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STUDY OF THE CYTOTOXIC ACTIVITY OF ALKALOID- CONTAINING FRACTIONS ISOLATED  
FROM CERTAIN PLANT SPECIES GROWING AND INTRODUCED IN GEORGIA

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საქართველოში ზოგიერთი მოზარდი და ინტროდუცირებული მცენარეებიდან მიღებული  
ალკალიოიდმცველი ფრაქციების ციტოტოქსიკური აქტივობის შესწავლა

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### რეზიუმე

საქართველოში მოზარდი მცენარეებიდან: *Ephedra procera* Fisch (ოჯ. *Ephedraceae*), *Taxus baccata* L. (ოჯ. *Taxaceae*) და საქართველოში ინტროდუცირებული *Mahonia aquifolium* (Pursh) Nutt. (ოჯ. *Berberidaceae*), სითხე-სითხოვანი ექსტრაქციის მეთოდით მიღებულია ალკალიოიდების ბიოლოგიურად აქტიური ჯამური სუბსტანციები. ფიტოქიმიური შესწავლის საფუძველზე დადგენილია, რომ დომინანტური ალკალიოიდები მიეკუთვნებიან აციკლურ, დიტერპენულ და იზოქინოლინის ჯგუფებს, შესაბამისად.

*In vitro* სპეციფიკური ბიოლოგიური ციტოტოქსიკური აქტივობების შეფასებისას, ალკალიოიდმცველმა ჯამურმა სუბსტანციებმა აჩვენეს: ძლიერი აქტივობა ფილტვის კარცინომის უჯრედული ხაზების წინააღმდეგ (A-549); საშუალო და სუსტი აქტივობა მსხვილი ნაწლავის კარცინომის უჯრედული ხაზების (DLD-1) და ადამიანის კანის ფიბრობლასტების უჯრედული ხაზების (WS-1) წინააღმდეგ, *Ephedra procera*-ს შემთხვევაში; *Taxus baccata* L.-ის ალკალიოიდების შემცველი გამდიდრებული ფრაქციის შემთხვევაში აღინიშნა მნიშვნელოვანი ციტოტოქსიკური ეფექტი A-549 და DLD-1 უჯრედების მიმართ, ამასთან მსუბუქი სპეციფიკურობა WS-1 უჯრედების შემთხვევაში; საშუალო ციტოტოქსიკური აქტივობა ნაჩვენებ იყო *Mahonia aquifolium*-ის ალკალიოიდების გამდიდრებული ფრაქციის შემთხვევაში ყველა უჯრედული ხაზის მიმართ - A-549, DLD-1 და WS-1, *Resazurine* და *Hoechst* მეთოდების მიხედვით.

Decoctions, tinctures, extracts and ointments of alkaloid containing plants have been used in folk and traditional medicine for the treatment of various diseases since time immemorial (antispasmodic, bacteriocidal, anti-cancer and etc.) As of today, there is great number of various medications on the pharmaceutical market – synthetic, semi-synthetic and natural. Under the aforementioned types, herbal medications are among the most popular. Modern experimental medicine uses up to 250 species of medicinal plants, with biomarkers of different chemical nature. Among these active substances alkaloids are one of the leading components. These are mainly optically active pure enantiomers. They are characterized by immense varieties of biological and pharmacological activities, mild therapeutic activity, high bioavailability and have fewer side effects [1,2,3].

It is a well known fact that cancer is a widely spread disease around the world. As of today oncological treatment include surgical, radiotherapy and chemotherapy. Among the chemotherapeutic medications an important part is occupied by plant based remedies or natural compounds and their derivatives which are extracted from them. Based on the above search for new sources of alkaloid containing plants can be considered highly relevant [4,5,6,7].

**The aim** of our research was the study of cytotoxic activity of alkaloid-containing fractions isolated from certain plant species growing and introduced in Georgia.

**Material and methods.** Research objects: *Ephedra procera* Fisch (Fam. *Ephedraceae*), *Taxus baccata* L. (Fam. *Taxaceae*), both growing in Georgia and introduced species - *Mahonia aquifolium* (Pursh) Nutt. (Fam. *Berberidaceae*).

*Ephedra procera* (0,2 kg) was harvested during flowering and fruiting phases at Akhaltsikhe (Rabathi castle) and areas surrounding Tbilisi. Purifications of aerial vegetative organs from non-alkaloid compounds were realized by extraction with hexane and petroleum ether. After preliminary alkalization with 12% ammonia, extraction was performed with chloroform. Combined extracts were purified with 5% hydrochloric acid. To the acidic phase 25% NH<sub>4</sub>OH was added and extraction was followed with chloroform. The yield of total substance was 0.3% based on air dry material of the plant.

*Taxus baccata*, was harvested during flowering in Terjola region. Air dried chopped needles (0,6 kg) were extracted with 96° ethanol. The combined and condensed extract was purified with petroleum ether, after that it was alkalized with 25% ammonia after which alkaloids were extracted with chloroform. The yield of total substance calculated based on air dry plant material was 0.33% in which the fractions obtained by polybuffer extraction were enriched with Taxol – 0,083%.

*Mahonia aquifolium*, introduced in Georgia, was harvested during flowering and fruiting phases in areas surrounding Tbilisi. Air dried pulverized flowers, bark and seeds as well as underground organs (roots) in the amount of 0,2kg and 0,1kg respectively preliminary alkalized was done with 12% NH<sub>4</sub>OH and alkaloids extraction was performed with chloroform. Combined extracts were purified by 10% H<sub>2</sub>SO<sub>4</sub>. The acidic fraction was processed with petroleum ether. Extracts were alkalized with aid of concentrated ammonia to a pH of 9 and extracted using chloroform. The yield of the alkaloid sum was: for flowers – 0,38%, for bark – 0,22%, for roots – 0,58%, for seeds – 0,3% of the air-dried mass of the plant.

**Results and discussion.** Phytochemical study on above mentioned plants was performed with GC/MS and TLC analysis to determine qualitatively and quantitatively composition. TLC conditions: Silicagel 254, Merck; Mobile phases: I-III - chloroform-methanol (9:1; 6:1; 4:1); IV -chloroform: methanol: 10% NH<sub>4</sub>OH (15:4:1); V - butanol: acetic acid: water (10:1:3); detection – ninhydrin, dragendorff reagent, iode chamber. With comparison to reference standards was determined, that identified compounds were attended to acyclic, diterpene and isoquinoline classes of alkaloids. Some of their chemical characteristics based on literature data as well as on the results generated by Department of Alkaloids of Iovel Kutateladze Institute of Pharmacochimistry (TSMU), is shown in table 1.

**Table1.** *Ephedra procera*, *Taxus baccata*, *Mahonia aquifolium*. dominant alkaloids identified in alkaloid-containing fractions

N	Alkaloids	Group of Alkaloids	Empirical formula	Molecular mass	Melting point	Specific rotation $[\alpha]_D^{20}$
<b><i>Ephedra procera</i> Fisch (fam. <i>Ephedraceae</i>)</b>						
1	L-ephedrine	acyclic	C <sub>10</sub> H <sub>15</sub> NO	165,23	73°-74° (EtOH)	-6,8(EtOH)
2	Pseudoephedrine	acyclic	C <sub>10</sub> H <sub>15</sub> NO	165,23	118°-119° (EtOH)	+53(EtOH)
<b><i>Taxus baccata</i> L. (fam. <i>Taxaceae</i>)</b>						
3	Taxol	diterpene	C <sub>47</sub> H <sub>51</sub> NO <sub>14</sub>	853(M+)	205°-208° (Water-C <sub>2</sub> H <sub>5</sub> OH)	-54(MetOH)
4	Karakoline	diterpene	C <sub>22</sub> H <sub>35</sub> NO <sub>4</sub>	377.2566	185°-187° (MetOH)	-21(Pyridine)
<b><i>Mahonia aquifolium</i> (Pursh) Nutt. (fam. <i>Berberidaceae</i>)</b>						
5	Berberine	isoquinoline	C <sub>20</sub> H <sub>18</sub> N <sup>+</sup> O <sub>4</sub> I <sup>-</sup>	336,1236	260°-262° (MetOH)	±0(Water)
6	Jatrorrhizine	isoquinoline	C <sub>20</sub> H <sub>20</sub> N <sup>+</sup> O <sub>4</sub> I <sup>-</sup>	338.1392	209°-210° (MetOH)	±0(Water)
7	Palmatine	isoquinoline	C <sub>21</sub> H <sub>23</sub> N <sup>+</sup> O <sub>4</sub>	352,1349	205°-206° (Chloride)	±0(Water)
8	Magnoflorine	isoquinoline	C <sub>20</sub> H <sub>24</sub> N <sup>+</sup> O <sub>4</sub> I <sup>-</sup>	608,2886	249°-252° (MetOH)	+100(Water)

*In vitro* Cytotoxic activity analysis was performed on three cell cultures: A-549 (Human lung cancer carcinoma cell culture ATCC#CCL-185); DLD-1 (cell culture of the rectum adenocarcinoma - ATCC#CCL-221) WS-1 (Human Dermal Fibroblasts) which are received from ATCC (American and Type Culture Collection - Manasa, USA). Cancer cells were cultivated in Earle salt and L-glutamine growth medium. (Earle's salts content: KCl, NaCl, NaH<sub>2</sub>PO<sub>4</sub> · H<sub>2</sub>O, D-Glucose, MgSO<sub>4</sub> · 7H<sub>2</sub>O, CaCl<sub>2</sub> · 2H<sub>2</sub>O, NaHCO<sub>3</sub>, red phenol), then was added 10% fetal calf serum (Hyklon, Logan, USA) vitamins (1X), Penicillin

(100 I.U/ml) and Streptomycin (100 mkg/ml), amino acids (1X), Sodium pyruvate (Mediaech Cellgro, VA). Incubation of cells was performed at 37°C temperature in humid atmospheric conditions CO<sub>2</sub> 5%.

Cytotoxic activity was determined by inhibitory concentration (IC<sub>50</sub>), which inhibits 50% growth of cells. Etoposide was used as a reference (Table 2).

**Table 2.** *In vitro* cytotoxic activity of total alkaloid fractions

№	Plant	Vegetative organ	Line cells and methods					
			Resazurin			Hoechst		
			A-549	DLD-1	WS-1	A-549	DLD-1	WS-1
1	<i>Ephedra Procera Fisch</i>	Aerial parts	43±3µg/ml	>200µg/ml	130±3µg/ml	36±5µg/ml	155±11µg/ml	114±4µg/ml
2	<i>Taxus baccata L.</i>	Needles	13±14µg/ml	16±12µg/ml	79±12µg/ml	4±5µg/ml	2±5µg/ml	>200µg/ml
3	<i>Mahonia aquifolium</i> (Pursh) Nutt.	Flowers	25±3µg/ml	17,8±0,2µg/ml	9,7±0,9µg/ml	15±2µg/ml	13,4±0,4µg/ml	9±2µg/ml
		Bark	23±2µg/ml	23±2µg/ml	9±1µg/ml	13±1µg/ml	15±1µg/ml	11,7±0,8µg/ml
		Roots	20±2µg/ml	20±2µg/ml	6,4±0,8µg/ml	8±2µg/ml	11±1µg/ml	15,0±0,6µg/ml
		Seed	39±6µg/ml	26±2µg/ml	14±1µg/ml	23±3µg/ml	18±2µg/ml	21±4µg/ml
<b>Etoposide</b>			2,3±0,2µM	2,8±0,4µM	19±3µM	1,18±0µM	1,0±0µM	>50µM

**Conclusion:** As a result of the conducted phytochemical studies, it has been determined that the total sum of the purified alkaloids, obtained by liquid-liquid extraction from the studied plants, are dominated by alkaloids of the following groups: *Ephedra procera* – acyclic (L-ephedrine and pseudoephedrine); *Taxus baccata* - diterpen (taxol and karakolin); *Mahonia aquifolium* – isoquinoline (berberine, jatrorrhizine, palmatine, magnoflorine).

Cytotoxic activity of the studied samples was done *in vitro* on three cell lines - A-549 (lung carcinoma), DLD-1 (rectal adenocarcinoma) and WS-1 (normal human fibroblast) using the *Resazurine* and *Hoechst* tests. The following results were obtained: the total alkaloid fraction isolated from *Ephedra procera* showed moderate cytotoxic activity against the A-549 cell lines, while the fraction was not active against DLD-1 cells. Weak activity was recorded against WS-1 cell. According to the *Hoechst* method, medium activity was recorded against the A-549 cell line. The tested fraction showed very weak cytotoxic activity against DLD-1 and WS-1 lines.

The total alkaloid sum isolated from *Taxus baccata* showed moderate cytotoxic activity against A-549 and DLD-1 cells according to the *Resazurine* model, while showing strong specific cytotoxic activity against both A-549 and DLD-1 cell lines, showing weak cytotoxicity against WS-1 cells in the *Hoechst* method.

The isolated alkaloid enriched fractions from different organs of *Mahonia aquifolium* showed moderate activity against all three studied cell lines (A-549, DLD-1, WS-1) in both the *Resazurine* and *Hoechst* methods without any specificity.

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**ИЗУЧЕНИЕ ЦИТОТОКСИЧЕСКОЙ АКТИВНОСТИ АЛКАЛОИДСОДЕРЖАЩИХ ФРАКЦИЙ,  
 ВЫДЕЛЕННЫХ ИЗ НЕКОТОРЫХ ВИДОВ РАСТЕНИЙ, ПРОИЗРАСТАЮЩИХ И  
 ИНТРОДУЦИРОВАННЫХ В ГРУЗИИ**

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**РЕЗЮМЕ**

Объектами исследования были растения, произрастающие на территории Грузии: *Ephedra procera* Fisch, (сем. *Ephedraceae*), *Taxus baccata* L. (сем. *Taxaceae*) и интродуцированная в Грузии *Mahonia aquifolium* (Pursh) Nutt. (сем. *Berberidaceae*). Биологически активные алкалоид содержащие субстанции были получены методом жидкость-жидкостной экстракцией. В вышеуказанных видах растений, доминирующие алкалоиды принадлежат ациклическим, дитерпеновым и изохинолиновым группам алкалоидов, соответственно.

Цитотоксическую активность *in vitro* оценивали на A-549 (клетки линий карциномы легкого), DLD-1 (клетки аденокарциномы толстой кишки) и WS-1 (нормальные клетки линий фибробластов человека). Сумма алкалоидов, полученных из *Ephedra procera*, проявила сильную активность в отношении A-549, среднюю и слабую против DLD-1 и WS-1. Обогащенная алкалоидами фракция, выделенная из *Taxus baccata* L., продемонстрировала выраженную специфическую цитотоксическую активность в случае клеток линий A-549 и DLD-1, а против клеток линий WS-1 проявила слабую активность. Фракции обогащенные алкалоидами, полученные из *Mahonia aquifolium*, проявили среднюю цитотоксическую активность по отношению всех клеток линий- A-549, DLD-1 и WS-1, согласно *Resazurine* и *Hoechst* методам.

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**STUDY OF THE CYTOTOXIC ACTIVITY OF ALKALOID- CONTAINING FRACTIONS ISOLATED FROM  
 CERTAIN PLANT SPECIES GROWING AND INTRODUCED IN GEORGIA**

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**SUMMARY**

The objects of the study were *Ephedra procera* Fisch (Fam. *Ephedraceae*), *Taxus baccata* L. (Fam. *Taxaceae*) and *Mahonia aquifolium* (Pursh) Nutt (Fam. *Berberidaceae*) growing or introduced in Georgia. Biologically active alkaloid-containing substances were obtained by liquid-liquid extraction. In all studied species, the dominant alkaloids belong to the acyclic, diterpene and isoquinoline groups of alkaloids, respectively.

*In vitro* cytotoxic activity was evaluated on A-549 (lung carcinoma cell lines), DLD-1 (colon adenocarcinoma cells) and WS-1 (human fibroblast normal cell lines). The sums of alkaloids obtained from *Ephedra Procera* showed selective cytotoxic activity against A-549 and DLD-1. An alkaloid enriched fraction isolated from *Taxus baccata* L. demonstrated significant specific cytotoxic activity against A-549 and DLD-1 cell lines. The sums obtained from *Mahonia aquifolium* exhibited pronounced activity against all cell lines - A-549, DLD-1 and WS-1 according to the *Resazurine* and *Hoechst* models.

**Keywords:** *Ephedra procera* Fisch, *Taxus baccata* L., *Mahonia aquifolium* (Pursh) Nutt., alkaloid, cytotoxic activity.