

## Study of Alkaloids from the species *Mahonia bealei* (Fort.) Carr. introduced in Georgia

Todua Nika<sup>1</sup>, Chincharadze David<sup>2</sup>, Vachnadze Nina<sup>3</sup>, Getia Malkhaz<sup>4</sup>

<sup>1</sup>Tbilisi State Medical University, Direction of pharmacognosy and pharmaceutical botany, PhD Student; <sup>2</sup>Tbilisi State Medical University, Department of pharmacognosy and pharmaceutical botany, Head of Department; <sup>3</sup>Tbilisi state medical university; Iovel Kutateladze Institute of Pharmacochemistry; Department of Phytochemistry, direction of Alkaloids& Standardization Principal research scientist; <sup>4</sup>Tbilisi state medical university; Iovel Kutateladze Institute of Pharmacochemistry; Department of Pharmaceutical Analysis & Standardization, *Principal research scientist*, ORCID 0000-0001-7729-8042

### Abstract

The aim of the work was to study the introduced plant on the Black Sea coast of Adjara *Mahonia bealei* (Fort.) Carr. for the content of alkaloids. A comparative analysis of the quantitative yield of the total amounts of alkaloids isolated from the aerial organs of *M. bealei*, collected in the phase of active flowering, showed the advantage of the amount obtained from flowers - 2.48%. Based on the results of the HPLC analysis, it was concluded not only about the percentage of the main protoberberine and aporphine alkaloids, but also in which fractions, which alkaloid is dominant (in leaves, three bases dominate at once: berberine (27%), jatrorrhizine (23%), magnoflorin (18%), in branches and flowers - dominated by one base - berberine (50%) and palmatin (57%), respectively, the rest are present in the form of traces).

The results of the preliminary investigation suggest that the alkaloid containing substances obtained from the aboveground vegetative organs of the plant demonstrate antioxidant and anti-inflammatory activities.

**Keywords:** *Alkaloids, berberine, liquid-liquid extraction, Mahonia, anti-inflammatory*

### Introduction

The study of introduced plant species of Georgia for the reliability of alkaloid-ness has been carried out since 1946, as a result of pharmacobotanical expeditions, plant samples were collected in the territories of: Svaneti, Racha-Lechkhumi, Samegrelo, Imereti, Guria, Adjara, Shida Kartli, Mtiuleti, Tush-Pshav-Khevsureti, Kakheti, Kiziki, Gare Kakheti, Trialeti, Kvemo Kartli, Javakheti. Local bioecological and soil-climatic zones of these regions, especially the Black Sea coast of Adjara, contributed to the introduction of valuable medicinal foreign plant species. For example, one of the representatives of the collection of the Batumi Botanical Garden is the genus *Mahonia* Nutt.,

represented by 5 introduced plant species belonging to the family *Berberidaceae* Torr. et Gray: *M. bealei* (Fort.) Carr., *M. japonica* (Thunb.) DC., which were naturalized back in 1913, and *M. fortunei* (Lindl.) Fedde, *M. lomariifolia* Takeda., *M. wagneri* Jouin.- in the 70s of the last century (information provided by employees of the Batumi Botanical Garden) [1,2,3,4].

In view of the fact that not a single species introduced on the territory of Georgia, Mahonia was studied for the content of biologically active bases, the object for the study was Mahonia bealei (Fort.) Carr., which was first studied for alkaloid content at the TSMU, Institute of Pharmacochimistry named after . Kutateladze, direction of alkaloids. This plant is native to the mountainous regions of southern China and is included in the Chinese Pharmacopoeia (2000). Its leaves, stems, and roots have long been used in Chinese traditional folk medicine to treat a variety of conditions, including colds, dysentery, jaundice, and pharyngolaryngitis, as well as to improve blood circulation.

The family *Berberidaceae* is known for its high content of isoquinoline alkaloids, which have a wide range of physiological activities. To date, according to the literature, alkaloid-containing total substances and individual compounds isolated directly from Mahonia bealei, according to *in vitro* and *in vivo* tests, are positively evaluated for anti-tuberculosis, antimicrobial, antioxidant, anti-inflammatory and many other activities [5,6,9].

*M. bealei*, is a low-growing evergreen shrub. The shoots are thick, erect. The leaves are alternate, compound, unpaired pinnate, composed of egg-shaped or elongated leaflets, dense, leathery, shiny, with a sharp-toothed edge. The flowers are bright yellow, collected in multi-flowered apical panicles and tassels. Numerous small fruits in the form of spherical oval berries, black-purple with a blue coating. Based on phenological observations, it was found that in the second half of February, the plant comes out of a dormant state. From the end of February to the beginning of March, the flowering phase is noted. In the second half of March - by the end of May, the growth of shoots begins and ends. Fruit ripening ends in June. All of these steps indicate the sequential and complete passage of all phenological phases by the introduced plant [3,7,8].

Since the phytochemical composition of Mahonia bealei introduced to the territory of Georgia has not been studied, this research intended to to analyse the plant's alkaloid content based on this the aim of the work was to study the introduced into Georgia plant Mahonia bealei for the content of alkaloids.

**Materials and Methods.** The object of research was the above-ground, vegetative organs of the plant (leaves, branches, flowers) collected in the Batumi Botanical Garden in the phase of active flowering.

**Qualitative and quantitative analysis of vegetative organs of the plant for the content of alkaloids.** 15 g of crushed leaves, stems, above-ground organs of the plant were extracted on water bath with ethyl alcohol at 100°C and insisted for 24 hours at the room temperature. The extract was filtered and evaporated under vacuum, dissolved in 2N HCl and alkaloid precipitation reactions were carried out with following reagents: Dragendorf, Sonnanstein, Valser, Mayer, silica tungsten and picric acids [10].

After a positive reaction to alkaloids, the raw materials were subjected to further analysis. The aerial vegetative organs of *M. bealei*, in an amount of 250 g (leaves-120 g, branches-80 g, flowers-50 g), were extracted with hexane, in order to remove lipophilic substances. The raw materials were extracted three times with ethyl alcohol, using liquid-liquid extraction and insisted at the room temperature. The extracts were combined and evaporated under the vacuum and the residue, as a sum of bases, was

treated with 5% HCl. Acid extracts were washed with chloroform, the sum was alkalinized with a 25% ammonia solution (while cooling) to pH 9-10 and simultaneously the alkaloids were extracted with chloroform. The chloroform extract was dried with anhydrous Na<sub>2</sub>SO<sub>4</sub>, filtered, the solvent was evaporated and the residue was dried. After the extraction of tertiary and quaternary bases from acidified (pH 3, 10% H<sub>2</sub>SO<sub>4</sub>) mother solutions, the quaternary bases were isolated by adding a saturated solution of potassium iodide.

Identification of alkaloids in fractions was carried out by using of TLC (thin layer chromatography), in the solvent system: Butanol - Acetic acid - Water (10 : 1 : 3) with the standards: berberine, palmatin, jatrorrhizine, magnoflorin (Boiron laboratory, France), on the plate Siliagel 254, Merck; Dragendorff's reagent and iodine vapor were used as detectors, For qualitative and quantitative analyses was used method of high-performance liquid chromatography (HPLC); Agilent Technologies 1260; column: Eclipse plus C18; 4.6 x 250mm, 5 µm; Column temp.: 20°C; Flow: 1 ml/min. Solvents: A - Water (0,1% HCOOC), B - ACN (0,1% HCOOC); Solv. B%: 10→100%; 20 min.

**Results and discussions.** while using technic of thin layer chromatography, total fractions containing quaternary bases, in the presence of standards, compounds were identified that, by a qualitative reaction with Dragendorff's reagent and iodine vapor with simultaneous mobility on TLC, gave a characteristic color: bright yellow - berberine, yellow-palmatine, pinkish red -jatrorrhizine, brown-magnoflorine . According to HPLC analysis in total fractions, the main quaternary alkaloids were identified and quantified. (table 1).

**Table 1. The results of the study of the vegetative organs of *Mahonia bealei*, for the content of alkaloids**

Species	Phenological stage	Vegetative organs of a plant	The total of alkaloids, % of the mass air/dry raw	Content of quaternary based in alkaloid containing total fractions, %			
				Aporphine alkaloids	Protoberberine alkaloids		
					Magnoflorin	Berberine	Jatrorrhizine
<i>Mahonia bealei</i> (Fort.) Carr.	Active flowering	leaves	0,07	18	27	23	2
		branches	1,33	trace	50	5	3
		flowers	2,48	trace	trace	trace	57

A comparative analysis of the total amounts of alkaloids revealed the advantage of the total substance obtained from flowers - 2.48%. Based on the results of the HPLC analysis, we can conclude not only the percentage of the main protoberberine and aporphine alkaloids in the fractions but also which alkaloid is dominant. For example, in leaves - three bases dominate at once: berberine (27%), jatrorrhizine (23%), and magnoflorin (18%), in branches and flowers - one base dominates - berberine (50%) and palmatin (57%), respectively, the rest are present in the form of traces.

In Table 2 are shown the formulas with the main physical and chemical characteristics of alkaloids: berberine, palmatin, jatrorrhizine and magnoflorin.

**Table 2.** Quaternary bases presented in total fractions obtained from the vegetative organs of *Mahonia bealei*.

#	name	Structure	Plant	Place of collection
1	<b>Berberine iodide</b> C <sub>20</sub> H <sub>18</sub> N <sup>+</sup> O <sub>4</sub> I <sup>-</sup> 336.1236 melting poit: 260°C -262°C [α] <sub>D</sub> ±0° R <sub>1</sub> =R <sub>2</sub> = CH <sub>2</sub> O <sub>2</sub> ; R <sub>3</sub> = H; R <sub>4</sub> =R <sub>5</sub> =OCH <sub>3</sub>		<i>Mahonia bealei</i> (Fort.) Carr	Botanical garden of Batumi
2	<b>Palmatin iodide</b> C <sub>21</sub> H <sub>22</sub> INO <sub>4</sub> 479,3 [α] <sub>D</sub> ±0° melting poit: 203°C -204°C R <sub>1</sub> =R <sub>2</sub> =OCH <sub>3</sub>			
3	<b>Jatroricin iodide</b> C <sub>20</sub> H <sub>20</sub> N <sup>+</sup> O <sub>4</sub> I <sup>-</sup> 338.1392 melting poit: 209°C -210°C [α] <sub>D</sub> ±0° R <sub>1</sub> =R <sub>4</sub> =R <sub>5</sub> =OCH <sub>3</sub> ; R <sub>2</sub> =OH; R <sub>3</sub> =H			
4	<b>Magnoflorin iodide</b> C <sub>20</sub> H <sub>24</sub> N <sup>+</sup> O <sub>5</sub> I <sup>-</sup> melting poit: 249°C-250°C [α] <sub>D</sub> ±100°			

HPLC analysis of the extracts derived from the vegetative organs of the *Mahonia bealei* and and main physical and chemical characteristics of the alkaloids, confirm the presence of the abovementioned bases.

**Conclusions:** As a result of assessing the content of total alkaloids in the vegetative organs in the analyzed naturalized form of *M. bealei*, growing in a subtropical climate, it was found that the most promising, in terms of quantitative yield, is the sum obtained from flowers. As a result of qualitative analysis, by TLC, in the presence of standards, protoberberine and aporphine bases belonging to the isoquinoline class of alkaloids were identified. In fractions containing quaternary bases, according to HPLC analysis methods, information was obtained not only on the qualitative ratio of bases, on the quantitative content of alkaloids also about the dominant - alkaloids. According to preliminary data, the amounts obtained from the aboveground vegetative organs of the plant exhibit antioxidant and anti-inflammatory activities.

## References

1. State Pharmacopoeia USSR XI (2), 1990: 115,116, 124, 125, 577, 579, 585;
2. Georgian flora. Tbilisi: "Metsniereba", 1983: 376;
3. Romanadze, M. Metreveli. Bioecology of genus Mahonia Nutt. species at Batumi Botanical Garden / S. 17th international conference: Environmental problems of the XXI century. Minsk, Collection of Proceedings Conf., 2017, v. 2, 42-43;
4. A. Makashvili – Botanical Dictionary, "Metsniereba", 1991. P.48
5. Ji, X., Li, Y., Liu, H., Yan, Y., Li, J. Determination of the alkaloid content in different parts of some Mahonia plants by HPCE. Pharmaceutica Acta Helvetiae 2000, 74, 387–391).
6. He J.-M. & Mu Q. The medicinal uses of the genus Mahonia in traditional Chinese medicine: An ethnopharmacological, phytochemical and pharmacological review. J. of Ethnopharmacology, 2015,175, 668–683.
7. Trees and shrubs of the Batumi Botanical Garden: an annotated list. Tbilisi, 2012, 184
8. Xiangying Zeng , Yulian Dong , Guoying Sheng , Xichang Dong , Xuehui Sun , Jiamo Fu. Isolation and structure determination of anti-influenza component from Mahonia bealei. J. Ethnopharmacology, 2006, 108(3), 317-319
9. Abdur Rauf<sup>1</sup>\* and all. Berberine as a Potential Anticancer Agent: A Comprehensive Review. Molecules 2021, 26(23), 7368.
10. State Pharmacopoeia USSR XI(2), 1990: 115,116, 124, 125, 577, 579, 585.

## საქართველოში კულტივირებული *Mahonia bealei* (Fort.) Carr. ალკალოიდების შესწავლა

თოდუა ნიკა<sup>1</sup>, ჭინჭარაძე დავით<sup>1</sup>, ვაჩნაძე ნინა<sup>2</sup>, გეთია მალხაზ<sup>3</sup>

<sup>1</sup>თბილისის სახელმწიფო სამედიცინო უნივერსიტეტი, ფარმაცოგნოზის და ფარმაცევტული ბოტანიკის მიმართულება

<sup>2</sup>თბილისის სახელმწიფო სამედიცინო უნივერსიტეტი, იოველ ქუთათელაძის ფარმაცოქიმიის ინსტიტუტი, ფიტოქიმიის დეპარტამენტი, ალკალოიდების მიმართულება

<sup>3</sup>თბილისის სახელმწიფო სამედიცინო უნივერსიტეტი, იოველ ქუთათელაძის ფარმაცოქიმიის ინსტიტუტი, ფარმაცევტული ანალიზისა და სტანდარტიზაციის დეპარტამენტი

კვლევის მიზანს წარმოადგენდა აჭარის შავი ზღვის სანაპიროზე ინტროდუცირებული მცენარის *Mahonia bealei* (Fort.) Carr. მიწისზედა ნაწილებში (ფოთლები, ყლორტები, ყვავილები) ალკალოიდების შემცველობის დადგენა. შედარებითი ანალიზის საფუძველზე დადგინდა, რომ *M. bealei*-ის მიწისზედა ორგანოებში ალკალოიდების მაქსიმალური შემცველობა აღინიშნება მცენარის აქტიური ყვავილობის ფაზაში - 2,48%. მაღალეფექტური სითხურ ქრომატოგრაფიული ანალიზით დადგინდა აპორფინის და პროტობერბერინის ჯგუფის ალკალოიდების შემცველობა. ფოთლებში: ბერბერინი (27%). იატრორიცინი (23%),

მაგნოფლორინი (18%). ყლორტებსა და ყვავილებში: ბერბერინი (50%) და პალმატინი (57%). სხვა ალკალოიდები წარმოდგენილია კვალის სახით.

წინასწარი ექსპერიმენტული კვლევების შედეგად დადგენილია, რომ მცენარის მიწისზედა ვეგეტატიური ორგანოებიდან მიღებული ალკალოიდებით მდიდარ ექსტრაქტებს გააჩნიათ ანტიოქსიდანტური და ანთების საწინააღმდეგო მოქმედება.

**საკვანძო სიტყვები:** ალკალოიდები, ბერბერინი, სითხე-სითხე ექსტრაქცია, *Mahonia*, ანთების საწინააღმდეგო