საქართველოს მეცნიერებათა ეროვნული აკადემიის მოამბე, ტ. 9, №3, 2015
BULLETIN OF THE GEORGIAN NATIONAL ACADEMY OF SCIENCES, vol. 9, no. 3, 2015

Organic Chemistry

Relation between Percentage Composition of Biologically Active Substances and the Age of Plants *Magnolia Obovata*, *Magnolia Grandiflora* and *Cocculus Lauripolius*

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ABSTRACT. Two species of Magnolia: Magnolia Obovata, Magnolia Grandiflora and a plant Cocculus Lauripolius were studied on the content of biologically active organic compounds. Total preparations were isolated from the mentioned plants and phenolic compounds, alkaloids and essential oils were obtained using modern methods of chromatography. On the basis of the spectral analysis individual alkaloids were identified. Changes in the contents of aglycone quartzetine and alkaloid liriodenine were detected in magnolia leaves. From the above mentioned two species of Magnolia the following alkaloids were separated: anonaine, remerine, liriodenine, lanuginozine, isolaurelin-N-oxide, remerine- N-oxide and dehydroremerine. *Cocculus Lauripolius* contains the following alkaloids: kokulin, kokulidin, koklaphyn, koklaurin, Izoboldin, Norizoboldin, Kokulidin-N-oxide. Phenolic compound such as izopheric acid was detected in large amount in the leaves of Magnolia Obovata. Separation and identification of biologically active substances were carried out by the use of modern chromatographic methods of analysis. Chemical compositions of leaves, shoots and flowers of evergreen Magnolia Grandiflora and deciduous Magnolia Obovata were thoroughly studied and compared. Different chemical processes important for living organisms were observed. Qualitative and quantitative changes in alkaloids, phenolic compounds and essential oils in different vegetation organs of all three plants were determined in different vegetation periods. Character of th dependence of the percent content of some important alkaloids on the age of plants was established. *© 2015 Bull. Georg. Natl. Acad. Sci.*

Key words: *alkaloids, phenolic compounds, extraction, vegetation organs, period of vegetation.*

The department of Organic Chemistry, I.Javakhishvili Tbilisi State University is studying the flora of Georgia on the content of chemical composition for many years. We studied plants Magnolia Obovata, Magnolia Grandiflora and

Cocculus Lauripolius growing in Batumi Botanical Garden, Ajara. Investigations were carried out on the content of alkaloids, phenolic compounds and other biologically active organic compounds in the mentioned plants.

We began studying magnolia plants on the content of alkaloids in 1990s in cooperation with the

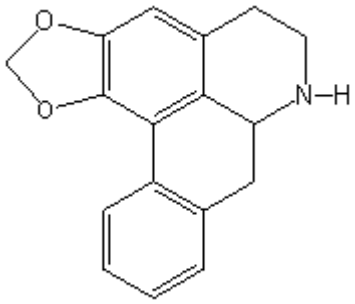
professors of the Tashkent State Agrarian University, Uzbekistan.

Accumulation of alkaloids in plants and their qualitative and quantitative composition mostly depend on environment, soil, athmosphere, so, it is reasonable to study repeatedly those plants, which have already been investigated [1]. Therefore, we expanded investigations of chemical compositions of the above mentioned three species and besides alkaloids we studied various biologically active organic compounds.

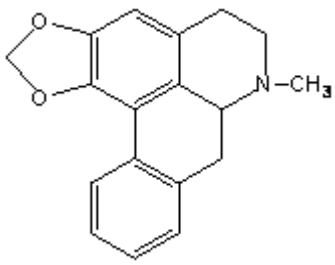
Investigations in I. Javakhishvili Tbilisi State Unversity and Tbilisi Medical Teaching University "Hyppocrates" were carried out in autumn, 2014 and in May-June, 2015. Objects under study were the flowers, leaves and shoots of Magnolia Obovata, Magnolia Grandiflora and *Cocculus Lauripolius* gathered in Batumi Botanical Garden.

The species Magnolia Obovata contains the following alkaloids:

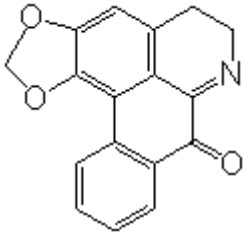
Anonaine $C_{17}H_{15}NO_2$ (1)



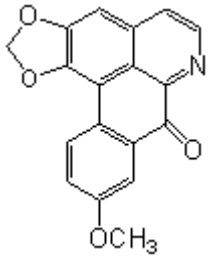
Remerine $C_{18}H_{17}NO_2$ (2)



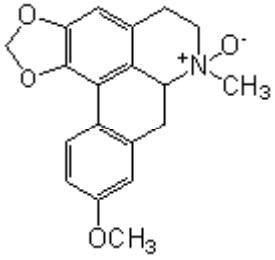
Liriodenine $C_{17}H_9NO_3$ (3)



Lanuginozine $C_{18}H_{11}NO_4$ (4)



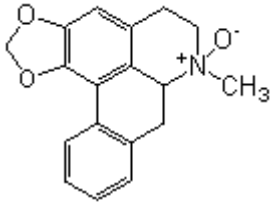
Isolaurelin-N-oxide $C_{19}H_{19}NO_4$ (5)



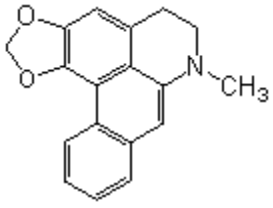
Magnolia Grandiflora contains:

Anonaine (1); Remerine (2); Liriodenine (3); Remerine-N-oxide (6); Dehydroremerine (7) [2];

Remerine-N-oxide $C_{18}H_{17}NO_3$ (6)

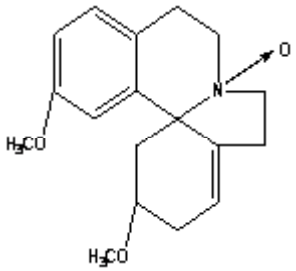


Dehydroremerine $C_{18}H_{15}NO_2$ (7)

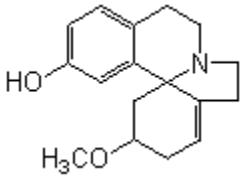


First, the total alkaloids from the leaves and shoots of the plant *Cocculus Lauripolius* were isolated, which were then divided into phenolic and non-phenolic parts. From the total non-phenolic alkaloids a new alkaloid kokulidin-N-oxide was isolated, a brutto formula of which is $C_{18}H_{23}NO_3$ [3]. Its good solubility and little intensity of molecular ion pic (3.4%) proved N-oxide character of this substance, though besides the new alkaloid, other known alkaloids were also detected in this plant, such as kokulin, kokulidin, koklaphyn, izoboldin, norizoboldin and koklaurin.

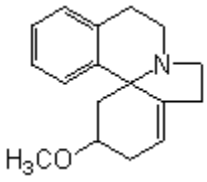
Kokulidin-N-oxide (8)



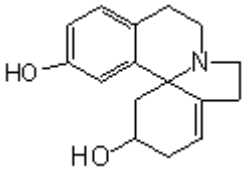
Kokulin (9)



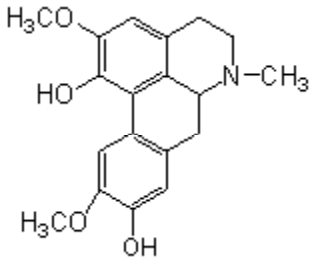
Kokulidin (10)



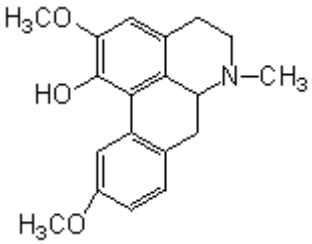
Koklaphyn (11)



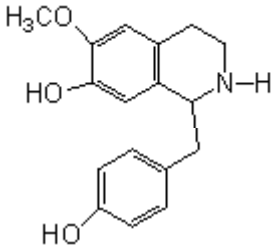
Izoboldin (12)



Norizoboldin (13)

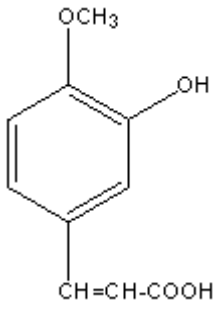


Koklaurin (14)



Besides the alkaloids the phenolic compounds were also separated from leaves of Magnolia Obovata. We identified isopheric acid $C_{10}H_{10}O_4$ [4].

Isopheric acid $C_{10}H_{10}O_4$ [4]



First, we chose the unique species of deciduous Magnolia Obovata for our investigation because it was introduced in Batumi as early as 1913 (100 years ago), there is its single copy in Georgia and it differs quite from the evergreen Magnolia Grandiflora, which is widespread in Georgia.

Magnolia Obovata is a deciduous frost-resistant and valuable ornamental plant. It flowers after leafing out from the end of May to July. It can be well propagated from seeds. The young shoots of Magnolia Obovata are light chestnut-coloured, the leaves are large green above, bluish-green below, roundish at the apex; 40 cm long and 20 cm broad they are held in whorls of 8 to 10 at the end of each shoot. The flowers are also large, cup-shaped, 15-18 cm diameter, with 9-12 creamy, fleshy tepals, red stamens; The fruit is an oblong-cylindric of 12-18 cm long.

Magnolia Obovata is native to Japan and the adjacent Kurile Islands.

Experimental

The method of continuous extraction was used to quantitate and isolate total alkaloids from leaves, shoots and flowers of Magnolia Obovata, Magnolia Grandiflora and *Cocculus Lauripolius*. This method allowed reaching the highest yield of the total alkaloids. The experiment was carried out in the device for continuous extraction.

The study of the dynamics of accumulation of the total alkaloids allows solving many important problems such as biogenesis, mutual transitions of alkaloids and determination of the best time for collecting the raw materials for alkaloids.

Our investigations in Autumn and Spring were coincided with two vegetative periods of the plants. In case of Magnolia Obovata those were the flowering and leaf-fall periods, and in case of evergreen Magnolia Grandiflora and *Cocculus Lauripolius* – the flowering and pre-fruiting periods.

The content of the total alkaloids was determined from air-dried plant, and the content of individual alkaloids in the total preparation was determined by the qualitative-chromatographic methods. Composition and construction of separated individual alkaloids were determined by the elemental analysis and the data of IR, UV, NMR- and Mas-spectra, and also comparing the results with the published literature data.

The substance purity control and determination of Rf-value were carried out by thin-layer chromatography on the Silufol UV-254 plate. Silica gel (brand L, Czech Republic) served as a sorbent. Granules of 5-40 mm were pasted with gypsum.

Systems:

1. Benzene-methanol (4:1)
2. Buthanol-1- acetic acid-water (4:1:5)
3. Ethylacetate-methanol (9:1)
4. Benzene-ethanol (9:1)
5. Chloroform-ethanol (9:1).

Spots became apparent with Dragendorff's reagent followed by iodine vapor in the UV area. For column chromatography the silica gel with granules of 40-100 mm was used as sorbent. Benzene-methanol (in different ratios) was eluent. IR spectra were recorded with UR-20 device (Carl Zeiss).

UR spectra in ethanol were obtained using a Specord spectrophotometer; NMR spectra in deuterchloroform were recorded using a spectrophotometer JNM-4H-100 (100 MHz). Hexamethyldisyloxan was applied as external standard. Mass-spectra were obtained with Mass-spectrometer MX-1310. Ionization energy - 40 eV, T=100-150 °C

The yields of individual compounds were calculated from total preparations, and the yields of total preparations from the gathered volume of the air-dried leaves.

Melting points are determined by the Celsius scale of temperature. Specific turnover of alkaloids, phenolic compounds and essential oils was determined using a Lipis-Landolt's polarimeter.

In 2014-2015 the percentage composition of biologically active substances was studied in the leaves, flowers and shoots of the mentioned species, their quantitative changes in different vegetation

Table. Content of active organic substances in different vegetation organs of plants at different vegetation periods

#	Species	Vegetation organ of a plant	Period of vegetation; time for taking a sample	Substances found	Sum of alkaloids, %	Phenolic compounds, %	Essential oils, %
1	Magnolia obovata	Leaf	Flowering 25.05.2015	Anonaine Remerine Liriodenine Isolauren-N-oxide Izopheric acid	0.65	1.14 0.3	0.12
		Flower	Flowering 25.05.2015	Lanuginozin Remerine Liriodenine	0.56	0.3	0.22
		Leaf	Leaf fall 20.09.2014	Lanuginozin Liriodenine Remerine Izolaurelin-N-oxide Izoferulic acid	0.58	1.8 0.25	0.15
2	Magnolia grandiflora	Leaf	Flowering 10.06.2015	Anonaine Remerine Liriodenine Rutine	0.54	0.01	0.58
		Flower	Flowering 10.06.2015	Liriodenine Anonaine Dehydroremerine	0.52	0.12	0.02
		Leaf	Autumn 12.10.2014	Liriodenine Remerine Remerine-N-oxide	0.46	2.15	0.5
3	Cocculus Lauripolius	Leaf	Autumn 12.10.2014	Kokulin Izoboldin Kokulidin N-oxide Koklaurin	0.50	0.12	0.25
		Shoot	Flowering 25.05.2015	Kokulidin Izoboldin Kokulin	0.48	0.1	0.17
		Leaf	Flowering 25.05.2015	Norizoboldin Kokulidin Kokulin Koklaphyn	0.52	0.05	0.12

periods were compared. The results are given in the Table.

During the study of biological activeness of aqueous and alcohol extracts of leaves of Magnolia Obovata it was detected that they reveal selective-biocide activity against test-microorganisms, therefore they can be used in agriculture against actinomicetes and phytopathogenic microorganisms such as *Bacilus substilis, Xanthomonas campestris, streptomyces albogriseolus* subsp. *Aragviensis* (Approved by the Assistant Director of N.Ketskhoveli Institute of Botany of Academy of Sciences of Georgia Sh.Chanishvili, by the 14th of May, 1998 statement). Initial studies showed that the content of alkaloids in plants depends on geographical factors and seasons of the year [5].

As a result of the studies carried out in 2014-2015, it was revealed that the percentage composition of alkaloids and phenolic compounds in the leaves and flowers of both species Magnolia Obovata and Magnolia Grandiflora increases with the age of the plants. The main constituent alkaloids of these species remerine and liriodenine were found in the leaves at any time of vegetation. It is also known that both alkaloids possess inhibitory effect against tumor. Due to this fact our study is of great importance.

ორგანული ქიმია

ბიოლოგიურად აქტიური ნივთიერებების პროცენტული შემცველობის დამოკიდებულება მცენარეების Magnolia Obovata-ს, Magnolia Grandiflora-ს და Cocculus Lauripolius-ის ასაკზე

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მცენარე მაგნოლიის ორი სახეობა: Magnolia obovata, Magnolia grandiflora და Cocculus lauripolius შესწავლილია ბიოლოგიურად აქტიურ ორგანულ ნაერთთა შემცველობაზე. აღნიშნულ მცენარეებიდან მიღებულ იქნა ჯამური პრეპარატები, რომელთაგან ქრომატოგრაფიის თანამედროვე მეთოდების გამოყენებით მიღებულია ფენოლური ნაერთები, ალკალოიდები და ეთერზეთები. სპექტრალური ანალიზის საფუძველზე იდენტიფიცირებულია ცალკეული ალკალოიდები. დადგენილია აგრეთვე Magnolia obovata-ს ფოთლებში აგლიკონ კვერცეტინის, ხოლო მაგნოლიის ორივე სახეობის ფოთლებში ალკალოიდ ლირიოდენინის შემცველობის ცვლილებები. მაგნოლიის ზემოაღნიშნულ ორივე სახეობიდან გამოყოფილი იქნა შემდეგი ალკალოიდები: ანონაინი, რემერინი, ლირიოდენინი, ლანუგინოზინი, იზოლაურელინის N-ოქსიდი, რემერინის N-ოქსიდი და დეჰიდრორემერინი, ხოლო მცენარე Cocculus lauripolius–ის შემადგენელი ალკალოიდებია: კოკულინი, კოკულიდინი, კოკლაფინი, კოკლაურინი, იზობოლდინი, ნორიზობოლდინი და კოკულიდინის N-ოქსიდი. Magnolia obovata-ს ფოთლების შემადგენლობაში დიდი რაოდენობით აღმოჩნდა ფენოლური ნაერთები, მაგალითად იზოფერულის მჟავა. ბიოლოგიურად აქტიური ნივთიერებების, ალკალოიდების, ფენოლური ნაერთებისა და ეთერზეთების გამოყოფა და იდენტიფიცირება წარმოებდა ანალიზის თანამედროვე ქრომატოგრაფიული მეთოდებით. მაგნოლიის ორი სახეობის და Cocculus lauripolius-ის საფუძვლიანი კვლევა ჩატარდა 2014 წლის შემოდგომისა და 2015 წლის გაზაფხულის პერიოდში, რადგან ვაკვირდებოდით და ვადარებდით მარადმწვანე მაგნოლიის სახეობის Magnolia grandiflora-ს და ფოთოლმცვენი მაგნოლიის Magnolia obovatas-ს ფოთლებისა, ყლორტებისა და ყვავილების ქიმიურ შემადგენლობას. კვლევის შედეგად აღმოჩნდა, რომ ვეგეტაციის სხვადასხვა პერიოდში, სხვადასხვა ვეგეტატიურ ორგანოში ალკალოიდები სხვადასხვა თანაფარდობით აკუმულირდება. ლაბორატორიაში ალკალოიდებზე ჩატარებულ ცდებში ადვილად ხდებოდა ალკალოიდების ურთიერთგარდასვლები. ეს ყოველივე ადასტურებს, რომ მცენარეშიც ვეგეტაციის სხვადასხვა პერიოდში მიმდინარეობს ჟანგვა-აღდგენითი პროცესები, რაც მნიშვნელოვან როლს ასრულებს ცოცხალ ორგანიზმებში. სამივე გამოსაკვლევი მცენარისათვის დადგენილ იქნა ალკალოიდების, ფენოლური ნაერთების და ეთერზეთების რაოდენობრივი და თვისობრივი ცვლელებები ვეგეტაციის სხვადასხვა პერიოდში სხვადასხვა ვეგეტატიურ ორგანოების მიხედვით. მიდინარე კვლევებმა გვაჩვენა, რომ ორივე სახეობის მაგნოლიების ფოთლებში ამ მცენარეთა ასაკის მატებასთან ერთად მათ შემადგენლობაში მყოფი ძირითადი ალკალოიდების ლირიოდენინის და რემერინის პროცენტული შემცველობა იზრდებოდა. თვით ეს ალკალოიდები ლირიოდენინი და რემერინი კი ხასიათდება სიმსივნის მაინჰიბირებელი თვისებებით, რაც განაპირობებს ამ გამოკვლევების აქტიურ სამეცნიერო-პრაქტიკულ ინტერესს და მნიშვნელობას.

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Received August, 2015