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RESEARCH ARTICLE

Far Eastern Species of the Genus *Atragene* L. (*Ranunculaceae*) – valuable Medicinal Plants

Luferov A.N.¹, Bokov D.O.^{1,2*}, Yakubov V.V.³, Samylina I.A.¹, Gildeeva G.N.¹,
Smolyarchuk E.A.¹, Nicheporchuk P.A.¹, Antsyshkina A.M.¹, Rodin M.N.¹, Bondar A.A.¹,
Durnova N.A.¹, Konovalchik D.A.¹, Prostodusheva T.V.¹

¹I.M. Sechenov First Moscow State Medical University (Sechenov University),
8-2 Trubetskaya str., Moscow, 119991, Russian Federation.

²Federal Research Center of Nutrition, Biotechnology and Food Safety,
2/14 Ustyinsky pr., Moscow, 109240, Russian Federation.

³Federal Scientific Center of the East Asia Terrestrial Biodiversity, Far Eastern Branch of the Russian Academy
of Sciences, 159 Prospect 100-let Vladivostoka, Vladivostok, 690022, Russian Federation.

*Corresponding Author E-mail: bokov_d_o@staff.sechenov.ru

ABSTRACT:

ABSTRACT. Species of *Atragene* contain biologically active compounds acting to treat dermal diseases, gastrointestinal tract diseases, nervous system diseases, immune system diseases as well as malignant tumors and other diseases, so thorough research of the plants is of great practical importance for medical practice. **Object of the research:** to estimate possibilities of using the Far Eastern species of *Atragene* in applied medicine and to prepare the sources to determine them. **Materials and Methods:** The plants were researched during expeditions to the Far East, the Sakhalin Region, the Khabarovsk Territory, and Primorsky Krai. The literature data and many herbarium collections were studied. Morphological and geographical methods of research as well as ecological, biological, and karyological data were the main method of the research. **Results** There was a review of the data on chemical composition and pharmacological properties of biologically active compounds of species of the *Atragene* flora of the Far East, as well as the perspectives to study them as a valuable source of medicinal raw material to make pharmaceutical substances and their possible using for official medicine. *A. speciosa*, which consists of anthraquinones, flavonoids, alcohol phenols, oxycoumarin, phenolcarboxylic acid, triterpene glycoside, diterpene alkaloids, amino acids, carotenoids, tanning materials, polysaccharides, and trace elements is mostly analyzed. The complex of the compounds is of nootropic, adaptogenic, antioxidant, wound healing, and other pharmacological effects. Stems, leaves, flowers, and fruits of the following species may be used as admixtures: *A. ochotensis*, *A. macropetala*, and *A. koreana*. There are also morphological characteristics and dichotomy key recommended to determine the Far East species of *Atragene*. **Conclusion.** *A. speciosa* is perspective for medical use: medicinal raw material is above-ground shoot, contains the rich chemical biologically active compound composition. The following morphological characteristics of vegetative and reproductive organs were suggested for using to identify the Far East *Atragene* genus: the form of the leaflets of compound leaves, presence or absence of downiness of the stems, sticks, blades, and perianths; color and spatial orientation of trichome; form, size, and form of tops of sepals and petals; downiness of fruitlets, i.e. nutlets; size, color, and spatial orientation of hair. With regard to these structure characteristics, species indicator of the *Atragene* flora of the Far East of Russia was worked out.

KEYWORDS: *Atragene*, species indicator, Far East of Russia, chemical composition, pharmacological properties of the substances, perspectives of using in medicine.

INTRODUCTION:

The *Atragene* (*Ranunculaceae*) genus species are used in the folk medicine of various countries as well as in folk

Tibetan medicine at all times. It is found that these plants contain biologically active compounds acting to treat dermal diseases, gastrointestinal tract diseases,

nervous system diseases, immune system diseases as well as malignant tumors, and other diseases. Lack of knowledge of the *Atragene* species locks down their wide use. Without a doubt, thorough research of the plants is of practical importance for medical practice.¹⁻⁴

Object of research is to estimate possibilities for using Far Eastern species of the *Atragene* genus in practical medicine and to prepare the key to determine them.

To achieve an object, it was necessary to decide the following

tasks:

1. Collect the information on the chemical compounds contained in the Far East species of *Atragene*.
2. Generating the information on pharmacological properties of biologically active compounds of these species of genus and perspectives of their use for official medicine.
3. Discovering of morphological characteristics of the structure of vegetative and reproductive organs of the plants, that are of great importance to determine species of *Atragene*.

Chemical composition, pharmacological activity, and possibilities of using medicine as a source of biologically active compounds were mostly studied for *A. speciosa*. In folk medicine, this species of the herb (above-ground shoots) is used to treat cardiovascular and gynecologic illnesses, as well as an antitumor, sedative, wound healing, antiarthritic, diuretic and bracing drug⁵⁻⁸.

The extracts of the above-ground parts of this species of the herb contain diterpene alkaloids (aconitine and delphinine)⁹, anthraquinones, flavonoids (quercetin, isoquercitrin, kaempferol), alcohol phenols, oxycoumarins (skimmin, scopoletin, umbelliferone, esculin, esculetin), phenolcarboxylic acids (caffeic and quinic acids), triterpene β -amyrin glycosides, carotenoid, tanning materials, polysaccharides^{10,11}, such amino acids as proline and isoleucine, as well as trace elements: Na, Fe, Ba, Cr, Br^{8,12}, as well as there is an extracted fraction containing 3,4-dihydroxyphenylethanol-2, and 2-O- β -D-glucopyranoside, and summarized glycosides of dihydroxybutanoic acid¹³.

The results of chemical and pharmacological analysis of biologically active compounds of *A. speciosa* make it possible to think that they regulate the activity of neurons and vascular systems of homoitherns and human organisms⁸. Pharmacological analysis makes it possible to prove nootropic, adaptogenic, and antioxidant activity of the complex of this species of chemical compounds^{8,10,14,15}.

The extract of the above-ground shoots of *A. speciosa* increased the efficiency of laboratory animals¹⁴. The patent for the invention "Pharmaceutical Composition of Nootropic Activity" has been proved¹⁶. The authors suggest a nootropic medicinal drug, which contains fluid alcohol extract of the above-ground part of *A. speciosa*.

The patent for an invention "The Way to Extract Biologically Active Substances from Cell Culture of *Atragene speciosa* Weinm. (Siberian Clematis)"¹⁷ includes information on the used method of cultivation of biomass of tylosis cell culture with the high level of triterpene saponins and flavonoids of this kind of *Atragene*. Extracted compounds are of adaptogenic and nootropic activity.

The patent for an invention "Collection of Antioxidant Medicinal Plants"¹⁸ contains information on the new medicinal antioxidant tea. The tea includes shoots of *A. speciosa* Weinm, green leaves of *Bergenia crassifolia* (L.) Fritsch, and the *Filipendula ulmaria* (L.) Maxim herb. It is noted that *A. speciosa* is of expressed antioxidant, antiradical, nootropic, anti-ischemic, anxiolytic, antistress, adaptogenic substance, and immunotropic action.

Flowers of *A. ochotensis* contain kaempferol¹⁹, it is discovered that above-ground shoots contain triterpene glycoside, carotin, and coumarin, and roots contain saponin⁶. Leaves of *A. macropetala* contain quercetin, flowers contain kaempferol¹⁹. The information on the chemical composition and pharmacological properties of biologically active compounds of *A. koreana* is currently absent.²⁰⁻²⁴

MATERIALS AND METHODS:

Areas of research. During 10 expeditions (1980, 1986-1993, 2015) to the Sakhalin region (the Sakhalin island, the Iturup island, and the Kunashir island), the Khabarovsk Krai, and the Primorsky Krai, plants were studied in natural cenosis, herbarium and the material fixed in ethanol were gathered. Binocular MBC-3, microscope MBR-3, and eyepiece screw micrometer MOB 1-15 were used to study details of the structure of vegetative and reproductive organs. The literature data and many herbarium collections kept in the LE, LECB, MHA, MOSM, MOSP, MW, MWG, NS, NSK, SAK,

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TU, VBG, VLA reserves, Bolshekhokhtsirsky Nature Reserve, Kedrovaya Pad, and Khingan Nature Reserve were studied.

Objectives of research: morphological and geographical research as well as ecological, biological, and karyological data were the main methods of the research.

RESULTS AND DISCUSSION:

Species of the *Atragene* L. genus are geoxyl, taproot, or mixed root system shrubbery or forbs: megaphanerophytes and nanophanerophytes with upright climbing long shoots and axillary short monocyclic monocarpic shoots²⁵. Leaves are oppositifolious, tricomponent, or biternate with spiniform gnarled parts. For species of *Atragene*, the most significant diagnostic features are: the shape of leaflets in compound leaves, the presence or absence of pubescence, and the distribution patterns of hairs on the adaxial (upper) and abaxial (lower) surfaces of the leaf blades. Flowers are bisexual and actinomorphic. The perianth is double. There are 4–5 sepals and 4–5 petals. Flowers are blue-violet or blue (*A. macropetala*, *A. ochotensis*) to yellowish-white, less frequently nearly white (*A. koreana*, *A. speciosa*).

It is essential to consider the ratio of sepal length to petal length. For example, in *A. macropetala*, the petals are oblong-lanceolate with an acute apex, while in other species, they are spatulate-expanded with a rounded apex. Petals also differ in pubescence: in *A. speciosa*, petals are glabrous or sparsely hairy at the apex, whereas in other species, pubescence is dense and more or less uniformly distributed across the entire surface²⁵. Multiple stamens have threads with downy edges and expanded tops. The gynoecium is apocarpous; multiple carpels have one ovule. Fruit is apocarpous. Nutlets of *A. macropetala* are ellipsoid and have long and accumbent hair, nutlets of other species are inversely egg, glabrous, or downy to varying degrees. Seeds are ellipsoid, black, brown, or bright²⁶.

Morphological characteristics of the Far Eastern species of *Atragene*, which we suggest for them to be diagnosed. The first time, structure and specifics of the downiness of such organs as blades, stocks, stems, and fruits were noted. It was suggested to diagnose morphological characteristics of vegetative and reproductive organs as well as characteristics of life forms and species of the plants. There were biomorphological characteristics of structure: type of root system and the cycle of development of above-ground shoots²⁵.

Species indicator of kinds of the *Atragene* flora of the Far East of Russia:

Nine species are most widespread in forests of the temperate zone of Eurasia and North America²⁷.

1. Petals are equal or a bit shorter than sepals, oblong-lanceolate, and have acuminate tops. Nutlets are elliptic with short accumbent hair. Styles have extensive downy long (from 4–5 up to 7 mm long), half accumbent, or squarrose white hair.

2. *A. macropetala*

Petals are two and more times shorter than sepals, tops are 6 mm long and spatulate wide. Nutlets are inversely egg, glabrous, or downy with other color hair. 2. Stems and stocks are glabrous or with short accumbent hair. The calyx is blue-violet or electric blue. Styles have long (of 2–5 mm long) fawn drab or light brown hair.

3. *A. ochotensis*

Stems and stocks have long distant or a bit accumbent hair. The calyx is ochroleucous or almost white. Styles have off-white hair. 3

3. Leaflets are oval or broad-ovary, both sides have extensive hair. Petals have round tops, external sides are extensively downy hair of 2–2.5 mm long. The ovary is short-fibred. Style have long hair (trichome are of 1.5–4 mm long) all along

1. *A. koreana*

– Leaflets are lanceolate-ovate or narrowly elliptical, tops are glabrous, down parts are a bit downy with hair up to 1.5 mm long. Petals are blunt and glabrous and have a little hollow, or 6 mm of the top are villous. Ovary and down parts of styles are glabrous, rarer, they have scattered and short (0.5–1 mm long) downiness, the rest of styles have multiple long (2–5 mm long) hair.

4. *A. speciosa*

Here are citations, synonymics, and descriptions of the Far Eastern species of *Atragene*:

1. *A. koreana* (Kom.) Kom. 1903, Papers of the St. Petersburg Botanic Garden, 22, 1 (Manchurian Flora. 2, 1): 278. – *Clematis koreana* Kom. 1901, the St. Petersburg Botanic Garden, 18, 3: 438.

Above-ground shoots are up to 1.5 (2.5) meters long. Stems are creeping or climbing, grooved, or downy with distant hair. Leaves are tricomponent or double tricomponent; leaflets are 4–12 cm long and of 2–6 cm wide, oval, broad-ovary, with large lobes, or whole, large-toothed, with acuminate sharp tops and almost heart-shaped bases. Stocks, small petioles, and pedicel are extensively distant-villous. 4–5 sepals are broad-lance, shortly acuminate, and downy with mild hair on the outside and the edges. 4–5 or rarely more petals are linear, tops are spatulate and dense haired. The ovary is inversely egg and accumbent downy. Styles are up to 4

cm long, with long distant or accumbent hair (fig. 1). $2n = 16^{28}$.

Spread. Russia: Primorsky Krai: south part of Khasansky District, North-East China, and Korean Peninsula. Open rollings of hills, edges of broad-leaved forests, among shrubbers, and on stony expositions. It flowers in June or in July, it bears fruits in August or in September²⁷. It is a rare species, that need to be protected.



Fig. 1: *Atragene koreana*. Russia, Primorski Krai, Chasanski district, 15.05.2011. Yakubov V.V.

2. *A. macropetala* (Ledeb.) Ledeb. 1830, Fl. Alt. 2: 376. – *Clematis macropetala* Ledeb. 1829, Icon. Pl. Ross. 1: 5.

Above-ground shoots are up to 4 (5) meters long. Stems are climbing or rarer creeping. Leaves are twice tricomponent or trice tricomponent, rarer trice pinnately compound; leaflets are oblong-elliptic, ovate, unequally-large-toothed, or often 2-3-incised. Stocks are long and enwrap support. 4 (sometimes 5-6) sepals are usually of 3-5 cm long, ovate-lanceolate, severe, blue-violet, extensively downy with mild short hair. 15-40 petals are oblong-lanceolate, acuminate, lavender, or light blue, extensively downy on the outside. External stamens have extended tops and downy, internal ones are thin and almost glabrous. The ovary is elliptic and short accumbent villous. Styles have long pinnate off-white downiness (fig. 2).



Fig. 2: *A. macropetala*. Russia, Amur Region, Blagoveshchensk, Amur Branch of the Botanical Garden-Institute of the Far Eastern Branch of the Russian Academy of Sciences, 30.05.2014. Darman G.

Spread. Russia: The Amur region, South part of Eastern Siberia (Transbaikal), Mongolia, and North China. Open or shadowy parts of coniferous or deciduous forests, on slopes of hills, and rocks. It flowers from May till June; it bears fruits from July till September²⁷.

3. *A. ochotensis* Pall. 1788, Fl. Ross. 1, 2: 69. – *Clematis ochotensis* (Pall.) Poir. 1812, Encycl. Meth. Suppl. 2: 298.

Above-ground shoots are up to 4 (8) meters long. Stems are climbing or rarer creeping. Leaves are double tricomponent, rarer tricomponent or trice tricomponent; leaflets are ovate-lanceolate, whole, 2-3-lobed or divided, gnarled, or a bit downy. Sepals are of 4, rarer 5-6, or 3-4 (5) cm long, elliptic, broad-lance, blue-violet, azure-blue, with villous edges and outside. Petals are like stamens or a bit longer, with round tops, and villous outside. The ovary is inversely egg, their bases are broad-wedged, downy half accumbent, and distant short hair. Styles have long orange-yellow hair (fig. 3). $2n = 16^{29}$.



Fig. 3: *A. ochotensis*. Russia, Amur Region, Bureinsky Nature Reserve, June 2009. Yakubov V.V.

Spread. Russia: all Far East regions besides Chukotka Autonomous Region, Komandorskiye Islands, and North part of Greater Kuril Chain, Eastern Siberia, Mongolia, and North-East China. In coniferous and deciduous forests, on edges of the forests, on rocky-lumpy exposition, it grows in mountains up to forest line. It flowers from May till June, it bears fruits from July till September²⁷.

4. *A. speciosa* Weinm. 1850, Bull. Soc. Nat. Moscou, 23, 1: 538; Serov, Jarvis, 1988, Taxon, 37, 1: 167. – *A. sibirica* L. 1753, Sp. Pl. 1: 543, nom. ambig.; Krashenninnikov, 1937, quot. write.: 308.

Above-ground shoots are up to 4 meters long. Stems are climbing or creeping, grooved, and quite downy. Leaves are usually tricomponent; leaflets are 2-5 cm long, 0.8-2 cm wide, ovate-lanceolate, elliptic, severe, or acuminate, unequally gnarled, with base rounded or broad-wedged. 4 or sometimes 5-6 sepals are of 2.5-5 cm. long, ovate-elliptic, or broad-lance on the outside with short downy edges. 4-12 or rarely more petals are linear, tops are

spathulate and short villous, 2-4 times shorter than sepals and almost equal to stamens. The ovary is inversely egg, glabrous or a bit downy. Styles are up to 5 cm long, with long erectopatent or off-white hair (fig. 4). $2n = 16^{30}$.

Spread. Russia: The Amur Region, the Khabarovsk Territory, taiga zone of European Russia, Weast and East Siberia, Kazakhstan, Central Asia, Mongolia, and north China. In coniferous and deciduous forests, on the edges of forests, on rocky hills, on rocks, along rivers and streams, in intergap ravines, up to substandard mountain belt where it is often in pinus pumila tangle and stone birch. It flowers from May till June, it bears fruits from July till August²⁷.

Boreal species is widely known in Eurasia and well-known in literature as *A. sibirica* L. It is established that this name is to be considered as nomen ambiguum³¹, because this kind of taxon is a herborized leaf of *A. macropetala* (Ledeb.) Ledeb., and the neighbored shoot with a flower belongs to one of the *Aquilegia* species, taxonomic characteristic of which is still needed to be checked up, in our opinion.



Fig. 4: *A. speciosa*. Russia, Irkutsk region, Bodaibo, the bank of the Bodaibinka river, 12.06.2008. Onishchenko L.

CONCLUSION:

According to the review of bibliographical notes, the Far East species of *Atragene* are analyzed unevenly. *A. speciosa* has a more rich chemical composition of biologically active compounds such as anthraquinones, flavonoids, alcohol phenols, oxycoumarins, phenolcarboxylic acids, triterpene glycoside, diterpene alkaloids, amino acids, carotenoids, tanning materials, polysaccharides, and trace elements.

The complex of the compounds is of nootropic, adaptogenic, and antioxidant activities. *A. ochotensis* and *A. macropetala* are less analyzed, their above-ground parts contain flavonoids and, obviously, most of the above-mentioned compounds. The chemical composition and pharmacological effect of biologically active compounds of *A. koreana* are still not studied.

For the first time, we have studied morphological characteristics of the structure of vegetative and reproductive organs to identify Far East members of the *Atragene* genus. It is established that the following morphological characteristics of vegetative and reproductive and reproductive parts may be used to identify the species: the form of the leaflets of compound leaves, presence or absence of downiness of the stems, sticks, blades, and perianths; color and spatial orientation of trichome; form, size, and form of tops of sepals and petals; downiness of fruitlets, i.e. nutlets; size, color, and spatial orientation of hair. Using the morphological characteristics makes it possible to make species indicator of kinds of the *Atragene* flora of Far East of Russia.

A. speciosa is the most perspective for medical use: medicinal raw material is above-ground shoots. Stems, leaves, flowers, and fruits of other species of *Atragene* may be used as admixtures.

AUTHORS CONTRIBUTION:

Luferov A.N. sets the goal and objectives of the study, studying plants in natural communities, analyzing the diagnostic significance of morphological features of the studied species and compiling a key for their determination, collecting information about the chemical composition, pharmacological activity of biologically active compounds and the prospects for their use in medicine. Samylina I.A. and Konovalchik D.A. developed a plan for writing the paper and participated in the discussion of the theoretical and practical significance of the results of the work. Bokov D.O. and Nicheporchuk P.A. translated the text of the manuscript. Yakubov V.V. participated in the collection of plants in natural communities and their photography. Antsyshkina A.M., Rodin M.N., Bondar A.A., Durnova N.A., Gildeeva G.N., Prostodusheva T.V., Smolyarchuk E.A. searched for bibliographic information and prepared the text of the manuscript.

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CONFLICT OF INTEREST:

The authors declare no conflict of interest.

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