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SEARCH OF THE PROMISING SPECIES OF SUBFAMILY *AMYGDALOIDEAE* AND *PYROIDEAE* USING THE CHEMOTAXONOMY

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Світова флора нараховує понад 1000 видів роду Crataegus L., до підроду Prunus L. належать понад 30 видів, рід Malus Mill. містить 100 видів, до підріду Cerasus Juss. відноситься понад 150 видів. Незважаючи на різноманіття видів та достатню сировинну базу, лише деяки представники цих родів достатньо досліджені та знайшли своє використання як джерела біологічно активних речовин (БАР).

Мета. *Провести хемотаксономічне дослідження представників родів Crataegus L., Prunus L., Malus Mill., Cerasus* Juss.*; встановити перспективні джерела БАР для одержання лікарських засобів.*

Методи дослідження. *Хемотаксономічне дослідження проводили з використанням методу граф-аналізу. Хемомаркерами служили фенольні сполуки та терпеноїди, ідентифіковані у генеративних та вегетативних органах представників родів Crataegus L., Prunus L., Malus Mill., Cerasus* Juss*. Ідентифікацію терпеноїдів та органічних кислот проводили хромато-мас-спектрометричним методом на хроматографі Agilent Technology 6890N з мас-спектрометричним детектором 5973N. Флавоноїди та гідроксикоричні кислоти визначали хроматографічно.*

Результати дослідження. *Встановлені хімічні профілі вегетативних та генеративних органів 34 видів роду Crataegus L., 5 видів роду Prunus L., 7 видів роду Malus Mill., 4 видів роду Cerasus Juss. Встановлені перспективні види глоду, які містять основну групу БАР роду.*

Висновки. *За результатами хемотаксономічного дослідженя представників підродів Amygdaloideae та Pyroideae встановлені перспективні джерела біологічно активних речовин (БАР) видів родів Crataegus L., Prunus L., Malus Mill. та Cerasus Juss. Встановлено, що хімічний профіль досліджених родів формують флавоноїди, терпеноїди та ароматичні кислоти. Перспективні види глоду були введені до складу фітокомплексу «Кратофіт»*

Ключові слова: *розоцвіті, глід, яблуня, вишня, слива, листя, квітки, плоди, таксон, хемотаксономія*

1. Introduction

The representatives of genus *Crataegus* L., *Malus* Mill., *Prunus* L. and *Cerasus Juss.* belong to the family *Rosaceae L. (Rose)* subfamily *Amygdaloideae* and *Pyroideae*. According to modern data *Cerasus* Juss. and *Prunus* L. belong to the *Amygdaeae*, genus *Crataegus L.* and *Malus* Mill. – *Pyreae* [1].

In the world of flora, the genus *Crataegus* L. has more than 1000 species, the *Prunus* L. – more than 30 species, the *Malus* Mill. – more than 100 species, the *Cerasus* Juss. – more than 150 species.

2. Formulation of the problem in a general way, the relevance of the theme and its connection with important scientific and practical issues

Despite the wide species composition, only some representatives of the genera *Crataegus* L., *Prunus* L., *Malus* Mill. and *Cerasus* Juss. thoroughly investigated. Most of the existing species are not sufficiently studied. The feature of the above genus is that their representatives are easily hybridize as within the genus and between families, which complicates their identification [2]. Because of this, the taxonomy of the

family and the genus is a subject of constant review and needs to be clarified.

3. Analysis of recent studies and publications in which a solution of the problem are described and to which the author refers

According to the characters of the morphological structure of generative and vegetative organs, modern systematics are point 25 botanical sections of the genus *Crataegus L.* the main of which are: *Henryanae* Sarg., *Pinnatifida* Zbl., *Sanguineae* Zbl., *Douglasii* Loud., *Pentagynae* Zbl., *Azaroli* Loud., *Oxyacanthae* Zbl., *Molles* Sarg., *Tenuifoliae* Sarg., *Rotundifoliae* Eggl., *Virides* Sarg., *Crus-Galli* Loud., *Punctatae* Loud., *Parvifoliae* Loud., *Flavae* Loud., *Macracantae* Loud., *Dilatatae* Sarg. 8 other sections are endemic species.

A chemotaxonomic study of the genus Crataegus L. using phenolic compounds of 12 species was previously carried out. As a result, it was found that for all investigated species the presence of hyperoside, quercetin and chlorogenic acid [3].

Modern systematics are separated *Malus* Mill. as an independent genus, although earlier it was a sub-genus of the genus *Pyrus* L. By the features of the morphological structure the genus *Malus* Mill. is divided into 2 sections: *Pumilae* Rehder. – *M. silvestris* Mill., *M. domestica* Borkh. Handb., *M. prunifolia.* (Willd.) Borkh., *M. orientalis* Ugl. = *M. pumila* Grossh.), *M. Sieversii* (Ldb.) M. Roem, *M. Niedzwetzkyana* Diek., *M. prunifolia* (Willd.) Borkh.) and *Baccatae* Render – *M. baccata* (L.) Borkh. Handb., M. Pallasiana Juz., *M. sachalinensis* Juz., *M. manshurica* (Maxim.) Kom. [4]. The most studied are species of sections of *Pumilae* Rehder. Representatives of the *Baccatae* Render. section have practically not been studied from a pharmacological point of view.

In Ukrainian flora two wild species are described – *M. silvestris Mill.* and *M. praecox* (Pall.) Borkh., and also 4 cultivated species – *M. domestica* Borkh., *M. baccata* (L.) Borkh., *M. prunifolia* (Willd.) Borkh. and *M. sachalinensis* Juz.

The genus *Cerasus* Juss. is consist of subgenus *Typocerasus* Koehne ex Kurt and *Microcerasus* Webb. To subgenus *Typocerasus* are belong sections:

1. *Mahaleb* Koehne – C. *Maximowiczii (Rupr.) Kom.*, C. *mahaleb* (L.) *Mill.*;

2. Pseudocerasus Koehne – C. sachalinensis Kom.;

3. *Hypadenium* Koehne – *C. glandulifolia* (Rupr. Et Maxim.) Kom., *C. avium* (L.) Moench., *C. fruticosa* (Pall.) G. Woron., *C. austera* (L.) Roem. та *C. colina* Lej. et Court. In modern genus system is devided a section *Eucerasus* Koehne., which include *C. avium* (L.) Moench., *C. fruticosa* (Pall.) G. Woron. and *C. vulgaris* Mill. [5].

The representatives subgenus *Microcerasus* divided into 2 sections:

1. *Spiraeopsis* Koehne – *C. glandulosa* (Thunb.) Lois.;

2. *Amygdalocerasus* Koehne ex Kurt. – *C. microcarpa* (C.A.M.) Boiss., *C. araxina* Poyark., *C. incana* (Pall.) Spach., *C. pseudoprostrata* Poyark., *C. erythrocarpa* Newski., *C. verrucosa* Newski., *C. Jacquemontii* (Hook.) Buser., *C. alaica* Poyark., *C. tianschanica* Poyark. and *C. turcomanica* Poyark.

In Ukrainian flora 6 species of *Prunus* Mill. genus are described – *P. spinosa* L., *P. stepposa* Kotov., *P. moldavica* Kotov., *P. domestica* L., *P. divaricata* Ldb., *P. insitilia* L. The most widespread in Ukraine of species the genus *Prunus* Mill. are: *P. domestica* L.; *P. spinosa* L.; *P. salicina* L.; *P. cerasifera* Ehrh.; *P. americana* Marsh.; *P. ussuriensis* K. et K.; *P. nigra* Ait; *P. angustifolia* Marsh.; *P. hortulana* Bail.; *P. munsoniana* W. and Hed.; *P. Simonii* Car. [6].

4. The field of research considering the general problem, which is described in the article

After analyzing the current state of research of representatives of the genus *Crataegus* L., *Prunus* L., *Malus* Mill. and *Cerasus* Juss., it can be concluded, that among species of the genus *Crataegus* L. 14 species have been studied, in genus *Prunus* L., *Malus* Mill. and *Cerasus* Juss. are sufficiently studied BAS of the species *Prunus domestica, Prunus spinosa, Malus domestica, Malus silvestris, Cerasus vulgaris, Cerasus avium* and their varieties. Other species of these genus were practically not investigated. We believe that the scientific interest is conducting a chemotaxonomic study of representatives of this subgenus to clarify their position in the genus system and to search for new sources of BAS, as well as to obtain substances for the creation of phytopreparations.

5. Formulation of goals (tasks) of article

To conduct a comparative chemo-taxonomic study of the genus *Crataegus* L., *Prunus* L., *Malus* Mill. and Cerasus Juss., to establish the chemical profiles of the genera, to establish promising for medicine species.

6. Presentation of the main research material (methods and objects) with the justification of the results

The objects of chemotaxanomic study were vegetative and generative organs of 34 species of genus *Crataegus* L.: *C. pinnatifida* Bge. (*C. pinnatifida var. psilosa* C. K. Schneid.), *C. Maximowiczii* C.K. Schneid (*C. altaica var. villosa* Lge.), *C. chlorosarca* Maxim. (*C. atrocarpa* E. Wolf), *C. schneideri* C.K. Schneid., *C. kansuensis* Wils., *C. almaatensis* Pojark., *C.pseudomelanocarpa* M. Pop., *C.laevigata* Loud., *C. oxyacantha* L. (*C. oxyacanta var. genuina* Rouy et Camus), *C. ambiguae* C. A. M. (*C. pseudoambiqua* A. Pojark.), *C. kyrtostyla* Fingerh., *C. pseudokyrtostyla* Klok., *C. subrotunda* Klokk., *C. curvisepala* Lindm., *C. fallacina* Klok., *C. turkestanica* A. Pojark., *C. monogyna* Jacq. (*C.monogyna va. intermedia (Schur) Jav.*), *C. canadensis* Sarg., *C. submollis* Sarg., *C. arnoldii* Sarg., *C.densiflora* Sarg., *C. festiva* Sarg., *C. flabellata* (Bosc) C.Koch, *C. rotundifolia* Moench., *C. punctata* Jacq., *C.rivularis* Nutt., *C. douglasii* Lindl., *C. prunifolia* (Poir.) Pers., *C. macracantha* Lodd., *C. pringlei* Sarg., *C. holmesiana* Ashe., *C. pedicelata* Sarg., *C. coccinoides* Asche., *C. cuneata* S. et Z.; 4 species of genus *Cerasus* Juss.: *C.avium* L., *C. fruticosa* Pall., *C. vulgaris* Mill., *C. glandulosa* (Thunb.) Lois.; 7 species of genus *Malus* Mill.: *M. silvestris, M. domestica* Borkh. Handb., *M. prunifolia* (Willd.) Borkh., *M. baccata* (L.) Borkh. Handb., *M. manshurica* (Maxim.) Kom., *M. cerasifera,*

M. coronaria; 5 species of genus *Prunus* L.: *Pr. domestica* L., *Pr. spinosa* L., *Pr. salicina* Lindl., *Pr. divaricata* Ldb., *Pr. americana.*

The research was carried out using the taxonomic analysis [7]. As chemo-markers used flavonoids, terpenoids, aromatic acids. Flavonoids and hydroxycinnamic acids identified chromatographically in a solvent system *n* - butanol - acetic acid - water (10: 2: 3) and 2 % solution of acetic acid [8]. Alcohol extracts of the studied species were chromatographed [9]. The results were evaluated by chromatography colored spots substances in daylight and UV- light before and after processing the chromatogram a chromogenic reagents (pair of ammonia, 10 % alcohol solution of sodium hydroxide) [10].

Terpenoids and aromatic acids in the raw material were determined by chromatographic mass spectrometry on a Agilent Technology 6890N chromatograph with a 5973N mass spectrometry detector [11]. Substances from the raw material were extracted with hexane. The content of the compounds was calculated relative to the internal standard (50 μg solution of tridecane in hexane). The identification of the compounds used data from the NIST05 and WILEY 2007 mass spectrum libraries with a total number of spectra of over 470000 in combination with programs for identifying AMDIS and NIST [12].

The compounds were determined in each species and added in the matrix table, after which for each species at the boundary of the genus, the coefficients of the pair affinity (Cpa) and the coefficient of group affinity (Cga) were determined on the basis of the chemical characteristics of the generative and vegetative organs.

In study a generative organs genus *Crataegus* L. was analyzed 4760 stages signs, genus *Malus* Mill. – 441, genus *Cerasus* Juss. – 212, genus *Prunus* L. - 414.

In study vegetative organs – genus *Crataegus* L. – 4760 stages of signs, genus *Malus* Mill. – 553, genus *Cerasus* Juss. – 252, genus *Prunus* L. – 384.

As a result of the study, chemical profiles of vegetative and generative organs of the genera were established. It should be noted that for identifying the characteristic compounds of genera were selected a chemical characteristics for which Cpa was from 75 % to 100 %.

For a generative organs the species of *Crataegus* L. general are compounds: terpenoids (eugenol, cis-linalool oxyde, trans-linalool oxyde, β-phenylethyl alcohol, squalen), flavonoids (quercetin, bioquercetin, hyperoside, rutin), organic acids (*p*-hydroxybenzoic acid, benzoic, phenylacetic, salicylic, 4-hydroxycinnamic, vanillinic, chlorogenic, coffeic, pherulic, neochlorogenic, gentisinic, lilac) [13].

The chemical profile of vegetative organs of hawthorn species is characterized by terpenoids and aldehydes (benzaldehyde, cis-linalool oxyde, trans -2-hexenal, eugenol, squalen, nerol), flavonoids (quercetin), aromatic acids (*p*-coumaric, benzoic, phenylacetic, salicylic, vanillinic, chlorogenic, coffeic, pherulic, neochlorogenic, gentisinic, lilac) [14]. The taxons, which have the highest values of Cga are characterize the hawthorn by the chemical composition of the vegetative and generative organs and form a chemical profile of the genus. The main group of species of the genus Crataegus, having largest information weight according the chemical composition of generative organs, represented by 10 taxons: *C. prunifolia* (Cga – 1523 %), *C. douglasii* (Cga – 1486 %), *C. cuneata* (Cga – 1485 %), *C. pseudokyrtostylla* (Cga – 1463 %), *C. almaatensis* (Cga – 1457 %), *C. chlorosarca* (Cga – 1437 %), *C. ambiquae* (Cga – 1437 %), *C. rotundifolia* (Cga – 1426 %), *C. flabellata* (Cga – 1363 %), *C. densiflora* (Cga – 1320 %) (Table 1).

Table 1

Cpa the main grous of taxons according chemical content of generative organs

Taxon	C. chlorosarca	C. almaatensis	C. ambiguae	C. pseudokyrtostyla	C.densiflora	C. flabellata	C. rotundifolia	C. douglasii	C.prunifolia	C. cuneata
	Cpa, %									
C. chlorosarca	100 %	51 %	51 %	59 %	33 %	37 %	47 %	49 %	49 %	50 %
C. almaatensis	51 %	100 %	43 %	47 %	48 %	45 %	64 %	51 %	65 %	59 %
C. ambiguae	51 %	43 %	100 %	54 %	36 %	37 %	41 %	44 %	44 %	45 %
C. pseudokyrtostyla	59 %	47 %	54 %	100 %	37 %	38 %	45 %	48 %	51 %	45 %
C.densiflora	33 %	48 %	36 %	37 %	100 %	66 %	50 %	62 %	42 %	43 %
C. flabellata	37 %	45 %	37 %	38 %	66 %	100 %	58 %	61 %	46 %	47 %
C. rotundifolia	47 %	64 %	41 %	45 %	50 %	58 %	100 %	50 %	50 %	51 %
C. douglasii	49 %	51 %	44 %	48 %	62 %	61 %	50 %	100 %	52 %	54 %
C.prunifolia	49 %	65 %	44 %	51 %	42 %	46 %	50 %	52 %	100 %	64 %
C. cuneata	50 %	59 %	45 %	45 %	43 %	47 %	51 %	54 %	64 %	100 %

The main group of species of genus *Crataegus* L. according vegetative characteristics include 14 taxons: *C. oxyacantha* (Cga – 1416 %*), C. chlorosarca (*Cga – 1411 %*), C. almaatensis* (Cga – 1411 %), *C. festiva* (Cga – 1380 %), *C. pseudokyrtostyla* (Cga – 1371 %), *C. subrotunda* (Cga – 1368 %), *C.prunifolia* (Cga – 1366 %), *C.pseudomelanocarpa* (Cga – 1354 %), *C. submollis* (Cga – 1354 %), *C. punctata* (Cga – 1351 %), *C. curvisepala* (Cga – 1348 %), *C. pinnatifida* (Cga – 1339 %), *C.laevigata* (Cga –1323 %), *C. cuneata* (Cga – 1311 %) (Table 2).

The main group of taxons that characterize the genus *Crataegus* L. by the chemical composition of generative and vegetative organs include *C. prunifolia* (Cpa – 1449 %), *C. chlorosarca (*Cga – 1424 %*), C. almaatensis* (Cga – 1434 %), *C. pseudokyrtostylla* (Cga – 1417 %), *C. cuneata* (Cga – 1396 %), *C. subrotunda* (Cga – 1374 %).

After analyzing the chemical composition of generative and vegetative organs of the genus *Crataegus* L., the basic chemical compounds that form the chemoprofile of the genus are established: cis-linalool oxyde, linalool, squalen, quercetin, hyperoside, rutin, *p*-hydroxybenzoic acid, chlorogenic acid, pherulic acid, vanillinic acid, lilac acid.

Table 2

Cpa the main grous of taxons according chemical content of vegetative organs

Taxon	*C. pinnatifida*	*C. chlorosarca*	*C. almaatensis*	*C.pseudomelanocarpa*	*C.laevigata*	*C. oxyacantha*	*C. pseudokyrtostyla*	*C. subrotunda*	*C. curvisepala*	*C. submollis*	*C. festiva*	*C. punctata*	*C.prunifolia*	*C. cuneata*
	Cpa, %													
C. pinnatifida	100 %	47 %	44 %	42 %	45 %	43 %	37 %	40 %	41 %	40 %	39 %	48 %	51 %	45 %
C. chlorosarca	47 %	100 %	71 %	72 %	38 %	72 %	77 %	80 %	68 %	71 %	73 %	41 %	41 %	38 %
C. almaatensis	44 %	71 %	100 %	65 %	37 %	65 %	70 %	68 %	68 %	65 %	66 %	43 %	40 %	37 %
C.pseudomelanocarpa	42 %	72 %	65 %	100 %	38 %	64 %	73 %	76 %	60 %	61 %	74 %	40 %	41 %	38 %
C.laevigata	45 %	38 %	37 %	38 %	100 %	36 %	39 %	38 %	33 %	32 %	41 %	71 %	82 %	82 %
C. oxyacantha	43 %	72 %	65 %	64 %	36 %	100 %	73 %	64 %	73 %	74 %	69 %	39 %	39 %	36 %
C. pseudokyrtostyla	37 %	77 %	70 %	73 %	39 %	73 %	100 %	81 %	69 %	68 %	79 %	40 %	42 %	39 %
C. subrotunda	40 %	80 %	68 %	76 %	38 %	64 %	81 %	100 %	63 %	68 %	77 %	40 %	41 %	38 %
C. curvisepala	41 %	68 %	68 %	60 %	33 %	73 %	69 %	63 %	100 %	72 %	61 %	42 %	36 %	33 %
C. submollis	40 %	71 %	65 %	61 %	32 %	74 %	68 %	68 %	72 %	100 %	64 %	40 %	35 %	32 %
C. festiva	39 %	73 %	66 %	74 %	41 %	69 %	79 %	77 %	61 %	64 %	100 %	43 %	45 %	41 %
C. punctata	48 %	41 %	43 %	40 %	71 %	39 %	40 %	40 %	42 %	40 %	43 %	100 %	77 %	73 %
C.prunifolia	51 %	41 %	40 %	41 %	82 %	39 %	42 %	41 %	36 %	35 %	45 %	77 %	100 %	89 %
C. cuneata	45 %	38 %	37 %	38 %	82 %	36 %	39 %	38 %	33 %	32 %	41 %	73 %	89 %	100 %

The chemical profile the generative organs of genus *Malus* Mill. are forming α-terpineol, geraniol, eugenol, aromatic acids (benzoic, phenylacetic, salicylic, vanillinic, lilac, chlorogenic, pherulic), flavonoids (hyperoside and rutin). For vegetative organs common compounds are cis-bisabolene epoxide, α-pharnezene, linalool, damascenon, limonen, squalen, organic acids (fumaric, phenylacetic, vanillinic, *p*-oxybenzoic, pherulic, *p*-coumaric, chlorogenic), rutin, naringenin, epicatechin.

For generative organs of the genus *Cerasus* Juss. the most characteristic BAS are: trans-linalool oxyde, linalool, lilac aldehyde, terpen-4-ol, α-terpineol, nerol, geraniol, damascenon, squalen, aromatic acids (benzoic, salicylic), rutin. In vegetative organs established nonanale, α-terpineol, trans-linalool oxide, cis-linalool oxyde, 4-vinile-2-methoxyphenol, damascenon, ionone-5,6-epoxide, squalen, hexacosane, aromatic acids (benzoic, *p*-coumaric, *p*-hydroxybenzoic, chlorogenic), flavonoids luteolin, vitexin, naringenin, gerniarin, catechin [15].

The chemical profile of generative organs of the genus *Prunus* L. is represented by nerol, limonene, squalene, quercetin, hyperoside and rutine. For vegetative organs, the most characteristic are squalene, chlorogenic acid, isoqueretine, quercetin, avicularine, rutine [16].

Practically in all investigated objects are identified fumaric, oxalic, malonic, levulinic, succinic, malic, ascorbic, citric acids.

7. Conclusions from the conducted research and prospects for further development of this field

The chemotaxonomic study of the genera *Crataegus* L., *Malus* Mill., *Cerasus* Juss., *Prunus* L.

was carried out and promising species were identified as a sources of BAS. According to the chemical composition of generative and vegetative organs the Cpa and Cga are determined. Established chemical profiles of vegetative and generative organs of the genera, as well as the main groups of taxons. The promising hawthorn species was edded to complex «Kratophyt».

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