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Articles

Search for new sources of bioactive compounds in the flora of Southern Siberia and evaluation of the potential for their use

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The paper addresses the issue of the search for plants containing bioactive compounds and evaluation of the potential for their use. A methodology and an algorithm have been developed to conduct comprehensive studies using methods of taxonomy and plant ecology, phytochemistry, botanical geography, and resource studies. The developed algorithm has been implemented for large taxa of the family *Asteraceae* Dumort. 1820 from the flora of Southern Siberia (tribe *Cynareae* Lam. ex DC. 1813 and genus *Artemisia* L., 1753). Four groups of plants were identified, and recommendations on their use were elaborated. The group of promising species included 20 representatives of the tribe *Cynareae* (from the genera *Saussurea* DC. 1979, *Serratula* L. 1753, *Centaurea* L. 1753, *Cirsium* Mill. 1754) and 15 species of the genus *Artemisia*. The algorithm enabled identification of 30 species of the tribe *Cynareae* and 8 species of the genus *Artemisia* that need special conservation measures.

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Keywords

Algorithm, *Artemisia* L., *Asteraceae* Dumort., *Cynareae*, bioactive compounds, promising species, Southern Siberia

Introduction

Among the problems facing humanity in the 21st century, the problem of health and life expectancy is most relevant. The spectrum of human diseases is currently expanding, and the number of cases and deaths is increasing. It is known that many plants contain bioactive compounds used as a basis for drugs to treat various diseases and maintain human health. Bioactive compounds isolated from plants are potential candidates for drugs against influenza and parainfluenza, respiratory syncytial virus, and severe acute respiratory syndrome (SARS). Bioactive compounds derived from plants show promising inhibitory effects against viruses. Plant bioactive compounds have become the basis for development of 93 antiviral drug candidates to provide cost-effective therapy (Nair et al. 2022; Adnikari et al. 2022).

The analysis of literature data showed the need for further chemical screening of unstudied and poorly studied species, and evaluation of the potential for using from natural resources.

Numerous researchers are engaged in the search for new medicinal plants and creation of drugs based on them, yet the results obtained do not always justify efforts. History shows that such work can be efficient only with the joint knowledge and efforts of researchers in various fields (botanists, phytochemists, pharmacologists, clinicians). During the Great Patriotic War, scientists from Tomsk united to conduct the research in this area. During wartime, the search for new medicinal plants to create effective drugs was of high relevance. This work brought together researchers from various fields in some or other way involved in medicine and studies into medicinal properties of plants. In a short time, working towards a common goal, a team of Tomsk scientists discovered a number of new medicinal plants in the flora of Siberia. The scientists were awarded for their achievements. For example, the Stalin Prize was awarded to V.V. Reverdatto (botanist), N.V. Vershinin (pharmacologist), and D.D. Yablokov (clinician).

At present, science has come a long way, researchers obtain outstanding results, yet scientists are experts in a very narrow field and are unable to conduct comprehensive studies to achieve the common goal. The authors of this paper have long been engaged in search for new sources of plants with bioactive compounds and attempted to develop a methodology and an algorithm for searching plants and evaluating their potential. The algorithm was tested on the example of some groups from the family *Asteraceae*.

Materials and methods

Southern Siberia was chosen as the study area since it is the territory with the richest and most diverse flora. The territory of Southern Siberia is bounded by parallels 49°–57° N and meridians 65°–120° E, and its border stretches for 850 km from north to south and for almost 4,000 km from west to east.

The family *Asteraceae* attracts attention of numerous researchers. Based on the literature data and field observations, the authors took species of the tribe *Cynareae* and the genus *Artemisia* as models for developing the methodology and the algorithm to search for new promising sources of plant raw materials. To identify the species composition, distribution and ecology of species, field studies were conducted in the southeast of the West Siberian Plain, in Gorny Altai, Khakassia, Tuva, and in Western Sayan. The study employed herbarium materials from P.N. Krylov Herbarium of Tomsk State University (TK), M.G. Popov Herbarium (NSK), and I.M. Krasnoborov Herbarium (NS) of the Central Siberian Botanical Garden SB RAS, and numerous literature data on floras and floristic reports (Flora of the USSR 1961; Flora of Kazakhstan 1966; Flora of Siberia 1997).

The study object was phenolic and steroid compounds, and essential oils chosen from a wide range of bioactive compounds. Chemical screening was performed, and the composition and content of bioactive compounds were determined using high-performance liquid chromatography methods. Data on essential oils were obtained from currently available studies (Berezovskaya et al. 1970, 1982, 1991; Khanina et al. 2000; Alyakin et al. 2011). For each species, the potential for its use as a source of bioactive compounds was evaluated, and the character of the range, the frequency of occurrence, the abundance, and the possibility of plant introduction were considered.

Result

To search for new sources of bioactive compounds and evaluate the potential for their use, the algorithm with several stages was developed. The procedure for organizing and conducting the study was elaborated as a road map (Fig. 1) that included 7 stages:

1. Search for data on the chemical composition and the use of plants from different systematic groups in a wide information field. Formulation of the working hypothesis, including definition of the systematic group, the group of chemical compounds, and the study area.

2. Analysis of literature data:

2a. Assessment of the abundance and diversity of the systematic group within the chosen

area;

2b. Evaluation of the content of bioactive compounds and the relationship of their presence with individual taxa.

3. Choice of materials and research methods.

4. Botanical and phytochemical study.

4a. Identification of species composition, geography and ecological-coenotic confinement of species;

4b. Chemical screening for chemical compounds, detection of promising groups of bioactive compounds, their identification and evaluation of their content.

5. Identification of relationships between the content of bioactive compounds in various taxa (chemotaxonomic aspect) and their dynamics in different ecological and cenotic conditions (ecological and physiological aspect).

6. Identification of groups of promising taxa and determination of their applications.

7. Elaboration of recommendations for further study and obtaining practical results.

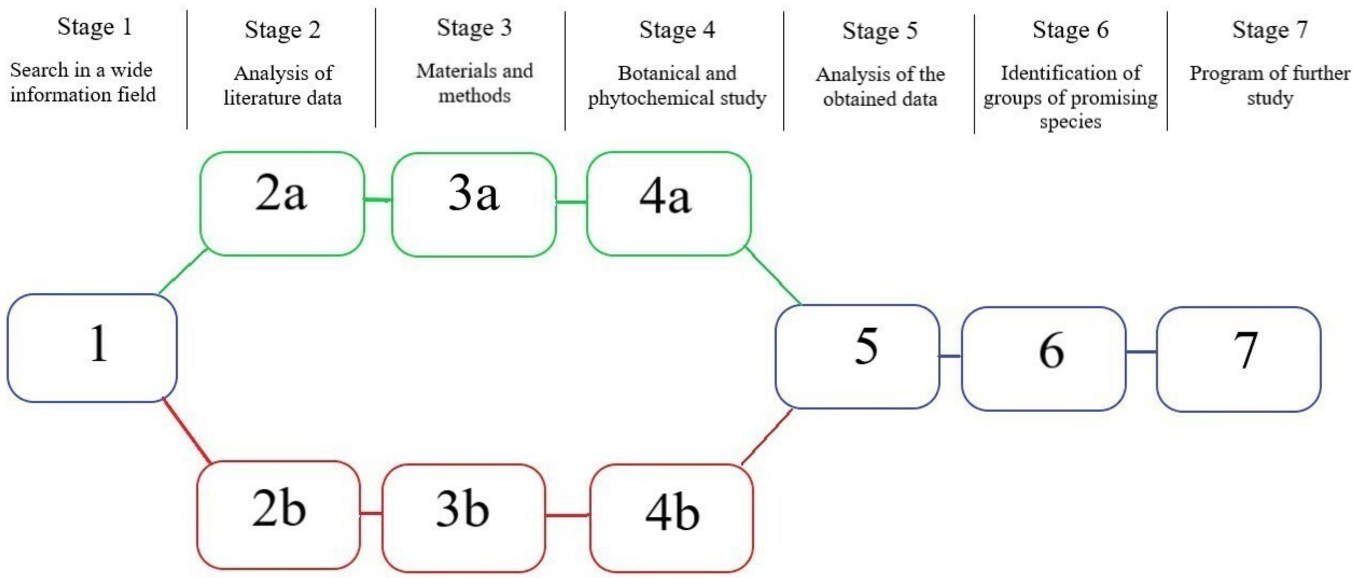

Figure 1. *Roadmap for evaluating the potential for using plants as sources of bioactive compounds.* ***1*** *– formulation of the working hypothesis,* ***2a*** *– assessment of the abundance and diversity of taxonomic groups,* ***2b*** *– evaluation of the content of bioactive compounds and their relationship with taxa,* ***3a*** *– selection of botanical material, territory, and methods of botanical analysis,* ***3b*** *– selection and development of methods of phytochemical analysis,* ***4a*** *– identification of species composition, systematic group, and geography of species,* ***4b*** *– content and identification of bioactive compounds,* ***5*** *– investigation of the relationship between the content of bioactive compounds and taxa and ecological groups,* ***6*** *– identification of promising plant groups,* ***7*** *– program of further study.*

The hypothesis regarding the potential for using species of the tribe *Cynareae* as sources of bioactive compounds dates back to 1988 to the study by Revina and Revushkin (1988). At the first stage of the study performed in line with the above algorithm, the literature data were reviewed, which showed that many species of the tribe *Cynareae* are known as medicinal plants; the species are widely used in traditional and official medicine. The unique biological activity of these taxa is due to a complex of compounds, which include phenolic glycosides, phenolcarboxylic acids, flavonoids, and ecdysteroids. The list of biological activities of tea, extracts, infusions and preparations from plants containing phenolic compounds and ecdysteroids is very diverse (Budantsev 2012). According to the literature data, a number of pharmacological preparations have been currently obtained based on phytochemical studies, which shows the need and demonstrates the potential for studying secondary plant metabolites. Thus, the medicinal preparation Sausfoli obtained from some species of the tribe *Cynareae* exhibits high anti-giardiasis and anti-opystarchosis activities (Krasnov et al. 2011).

Thus, based on the analysis of literature data and currently available studies (stages 1 and 2), the working hypothesis was formulated about the potential for using plant species from the tribe *Cynareae* growing in Southern Siberia as sources of phenolic compounds and ecdysteroids.

At stage 3, the species composition, geography, and ecological and coenotic confinement of species of the tribe *Cynareae* in the flora of Southern Siberia were analyzed. The boundaries of Southern Siberia were determined, and the study area was divided into areas. In accordance with the floristic zoning proposed by Takhtadzhyan (1978), Southern Siberia includes the southern part of the West Siberian, Altai-Sayan and Transbaikal provinces. This territory is diverse in its relief, climate and landscapes, and it is subdivided by the authors into 5 longitudinal areas: West Siberian (WS), Altai (Al), Sayano-Tuva (ST), Transbaikalian (Tb), Daurian (Da).

Stage 4 involves the botanical and phytochemical study of species. It was revealed that the tribe includes 103 species. The genus *Saussure*a is represented by a large number of species (49 species), followed by *Cirsium* L. (15 species), *Centaurea* L. (14 species), *Arctium* L. and *Fornicium* Cass. (4 species each) *Carduus* L. and *Jurinea* Cass. 1821 (3 species each), *Serratula* (8 species), *Alfredia* Cass (2 species) and one species was found for the genera *Ancathia* (Spreng.) DC. 1823 and *Alfredia*. The abundance of the tribe in Southern Siberia varies sufficiently. The largest number of species of the tribe grows in the Al area (54 species), the next are the ST area (50 species), the WS area (45 species), the Tb area (40 species), and the smallest number of species of the tribe grows in the Da area (36). Chorological analysis of the distribution of species of the tribe *Cynareae* growing in Southern Siberia revealed the predominance of the South Siberian group (33.7%), including 25.7% of endemics. The study of the belt-zonal structure of the tribe *Cynareae* in the flora of Southern Siberia as a whole showed that the predominant groups are the steppe group (21.3%), the high-mountain group (19.8%), the mountain-steppe group (17.8%), the mountain-forest group (14.8%), the forest group (12.9%), and the plurizonal group (11.9%).

Chemical screening was performed for 23 species of the tribe *Cynareae* for ecdysteroids and phenolic compounds (PC) (Kasterova et. al 2019). A trace amount of ecdysteroids was found in only 3 species of the genus *Serratula*: *S. algida* Iljin. 1934 (0.08%), *S. kirghisorum* Iljin. 1934 (0.01%), and *S. centauroides* L. 1808 (0.12%). Phenolic compounds were found in all 23 studied species.

At stage 5, the obtained data were analyzed. It was revealed that the species *Centaurea* 1937, *C. ruthenica* Lam. 1785, *C. scabiosa* L. 1753, and *C. sibirica* L. 1753 are promising raw materials for preparing flavonoid-containing drugs and broad-spectrum drugs. The highest content of phenolic compounds was found in *C. ruthenica*; analysis of this species from two geographically distant populations showed the dynamics of the studied secondary metabolites in the range from 2.49 to 6.74%. Promising sources of phenolic compounds were found among representatives of the genus *Saussurea*, which included 11 of the 12 studied species; a low content of phenolic compounds was found only in *S. amara* (L.) DC. 1810 (0.92%). The most promising sources of flavonoid-containing raw materials are the species *S. alpina* (L.) DC. 1810, *S. controversa* DC. 1810, and *S. parvirlora* (Poir.) DC. 1810 (Kasterova et. al 2021). Among the representatives of *Serratula*, the highest content of phenolic compounds (1.86%) was observed in *S. centauroides*. The data obtained indicate prospects for further in-depth study of the above plant species.

The study of the content of bioactive compounds in various ecological and cenotic conditions showed that plants growing in conditions of sufficient moisture exhibit the highest content of phenolic compounds. This yields the conclusion that it is important to take into account and maintain the required level of humidity in plant habitats to provide a high content of bioactive compounds when introducing promising species.

At stage 6, a comprehensive assessment of the obtained data was carried out in order to divide the studied species into groups by nature and by potential for their use:

1. Economically harmful species.

2. Promising species for use.

2a - species with the potential for their use from natural resources;

2b - species with the potential for introduction;

2c - species that require tissue culture.

3. Species of little promise and unpromising species.

4. Species that require special conservation measures.

Group 1 includes synanthropic (weedy, ruderal, invasive, potentially invasive) species, as well as species that tend to spread quickly in Siberia. Many of them contain bioactive compounds, therefore it is necessary to control the state of natural populations and create tissue culture. The group includes 14 species: *Arctium lappa* L. 1753, *A. leiospermum* Juz. et C. Serg. 1800, *A. minus* (Hill) Bernh. 1800, *A. tomentosum* Mill. 1768, *Carduus crispus* L. 1753, *C. nutans* L. 1753, *Cirsium incanum* (S.G. Gmel.) Fisch. 1820, *C. palustre* (L.) Scop. 1771, *C. oleraceum* Scop. 1771, *C. serratuloides* Hill. 1868, *C. serrulatum* (Bieb.) Fisher. 1812, *C. setosum* (Willd.) Besser, 1829, *C. vulgare* (Savi) Ten. 1835, *Centaurea cyanus* L. 1853.

Group 2 includes 20 species that were found to contain sufficient amount of bioactive compounds. Subgroup 2a includes the species *Saussurea controversa, Centaurea scabiosa, C. sibirica, C. modesti, C. phyllopoda,* and *C. ruthenica*. Subgroup 2b includes *Serratula algida, S. kirghisorum, S. centauroides,*and *Cirsium esculentum* (Siev.) C.A. Mey. 1845.

At present, employees of the Siberian Botanical Garden, National Research Tomsk State University (Tomsk), are evaluating the potential of species of the genus *Serratula* for introduction. Studies into the introduction of *Serratula* in Tomsk region have been carried out since the 90s of the 20th century, and species of this genus are successfully introduced into culture. Introduced species can be used as a source of medicinal plant raw material, as well as for restoration of endangered natural populations (reintroduction). The above species are important for species and intraspecific diversity conservation, and can serves as a source of seed material for restoration of depleted phytocenoses. The stability of most of these species in culture shows prospects for obtaining the required biomass of the studied species by introduction.

Due to low abundance and poor seed productivity, the studied species of the genus *Saussurea* were included in subgroup 2c (*Saussurea baicalensis* (Adams) B.L.Rob. 1911, *S. parviflora, S. pricei* N.D.Simpson,1913, *S. salicifolia* (L.) DC. 1810, *S. salsa* (Pall. ex Pall.) Spreng. 1826, *S. frolovii* Ledeb. 1833, *S. alpina* (L.) DC. 1810, *S. jadrinzevii* Kryl. 1915, *S. krylovii* Schischk. et Serg. 1944, and *S. schanginiana* Fisch. ex Herder 1868). If species cannot be introduced, plant cell, tissue and organ culture is required. This method can be used to quickly obtain sufficient biomass in aseptic conditions. Isolated plant cells retain the ability to produce compounds of secondary synthesis valuable for medicine: flavonoids, alkaloids, steroids, glycosides, hormones, essential oils, etc. Due to cell selection, the productivity of cultivated cells can significantly exceed the productivity of whole plants. This feature is widely used to create technologies for the industrial production of bioactive compounds.

Group 3 includes 2 species (*Saussurea amara, Ancathia igniaria* (Spreng.) DC. 1833) which did not show a high content of bioactive compounds, and those that have not yet been studied or poorly studied are conditionally included in the group.

Group 4 includes rare, endangered species. The Red Books of various Siberian regions report that species of the genus *Saussurea* are most endangered among the representatives of the tribe *Cynareae*. This can be due to the narrow ecological amplitude of some species of the genus, the relict nature of the species, and poor seed productivity.

The study of literature data revealed that many species of the genus *Artemisia* are medicinal and are widely used in medicine due to a high content of bioactive compounds. The taxa of the genus *Artemisia* were found to contain sesquiterpene lactones, coumarins, fatty acids, phytosterols, phenolcarboxylic acids, ascorbic acid, alkaloids, saponins, artemisinin, arglabin, essential oils, etc. The most valuable bioactive compounds found in species of the genus *Artemisia* include essential oils, which is characteristic of this genus (Berezovskaya et al. 1969; Belikov and Schreiber 1970; Khanina 1999). The main components of essential oils include compounds of secondary metabolism, namely terpenoids (Atazhanova 2006). Essential oils are highly valued for their antimicrobial, antibacterial, nematocidal, and fungicidal effects. A high content of bioactive compounds in wormwood determines its biological significance, including antiparasitic, antiasthmatic, insecticidal, and antiviral effects, and its biological activity against COVID-19 (Bisch 2022; Nair 2022). Wormwood is mainly used for making extracts and infusions that have analgesic, anti-inflammatory, and wound-healing effects (Buzuk and Elyashevich 2009). An active component artemisin, which is an excellent antimalarial agent, was isolated from *Artemisia annua* L. 1753. Later, a broad-spectrum drug Artemisin M was produced from this component.

The study of the species composition, geography and ecological-cenotic confinement of species of the genus *Artemisia* in the flora of Southern Siberia yielded the following results: the genus *Artemisia* in the flora of Southern Siberia includes 82 species, it is represented by 3 subgenera (*Artemisia, Dracunculus* Bess. 1835*, Seriphidium* (Besser ex Less.) Fourr. 1869) and 7 sections. The subgenus *Artemisia* includes 54 species, the subgenus *Dracunculus* has 19 species, and the smallest subgenus *Seriphidium* includes only 11 species. In Southern Siberia, the genus *Artemisia* is represented non-uniformly. In total, 28 species grow in the WS area, 41 species in the Al area, 50 species in the ST area, 44 species in the Zb area, and 41 species in the Da area.

Chorological analysis of the genus *Artemisia* revealed the predominance of Asian species (68.29%, 56 species), among which a high proportion of species from Southern Siberia (35.71%, 20 species), Mongolia and Southern Siberia (23.21%, 13 species) was found. There are significantly fewer Eurasian species (10%, 9 species), including North American-Asian and Holarctic species (3.7% each). The level of wormwood endemism in Southern Siberia attains 40.24%.

Based on a comprehensive assessment of the data obtained at the last stages, the studied species of the genus *Artemisia* were divided into groups according to nature and potential for their use.

Group 1 includes weed, ruderal plants. Many of them contain bioactive compounds and are used both in the economy as well as in the official and traditional medicine. These plants include 6 species of wormwood (*A. vulgaris* L. 1753*, A. absinthium* L. 1753*, A. sieversiana* Ehrn. ex. Willd. 1845, *A. annua*, *A. dracunculus* L. 1753, *A. scoparia* Waldst. et Kit. Pl. 1801).

Group 2 is represented by plants promising for medicine (as sources of bioactive compounds), which grow in Southern Siberia. The most common species include *A. pontica* L. 1753*, A. gmelinii* Web. ex Stechm. 1775*, A. santolinifolia* Turch. ex Bess. 1834*, A. austriaca*, *A. sericea* Web. ex Stecm. 1773*, A. latifolia* Ledeb. 1815*, A. macrobotrys* Ledeb. 1836, and *A. glauca* Pall. in Willd.1831.A number of species from the flora of Southern Siberia were considered promising for medicine: *A. macrocephala* Jacq. 1836*, A. jacutica* Drob. 1831*, A. altaiensis* Krasch. 1914, *A. martjanovii* Krasch. ex Poljak. 1949, *A. subviscosa* Turcz. ex Besser. 1836, *A. nitrosa* Web. ex Stecm. 1775. These species are promising due to the content of bioactive compounds, and due to their high biological activity and a wide range of pharmacological activities. The above species accumulate a significant amount of essential oil and coumarins (Berezovskaya et al. 1991).

Since wormwood shows high potential for medicine, the raw material base of medicinal plants can be expanded due to species of the genus *Artemisia* growing in the Southern Siberia, as well as through the introduction and cultivation of the most valuable species. The subgroup with the potential for using plants from natural resources includes 9 species (*A. vulgaris, A. absinthium, A, sieversiana, A. annua, A. frigida* Willd. 1838*, A. austriaca*, *A. dracunculus*, *A. glauca*, *A. scoparia)*. The subgroup with the potential for plant introduction includes 24 wormwood species *(A. vulgaris, A. abrotanum, A. absinthium, A. obtusiloba*, *A. martjanovii, A. austriaca*, *A. frigida, A. gmelinii, A. jacutica, A. laciniata*, *A. lagocephala* (Besser) DC. 1837, *A. latifolia*, *A. macrantha*, *A. rupestris* L., *A. mongolica* (Bess.) Fisch. ex Nakai 1917, *A. santolinifolia, A. tanacetifolia, A. pontica, A. sericea* Web. ex Stechm. 1775*, A. dracunculus, A. glauca*, *A. commutata* Bess. 1835, *A. dolosa* Krasch. 1949, *A. nitrosa*.). At present, the introduction of the genus and the study of introduced species are carried out in all botanical gardens of Siberia. In general, all introduced species show high resistance in culture, which allows their use as a source of medicinal plant materials, as well as for restoration of endangered natural populations (reintroduction) (Agafonova et al. 2017). Due to low abundance and poor seed productivity in culture, a number of species of the genus *Artemisia* entered the subgroup where introduction is impossible, and therefore tissue culture is required (*A. macrocephala, A. altaiensis, A. subviscosa*).

Group 4 includes rare, endangered species. The Red Books of various regions in Southern Siberia report that 8 species require special conservation measures: *A. xerophytica* Krasch. 1922, *A. m artjanovii, A. rutifolia, A. santolinifolia, A. macrantha, A. laciniata, A. gmelinii, A. compacta* Fisch. ex DC. 1838, *A. sublessingiana* (Kell) Krasch. ex Poljakov, 1954.

As a result of the study and analysis of the potential and rational use, species of the tribe *Cynareae* and representatives of the genus *Artemisia* were divided into groups that indicate their potential for use.

Conclusion

To search for and evaluate the potential for use of new sources of bioactive compounds, the algorithm has been developed that includes several stages and is designed as a road map for organizing and conducting studies. The study and approbation of this algorithm yielded the working hypothesis about the potential of using plants of the tribe *Cynareae* and the genus *Artemisia* growing in Southern Siberia as sources of bioactive compounds (phenolic compounds, ecdysteroids, essential oils). The species composition, geographical distribution, and chemical composition of the species were determined for the tribe *Cynareae* and the genus *Artemisia*.

In the flora of Southern Siberia, the tribe *Cynareaee* is represented by 103 species belonging to 11 genera (*Saussurea, Cirsium, Centaurea, Serratula Arctium and Fornicium, Carduus, Jurinea, Ancathia, Alfredia, Olgaea*). The genus *Artemisia* includes 84 taxa and is mainly represented by 3 subgenera (*Artemisia* Less., *Dracunculus* Bess., *Seriphidium* (Besser ex Less.) Fourr.).

Based on the results of evaluating the potential for the economic use, the species under study were divided into 4 groups. Group 1 includes synanthropic and economically harmful plants (14 species of the tribe *Cynareaee*, 6 species of *Artemisia*). Group 2 includes species promising for use (20 species of the tribe *Cynareaee* and more than 15 species of the genus *Artemisia*). Only 3 species are noted for the tribe *Cynareae*with the potential for using from natural resources, while the genus *Artemisia* has a larger supply of raw materials and includes 9 species. Four species of the tribe *Cynareae* are proposed for introduction: *Serratula algida, S. kirghisorum, S. centauroides, Cirsium esculentum,* and 24 wormwood species. Due to low abundance and poor seed productivity, 10 species of the genus *Saussurea* (the tribe *Cynareae*), and 3 species of the genus *Artemisia* are proposed for cell culture. Group 4 includes rare species that require conservation measures: 30 species of the tribe *Cynareae* and 8 species of the genus *Artemisia*.

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