Rowan species *Sorbus amurensis Koehne* as a prospective source of plant raw materials for the pharmaceutical and food industries

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Abstract. Rowan fruit is widely used in Russia as a stock for pharmaceutical and food industries. The only officinal species which fruits are harvested for pharmaceuticals is *Sorbus aucuparia L*. In the Far East it is substituted by the vicarious species of rowan *Sorbus amurensis Koehne*. The paper presents data on discriminating the fruits of both species of rowan based on macroscopic and microscopic identification, and on the content of biologically active compounds. The studies have proved no fundamental difference between the species. It was shown that there is no significant difference in the average values of ascorbic acid, organic acids and oxidizable substances in representative fruits samples of both rowans. The average content of flavonoids and hydroxycoric acids is reliably higher in the fruits of *S. amurensis Koehne*, although incidentally. It is shown that *S. amurensis Koehne* is not inferior to *S. aucuparia L*. in its main characteristics and can be considered a promising species to be used in the pharmaceutical and food industries along with the officinal species of rowan.

1. Introduction

Rowan is popular in Russia for medicinal, food and ornamental use. In medicine, the fruits of rowan are used as a multivitamin or as a part of pharmaceutical compositions [1]. They are included in the composition of food products for functional, preventive, and therapeutic nutrition [2, 3]. The tree is also used as an ornamental plant in squares, on boulevards, in parks.

Over the past decade, the study of rowan fruits has been carried out in various directions, including the studying of its chemical composition [4, 5, 6, 7] and biological activity [8, 9, 10, 11, 12].

The only medicinal species which fruits are harvested for pharmaceuticals is *Sorbus aucuparia L*. It is widely spread over the Western part of Russia. Whereas in the Far East it is substituted by the vicarious species of rowan *Sorbus amurensis Koehne*. In the Russian Far East *S. amurensis Koehne* is met is Primorsky and Khabarovsky territories. It is also present in Japan, Korea, and China.

*S. aucuparia L*. and *S. amurensis Koehne* are vicar (closely related) species, possess an incredibly significant morphological similarity and comparable area ranges [13]. Biological reserves of wild *S. amurensis Koehne* in the Far East of Russia are estimated at 9 thousand metric tons, 1.5 thousand metric tons are in production fund [14]. The local population uses *S. amurensis Koehne* in a similar way to *S. aucuparia L*. This, however, is not enough to have the fruits of *S. amurensis Koehne* included in the state register of medicines allowed for use in Russia.
For several years, the Far Eastern State Medical University has been studying S. amurensis Koehne as a prospective source of crude drug. The study is aimed to validate the use of S. amurensis Koehne fruits in the pharmaceutical and medical practice in Russia along with S. aucuparia L. fruits. The paper presents data on discriminating the fruits of both species of rowan based on macroscopic and microscopic identification, and on the content of biologically active compounds.

2. Materials and methods
The study was carried out on 38 samples of S. amurensis Koehne collected at various places of the range of population, mainly in the Khabarovsk and Primorsky territories, with samples taken from wild as well as cultivated plants. Rowan samples of S. aucuparia L. were mainly commercial samples (10 samples), a few were granted by the colleagues from the European part of Russia (4 samples).

Representativeness of samples for comparative purposes was ensured by harvesting the samples of S. amurensis Koehne throughout the range of population over several years, as well as including the samples of various commercial series. Standardization of samples was based on collecting fruits in the phase of maturity, and on verifying for compliance with the state standard of Russia, Pharmacopoeia article Rowan fruits [15, 16].

To exclude the influence of such factors as weather conditions of the sampling year, data was examined for homogeneity by statistical processing of results, as well as for the absence of statistically significant difference in the content of bioactive compounds in samples relating to individual years [18]. The absence of significant difference in bioactive compounds content in samples of different years was checked by means of variance analysis and Student's t-test and Fisher's ratio test. All calculations were performed with a confidence level of P = 95%.

Content of the biologically active compounds in two species of rowan (S. amurensis Koehne and S. aucuparia L.) was also examined via Student's t-test. The variability of bioactive compounds was estimated by the coefficients of variation (V, %), which is the ratio of the average quadratic deviation to the average arithmetic taken per cent.

Comparative macroscopic and microscopic identification of the fruits of both S. aucuparia L. and S. amurensis Koehne was carried out concurrently in accordance with the Russian standard on rowan fruits. Analysis of the anatomical structure of the fetuses was carried out using a microscope Olympus XSZ-107BN. Pictures of micropreparations were taken by NIKON D3100 camera, AF-S DX NIKKOR lens.

Comparative chromatographic analysis was tested on Merck Kieselgel 60 F254 10x20 plates in chloroform:acetone:formic acid (9:2:1) and toluene:ethyl acetate:formic acid (2:2:1). The plates were viewed in UV light at wavelengths of 254 and 365 nm before and after treatment with aluminum chloride solution.

Samples of both species of rowan were examined for content of ascorbic acid, total organic acids, flavonoids, hydroxycoric acids and oxidizable compounds. The amount of ascorbic acid, organic acids and oxidizable substances was determined by specified methods [15, 17]. The amount of flavonoids was determined using the method developed by the authors [18]. The hydroxycoric acid content was evaluated by direct spectrophotometry at 326 nm and conversion in equivalent to coffee acid. The number of samples used in the corresponding assay is shown in table 2. The number of repetitions of each analysis ensured sufficient accuracy to purposes of the study.

3. Research results and discussion
The macroscopic identification showed that all samples comply to the specific characteristics of rowan fruit described in the Pharmacopoeia article. The data in table 1 shows that the fruits of the two species have no difference in color, size, number of seed chambers.

Microscopic identification also revealed no significant difference in structure of the fruits of vicar species. The fruits of S. amurensis Koehne comply to the description of Microscopy section of Pharmacopoeia articles. This is confirmed by the following pictures of micropreparations (figures 1-10) given as an example.
Table 1. Comparison of macroscopic features of the rowan fruits *S. amurensis Koehne* and *S. aucuparia* L.

| Sample | Coloring of fruits | Diameter of fruits | Number of seed chambers/seeds |
|--------|--------------------|--------------------|------------------------------|
| 1      | Brownish red       | 0.70±0.22          | 4.0/4.7                      |
| 2      | Reddish-orange     | 0.67±0.12          | 2.8/2.5                      |
| 3      | Brownish red       | 0.69±0.15          | 4.5/4.3                      |
| 4      | Reddish-orange     | 0.67±0.14          | 3.8/3.2                      |
| 5      | Yellowish-orange   | 0.67±0.17          | 2.9/3.5                      |
| 24     | Brownish red       | 0.74±0.14          | 3.8/4.7                      |
| 25     | Reddish-orange     | 0.77±0.24          | 3.4/5.2                      |
| 26     | Brownish red       | 0.70±0.18          | 3.3/4.4                      |
| 27     | Reddish-orange     | 0.61±0.10          | 3.5/5.0                      |
| 28     | Brownish red       | 0.70±0.11          | 2.5/4.5                      |

*Figure 1.* Epidermis fragments of *S. aucuparia* L. fruits. Scale x400. Epidermis cells with droplets of fatty oil with carotenoid inclusions.

*Figure 2.* Epidermis fragments of *S. amurensis Koehne*. Scale x400. Epidermis cells with droplets of fatty oil with carotenoid inclusions.

*Figure 3.* Epidermis fragments of *S. aucuparia* L. fruits. Scale x400. Simple pachypleurous non-glandular hair.

*Figure 4.* Epidermis fragments of *S. amurensis Koehne*. Scale x400. Simple pachypleurous non-glandular hair.
Figure 5. A fragment of *S. aucuparia* L. fruit mesocarp. Scale x400. Mesocarp cells, vascular bundles, stone cells, clusters of calcium oxalate.

Figure 6. A fragment of *S. amurensis Koehne* fruit mesocarp. Scale x400. Mesocarp cells, vascular bundles, stone cells, clusters of calcium oxalate.

Figure 7. A fragment of *S. aucuparia* L. seed. Scale x400. Sclerenchyma cells.

Figure 8. A fragment of *S. amurensis Koehne* seed. Scale x400. Sclerenchyma cells.

Figure 9. A fragment of *S. aucuparia* L. seed. Scale x400. Structure of testa.

Figure 10. A fragment of *S. amurensis Koehne* seed. Scale x400. Structure of testa.

Rowan species *S. aucuparia* L. is known to contain a variety of biologically active compounds, such as vitamins, organic acids, phenolic compounds. Flavonoids, catechins, anthocyanins, leukoanthocyanidins were found in the fruits of *S. aucuparia* L. [19, 20, 21]. The occurrence of the same range of biologically active compounds in the fruit of *S. amurensis Koehne* was confirmed by phytochemistry reactions and chromatographic studies. Figure 11 shows a sample chromatogram of...
S. aucuparia L. and S. amurensis Koehne samples. As can be seen from the figure, there is no fundamental difference in the tracks of samples of both species; it is obvious that interspecific difference does not exceed intraspecific one. Chromatographic method shows that samples of both species correspond to the standard chromatogram (figure 12).

![Figure 11. Chromatogram of S. aucuparia L. (1,2,3) and S. amurensis Koehne (4,5,6) samples (wavelength 254 nm). Chloroform:acetone:formic acid (9:2:1) system.](image1)

![Figure 12. Standard chromatogram of S. aucuparia L. after treatment with aluminum chloride solution (wavelength 365 nm). Chloroform:acetone:formic acid (9:2:1) system.](image2)

The amount of biologically active compounds found in the fruits of S. aucuparia L. and S. amurensis Koehne with statistical analysis of data is given in table 2.

**Table 2.** The amount of biologically active compounds found in the fruits of S. aucuparia L. and S. amurensis Koehne with statistical analysis.

| Statistical parameters | flavonoids | ascorbic acid | organic acids | hydroxycoric acids | the oxidized substances |
|------------------------|------------|---------------|---------------|---------------------|------------------------|
|                        | S. amur<sup>a</sup> | S. aucup<sup>b</sup> | S. amur<sup>a</sup> | S. aucup<sup>b</sup> | S. amur<sup>a</sup> | S. amur<sup>b</sup> | S. aucup<sup>a</sup> | S. aucup<sup>b</sup> |
| n                      | 38 | 14 | 20 | 11 | 22 | 14 | 37 | 11 | 22 | 13 |
| $\bar{x}$              | 0.203 | 0.150 | 0.24 | 0.20 | 2.97 | 3.77 | 1.29 | 0.73 | 2.83 | 2.17 |
| $\Delta \bar{x}$       | 0.025 | 0.023 | 0.03 | 0.04 | 0.41 | 0.42 | 0.09 | 0.16 | 0.22 | 0.51 |
| $x_{\text{min}}$       | 0.098 | 0.098 | 0.13 | 0.09 | 1.50 | 2.86 | 0.71 | 0.55 | 1.97 | 1.63 |
| $x_{\text{max}}$       | 0.373 | 0.262 | 0.38 | 0.28 | 5.22 | 5.58 | 1.91 | 1.38 | 3.77 | 3.10 |
| $S^2$                  | 0.0060 | 0.0016 | 0.0040 | 0.0036 | 0.8357 | 0.5409 | 0.0750 | 0.0559 | 0.2536 | 0.1785 |
| $V$                    | 38 | 26 | 26 | 28 | 30 | 19 | 21 | 31 | 17 | 19 |
| $t_{\text{score.}}$    | 3.21<sup>c</sup> | 0.64 | 0.92 | 2.12<sup>c</sup> | 1.37 |
| $t_{\text{value}}$     | 2.03 | 2.05 | 2.03 | 2.03 | 2.03 |

<sup>a</sup> S. amur - S. amurensis Koehne,
<sup>b</sup> S. aucup - S. aucuparia L.
<sup>c</sup> statistically significant difference was noted (P = 0.95)
The data in table 2 shows that the amount of biologically active compounds in the fruits of both species fluctuates greatly: the maximum and minimum values of ascorbic acid differ by three times and more. The range of flavonoids and organic acids content in *S. amurensis Koehne* differs by more than three times. In general, fluctuations in the content of bioactive compounds range from moderate to significant; with variation characteristics of both species being at coequal levels.

When comparing the average values of biologically active compounds content, the fruits of *S. amurensis Koehne* and *S. aucuparia L.* have no statistically significant difference in terms of ascorbic acid, organic acids and oxidizable substances ($t_{\text{score}} < t_{\text{value}}$). The average content of total flavonoids and hydroxycoric acids is significantly higher in the fruits of *S. amurensis Koehne* ($t_{\text{score}} > t_{\text{value}}$).

4. Conclusion
The studies have shown that the fruits of *S. amurensis Koehne* and *S. aucuparia L.* have no meaningful difference in their macro and micro characteristics and fully comply with Russian regulatory document for medicinal rowan fruits. In terms of biologically active compounds content the fruits of *S. amurensis Koehne* are as potent as the fruits of *S. aucuparia L.* The content of total flavonoids and hydroxycoric acids in the first species even exceeds the latter. Although this conclusion should be considered more of an academical interest since intraspecific variability is higher than interspecific.

Thus, based on such characteristics as close systematic kinship, extensive range of population, significant reserves of plant raw material, as well as equivalence in the content of main biologically active compounds the fruits of rowan species *Sorbus amurensis Koehne* is a prospective source of crude drug for pharmaceutical practice along with the fruits of *Sorbus aucuparia L.*

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