K powder and Taxol positive control for 48 hours, and change the concentration with 10 values range from C1 to C10, the results of optical absorbance (D), the proliferation ratio (A%) and IC_{50} value are shown in Table 2 and Table 3.

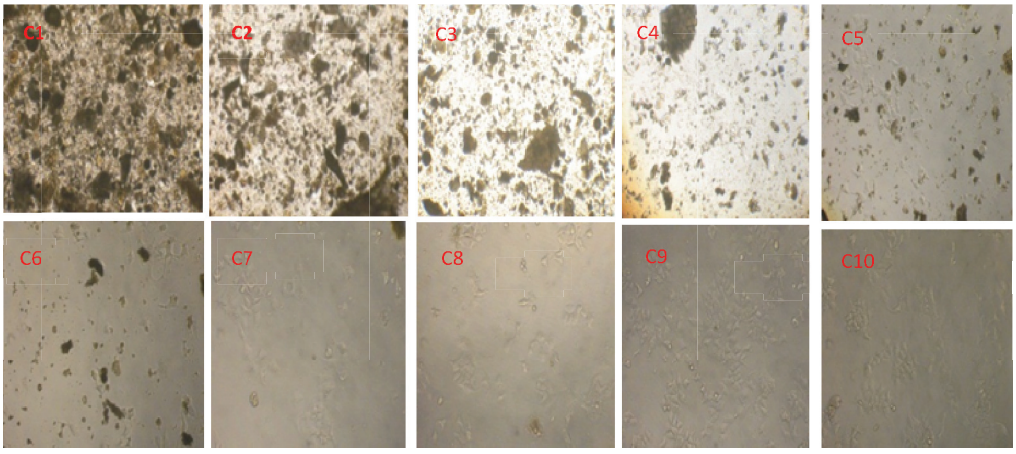

***Figure 3**. Image of Hela cells under the effect of inoculant at different concentrations (C1 to C10) as shown in Table 1 (100 × 4,6)*

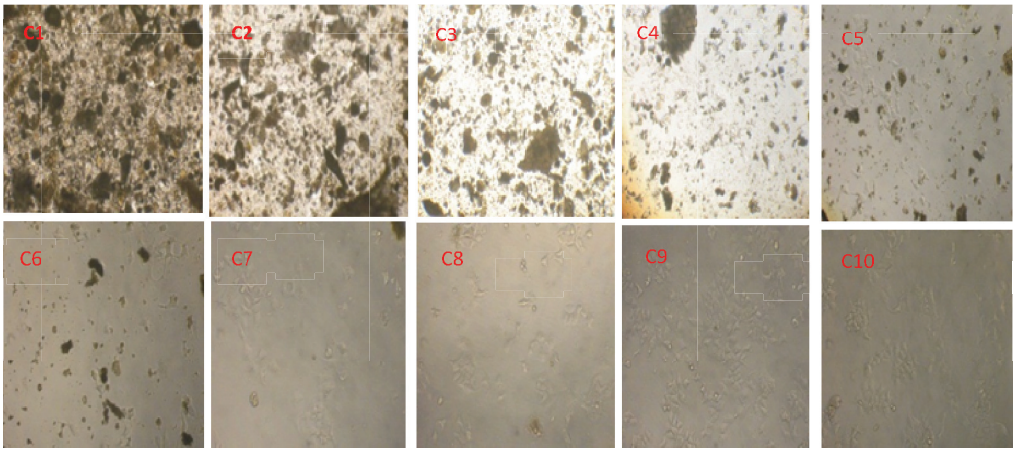

***Figure 4**. Image of Hela cells under the effect of Taxol positive control at different concentrations (C1 to C10) as shown in Table 1 (100 × 4.6)*

For the Hela cervical cancer cell line: The results are shown in Figure 3 and Figure 4. Under the impact of K powder with the same concentration range, the obtained image revealed that the product has a toxic effect on Hela cells but has been slower and less powerful than the MCF7 line. For Hela cell line, at 31.25 µg/mL concentration (C5), cells had a change in shape, decreased slightly in quantity, and showed the adhesion shape in morphology. At 62.5 µg/mL

concentration (C6), the cell has a significant change in morphology although it has a semi-adhesive shape but quite weak. The more concentrated K powder, the more change of cell morphology and its adhesion shape were observed. Consequently, at C7 concentration (125 g/mL), cells did not have the original shape anymore, the amount of cell decreased sharply, at A% corresponding to 45.45%. The D optical density, which was corresponding to the IC_{50} value in the Hela cell line, was 51.35 ± 1.76 µg/mL (R^2 = 0.988).

The obtained IC_{50} value of K powder in Hela and MCF7 cell lines were 50.1 µg/mL and 31.62 µg/mL, respectively. As a result, K powder had more effective in MCF7 breast cancer cell line than in Hela cervical cancer cell line.

***Table 2**. Optical density (D), proliferation rate (A%) of K powder in Hela cells*

Reagent concentration (µg/mL)	***K powder***		***Taxol positive control***	
	D_{490}	A%	D_{490}	A%
DCDM	**0.571**	**100**	**0.571**	**100**
1000	0.138	27.74	0.098	17.18
500	0.165	28.92	0.099	17.22
250	0.174	29.92	0.121	21.13
125	0.919	30.65	0.163	28.52
62.5	0.272	33.61	0.258	45.31
31.25	0.279	47.81	0.268	47.02
15.62	0.359	48.90	0.376	65.99
7.8	0.386	62.92	0.377	66.17
3.9	0.428	75.1	0.385	67.57
1.95	0.462	81.02	0.424	74.36
	IC_{50} = 51.35 ± 1.76 µg (R^2 = 0.988)		**IC_{50} = 1.15 µg (R^2 = 0.988)**	

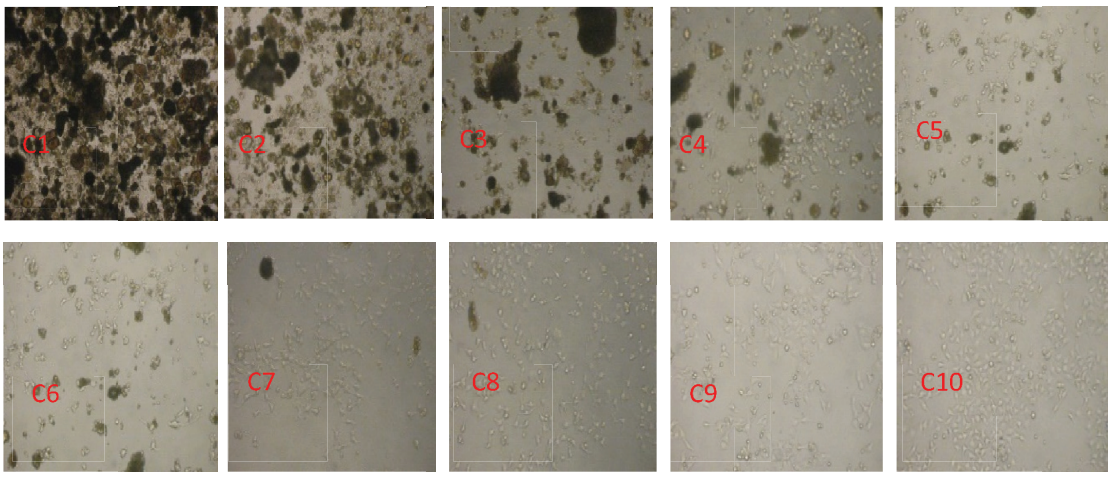

***Figure 5**. Image of MCF7 cells under the effect of K powder at different concentrations (C1 to C10) as shown in Table 1 (100 × 4,6)*

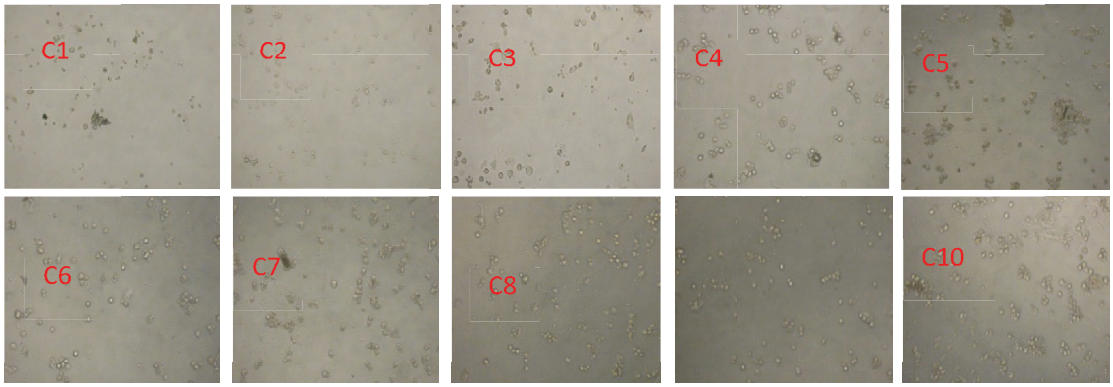

***Figure 6**. Image of MCF7 cells under the effect of Taxol positive control at different concentrations (C1 to C10) as shown in Table 1 (100 × 4.6)*

For MCF7 breast cancer cell line, results are shown in Figure 5 and Figure 6. After incubating with concentration as shown in Table 1 for 48 hours, it was observed that K powder had toxic effects to the cells, but the toxicity was not as high as Taxol positive control. At the lowest concentration (0.06 µg/mL) (C1), Taxol had a relatively high toxic effect. As a result, this caused the cells to shrink to 90%, and D optical density corresponding to IC_{50} value was low (only 0.013 µg (R^2 = 0.988). However, for K powder at C4 concentration (15.62 µg/mL), although the amounts of cells have been decreased, the basal cell shape was still quite firm; D optical density corresponding with IC_{50} value was 30.48 ± 1.6 µg/mL (R^2 = 0.958).

***Table 3**. Optical density (D) and proliferation rate (A%) of K powder in MCF7 cells*

Reagent concentration (µg/mL)	*K powder*		*Taxol positive control*	
	D_{490}	*A%*	D_{490}	*A%*
DCDM	**0.467**	**100**	**0.467**	**100**
1000	0.165	35.34	0.111	23.74
500	0.178	38.14	0.123	28.13
250	0.18	38.55	0.181	38.82
125	0.212	45.45	0.185	39.57
62.5	0.213	45.56	0.186	39.67
31.25	0.22	47.05	0.25	53.47
15.62	0.33	71.28	0.26	53.90
7.8	0.411	87.96	0.337	72.19
3.9	0.415	88.84	0.369	78.93
1.95	0.417	89.35	0.373	79.89
	IC_{50} = 30.48 ± 1.6 µg (R^2 = 0.958)		**IC_{50} = 0.13 µg (R^2 = 0.961)**	

4. CONCLUSION

Our study has extracted K powder from tea leaves. This powder has been used to test toxicity in MCF7 breast cancer cell lines and Hela cervical cancer cell line. The results revealed

that K powder has the ability to inhibit both MCF7 and Hela cancer cell lines but brought better results for MCF7 breast cancer cell line. Overall, our findings are the premise in order to conduct further researches for cancer treatment products from Vietnamese tea leaves.

5. REFERENCE

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Nghiên cứu đánh giá độc tính và xác định giá trị IC_{50} của chế phẩm chiết từ lá chè trên dòng tế bào ung thư cổ tử cung Hela và dòng tế bào ung thư vú MCF7 nuôi cấy in vitro

Nguyễn Thị Vân Anh[1], Nguyễn Đức Thanh[2]

[1]Học viện Y Dược học cổ truyền Việt Nam

[2]Học viện Quân Y

Tóm tắt

Lá chè được chiết bằng methanol 80% (acid hóa bằng HCl), dịch chiết được tinh khiết hóa qua hai giai đoạn: giai đoạn một sử dụng cột silicagel 60 với chế độ rửa giải gradient, giai đoạn hai tiếp tục phân lập trên cột Sephadex LH-20 với chế độ rửa giải đẳng dòng. Thu được bột K. Tiến hành thử độc tính của bột K trên hai dòng tế bào ung thư vú MCF37 và ung thư cổ tử cung Hela thu được kết quả: giá trị IC_{50} của chế phẩm với dòng MCF37 là 31,62 µg/mL và với dòng Hela là 50,1 µg/mL. So sánh với chất đối chứng dương là Taxol cho thấy hiệu lực của sản phẩm thu được còn thấp hơn, tuy nhiên đây là một hướng mới trong việc sử dụng thuốc có nguồn gốc thực vật nói chung và lá chè nói riêng.

***Từ khóa*:** *Lá chè, ung thư tử cung, ung thư vú, Hela, MCF7, in vitro.*