Differentiation of *Pimpinella saxifraga* L. s.l. in comparative, morphological and phytochemical analyses

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Summary

The paper presents study results on *Pimpinella saxifraga* L. s.l. which belongs to critical species whose taxonomy has not been finally determined, so far. Because of the fact that in this genus, there occur species with acknowledged usefulness (*P. anisum* L., and *P. anisetum* Boiss. et Ball) and possess therapeutic importance [among others: *P. saxifraga* L., *P. tragium* Vill., *P. major* (L.)Huds.], the presented studies can be helpful in the determination of their natural affinity. Burnet saxifrage, *P. saxifraga*, was the research object treated as an aggregation of forms which are related to each others because of a high degree of structural polymorphism. Morphological studies were carried out on herbarium material collected in 34 localities in West Poland (Wielkopolska-Kuyavia Lowland). Results of this part of studies that in that have shown terrain, there occur two separate forms ("nigra" and "saxifraga"), whereby the "nigra" form occurs significantly more frequently than the "saxifraga" one. The ranges of biometrical measurements of some features are overlapping indicating that there occur plants with an intermediate character (hybrids?).
In the phytochemical analysis of roots, it was that there occurred essential oil which contained eight components. The domination of the "nigra" form has been confirmed. Its essential oil different than the oil of the "saxifraga" form, it has a different colour

and its total content is usually increased. In the composition of both forms ("nigra" and "saxifraga"), there definitely dominate myrcene and limonene. The "nigra" form is characterized by the absence of cymene, while "saxifraga" form has no γ-terpinene and only some traces of cymene and sabinene.

Key words: *Apiaceae, Umbelliferae, Pimpinella saxifraga L., P. nigra Mill., taxonomy, variability, morphological features, phytochemical analysis, roots, essential oils*

INTRODUCTION

In spite of a significant advancement of science, the systematics of carrot family (*Apiaceae=Umbelliferae*) has not been completely elaborated, so far. Such situation in this group of plants is the result of a very uniform morphological and anatomic structure, as well as because of the common presence of secondary metabolites in the form of essential oils [1, 2]. Also the results of advanced chemotaxonomic analyses have not introduced any significant changes [3].

Therefore, generally, the Drude's classification is accepted, which, with minor changes, has survived until the present time [4]. That is why for the creation of a natural, prognostic system of the *Umbelliferae*, further studies are necessary.

New and justified proposals referring also to the whole family, are expected to confirm the determination of the limits for the units in the rank of species and genus. *Pimpinella* genus can serve as an example which together with the remaining ones (among ohers: *Aethusa, Ammi, Apium, Carum, Cicuta, Cuminum, Falcaria, Foeniculum, Ligusticum, Oenanthe, Petroselinum, Sium, Seseli*) represent a typical sub-family (*Apioideae*) and a tribe (*Apieae*).

Pimpinella genus, regarding its taxonomic riches among the *Umbelliferae*, belongs to the most numerous ones. According to reliable sources, there occur from 150 to 200 species in its composition [5, 6]. The significant range in the estimation of biodiversity is connected with a high variability occurring in some species. In Europe, this group includes *P. saxifraga* L., *P. major* (L.) Huds. and *P. tragium* Vill. The particularly critical ones include: *P. saxifraga* within which there are no explicitly defined taxonomic categories. There are two widespread conceptions. An earlier conception reduced the mentioned variability to the intraspecific differentiation of ssp. *saxifraga* and ssp. *nigra* (Mill.) Gaudin [2, 7]. A later proposal [8] with a wider popularity acknowledged species separateness of *P. saxifraga* L. s. str. and *P. nigra* Mill. [9-15]. The not fixed taxonomic position in connection with the potential phytotherapeutic utility of the title species gave the incentive to undertake the presented studies. The main objective of this work was the verification of the taxonomic affiliation of the herbarium collection using the basis morphological method and the phytochemical analysis of the essential oil.

MATERIAL AND METHODS

Morphological analysis was carried out with the use of the herbarium collection gathered on the area of Wielkopolska-Kuyavia Lowland and kept in the Department of Plant Taxonomy of Adam Mickiewicz University in Poznań. On the basis of literature data and on the initial herbary analyses, 7 quantitative features have been separated (plant high, number of petioles (rays) in the main umbel and in the side umbels, number of leaflets in the basal leaves, length of schizocarp measured together with the nectary-disk, length of pistil neck in the flowers and on ripe fruits), as well as 2 qualititative features (colour of the external surface of root with its blue colour appearing after root breaking and the pubescence degree of stalks in a 3-degree scale). Totally, 80 herbarium sheets with plant specimens collected in 34 localities were studied. Individual measurements referring to the length of fruits and necks were carried out with the use of an MPB, type 2, microscope. The pubescence degree of stalks was determined using a stereocscopic microscope. The investigated collection has been included to *P. nigra* and *P. saxifraga* forms, while the specimens in which untypical features were found in reference to both species have been classified as intermediate species. (*P. nigra/P. saxifraga*).

Numerical data from all measurements have been characterized by the basic statistical indices.

Roots constituted the raw material for phytochemical studies. Totally, 36 root samples were studied which had been collected in August and September of 2007 and 2008 in natural habitats in the surroundings of Poznań (Poznań-Morasko, Bąblinek, Brączewo, Kiszewo, Krąplewo, Owińska-Annowo, Radojewo, Wiórek). The number of collected raw materials for phytochemical studies depended on the size of the encountered population. Therefore, the number of samples obtained in the particular localities was not equal and it oscillated between one sample (Morasko 2, Bąblinek) to seven (Wiórek 1).

Basing on verified studies of morphological features, the particular samples were qualified to two species: *P. nigra* and *P. saxifraga*. Populations showing untypical features have been classified to transitional species *P. nigra/P. saxifraga*.

Fresh roots collected in the field studies were rinsed in water, then they were cut into pieces and dried for 3-4 weeks. Subsequently, they were kept in paper bags, in a dry place, at room temperature. The procedure of phytochemical analysis including extraction and qualitative studies have been described in details in a methodical work [16].

RESULTS

Interpopulational comparative morphological analysis has shown that:

- regarding plant heigh, there are no distinct differences; the arithmetic average value of *P. saxifraga* s.str. is within the interval (45) of 50–80 cm, while i *P. nigra*, it shows (40) 50-90 (100) cm;

- arithmetic average of the number of petioles (rays) in the main umbel for *P. saxifraga* s.str. oscillates in the range of 10-16, while for *P. nigra*, the range is 11-16; analogical indices for side umbels are 5-16 and 7-15, respectively;
- similar indices are shown by the number of leaflets in the basal leaves; the average for both species is 7–11;
- the mean length of schizocarp for *P. saxifraga* is 1,7-2,5 mm, while for *P. nigra*, it is 1,5-2,2 mm;
- pistil neck lengths in flowers and also observed in fruits do not change their dimensions, the average values are contained in the same interval, from 0.4 to 0.7 mm.

The above presented quantitative features in reference to *P. saxifraga* s. str, *P. nigra* and reference to individuals with a transitional character (*P. saxifraga*/*P. nigra*) show the following statistical characteristics (tab. 1).

Table 1.

Comparison of quantitative morphological features in the studied aggregations of *P. saxifraga* L. s.l. and its coefficient

feature/indicator	*P.s.*	*P.n.*	*P.s./P.n*
plant height			
arithmetic average	61.0	65.6	52.8
standard deviation	13.1	18.3	17.6
variation coefficient	21.5	27.9	33.3
number of leaflets in basal leaf			
arithmetic average	7.5	7.9	7.2
standard deviation	2.2	2.5	3.0
variation coefficient	33.7	43.5	106.3
number of petioles (rays) in main/side umbels			
arithmetic average	12.8/10.4	18.6/9.8	13.4/10.4
standard deviation	2.6/2.3	2.1/1.8	2.7/1.8
variation coefficient	12.9/22.1	16.7/18.5	20.0/16.4
fruit length			
arithmetic average	2.1	1.7	2.0
standard deviation	0.26	0.29	0.12
variation coefficient	12.4	17.4	5.7
length of necks in the flower/on fruit			
arithmetic average	0.49/0.51	0.25/0.48	–
standard deviation	0.19/0.06	0.18/0.11	–
variation coefficient	82.9/10.9	66.5/24.1	–

P.s – Pimpinella saxifraga L. s.str.
P.n. – Pimpinella nigra Mill.
P.s/P.n – Pimpinella saxifraga L. s.str./*Pimpinella nigra* Mill..

Pubescence of stalks, particularly in their lower parts is distinctly different in both species; in *P. saxifraga,* the stalks almost always are naked, while in *P. nigra*, they are densely pubescent; less frequently, the stalks are poorly pubescent.

Taking into consideration the organoleptic features of roots, the herbarium collection can be divided into: *P. saxifraga* whose root surface is of light-yellow-brown colour, while *P. nigra* shows a black root surface and in fresh state, usually after breaking the root, the colour changes to bluish (this feature was observed during plant collection!); it is worth noting that the two latter characteristics were regarded as diagnostic features during raw material collection for phytochemical studies.

Essential oil prepared from adequately treated roots (cleaned, air dried and broken up) were obtained by the method of distillation in Deryng's apparatus. Analysis of the essential oil composition was carried out using gas chromatograph.

Total content of essential oil in the roots studied samples ranged from 0.44 to 1.76%. The particular values of this parameter slightly differed in both studied taxa because they showed for *P. nigra* – 0.77%, while for *P. saxifraga* s. str. – 0.9%. However, it must be noted that the indices do not distinctly separate the samples of the particular species because the obtained results showed 0.44–1.76 and 0.99–1.13, respectively.

It is interesting that the amount of essential oil in the roots of *P. saxifraga* s. str., originating from Turkey, similarly as in several related species, was significantly lower – 0.17% [17].

Also in the chemotaxonomic studies of *P. saxifraga* s.l. – ssp.ssp. *saxifraga*, *alpestris*, *nigra* population from the surroundings of Riva del Garda (Italy), many times smaller amounts of myrcene and limonene and an absence of sabinene were found [20].

Chromatographical analysis showed the occurrence of 8 components in the roots which generally were repeated in both studied species (tab. 2). Regarding the amount of essential oil, the leading component was myrcene (32–39%) found in 17 samples, while in there remaining samples, it did not occur. Another important component was limonene which almost in all samples showed a high stability (13–14%). Only in case of one analysis (Wiórek 3, sample no. 18), trace amounts of this compound were found. sabinene occurred in smaller amounts (2–5%) and only the population from Radojewo 2 (sample no. 33) differed in this respect by showing only a trace amount of this compound.

Table 2.

Origin of plant material (roots) of *Pimpinella saxifraga* L. s.l. collected in the surroundings of Poznań and share of the identified essential oils

locality and sample number	compound							
	α-pinene	β-pinene	myrcene	α-phellandrene	limonene	γ-terpinene	cymene	sabinene
				Pimpinella nigra Mill.				
Morasko 1								
• No 1	–	–	–	0.02	13.59	0.03	–	2.46
• No 2	–	–	–	0.02	13.03	0.11	–	2.05
• No 3	–	–	38.30	0.03	14.52	0.33	–	3.00
• No 4	–	–	38.42	0.03	14.55	0.27	–	3.21
• No 5	–	–	37.49	0.03	14.24	0.24	–	3.40
Morasko 2								
• No 6	–	–	37.30	0.03	14.06	0.09	–	5.55
Wiórek 1								
• No 7	–	–	–	–	14.15	–	–	3.66
• No 8	–	–	–	0.03	14.48	0.10	–	3.20
• No 9	–	–	–	0.02	14.00	0.13	–	4.66
• No 10	–	–	–	0.03	14.35	0.07	–	3.72
• No 11	–	–	–	0.03	14.20	0.07	–	3.82
• No 12	–	–	35.84	0.03	13.53	0.05	–	3.67
• No 13	–	–	–	–	13.76	0.06	–	4.05
Wiórek 2								
• No 14	–	–	36.78	0.03	14.16	–	–	2.37
• No 15	–	–	34.14	0.02	13.21	–	–	3.02
• No 16	–	–	–	–	14.25	–	–	3.49
• No 17	–	–	–	0.02	14.23	0.05	–	3.35
Wiórek 3								
• No 18	–	–	32.01	0.03	–	–	–	4.27
• No 19	–	–	–	0.02	14.34	0.05	–	3.26
Brączewo								
• No 20	–	–	–	0.02	–	–	–	3.01
• No 21	–	–	–	0.03	14.47	0.03	–	2.58
Krąplewo 1								
• No 22	–	–	–	0.02	14.20	–	–	2.12
• No 23	–	–	–	0.03	14.57	–	–	2.47
• No 24	–	–	–	0.03	14.38	–	–	2.31
Krąplewo 2								
• No 25	0.03	0.63	–	0.01	13.72	–	–	4.14
• No 26	0.03	0.64	–	–	13.60	–	–	4.37
Owińska– Annowo								
• No 27	–	–	36.18	0.02	13.85	0.06	–	3.31
• No 28	–	–	36.66	0.02	13.97	0.13	–	3.13
• No 29	–	–	37.49	0.03	14.24	0.24	–	3.39
Radojewo 1								
• No 30	–	0.07	38.50	0.03	14.58	–	–	4.10
• No 31	–	0.06	38.45	0.03	14.51	–	–	3.10
Radojewo 2								
• No 32	–	–	39.12	0.03	14.84	–	–	3.95
• No 33	–	–	–	0.02	13.57	–	–	0.02

				Pimpinella saxifraga L. s.str.				
Kiszewo								
• No 34	–	–	37.79	0.03	14.34	–	0.01	0.02
• No 35	–	–	38.70	0.03	14.73	–	–	0.07
Bąblinek								
• No 36	0.02	0.22	37.31	0.03	14.13	–	0.03	0.03

Analysis of the occurrence of pinene, as well as cymene and sabinene can have the value of a phytochemical marker. The absence of cymene and α- and β-pinene characterized *P. nigra*, while the presence of these compounds was characteristic of *P. saxifraga* s.str. On the other hand, distinct deviations from the quantitative parameters may indicate an intermediate taxonomic character of a sample, in spite of the presence of the morphological diagnostic features in the given populations, which was signaled already in earlier literature [18, 19].

a b

Figure 1. Samples of cut and dried roots used in phytochemical analysis; a – *P. saxifraga* L. s. str.; b –. *P. nigra* Mill.

CONCLUSIONS

Pimpinella saxifraga L. s.l. is a polymorphic taxon on the area of Wielkopolska-Kuyavia Lowland shows a differentiation into two species: *P. nigra* Mill. and *P. saxifraga* L. s.str. The first taxon occurs frequently in the central part of the region, while the second one occurs rather sporadically. This fact shows an interesting phenomenon that the diploid form (*P. nigra*) is the expansive species, while the tetraploidal form (*P. saxifraga* s.str.) shows a rare occurrence. This fact is a contradiction with the common opinion that polyploids are characterized by an intensified vitality and commonly [20-23].

For taxonomic identification, one should use a set of morphological features (external colour of the root and colour of fresh root immediately after its cross-cutting, stalk pubescence, leaves and inflorescences).

The quantitative and qualitative components of essential oils in roots (24, 25) possess a chemotaxonomic value, whereby a comparative analysis contributes to the diagnostic optimization. Results of phytochemical analyses obtained in our elaboration indicate a variability connected with the geographical gradient.

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ZRÓŻNICOWANIE *PIMPINELLA SAFIFRAGA* L. S.L. W PORÓWNAWCZEJ ANALIZIE MORFOLOGICZNEJ I FITOCHEMICZNEJ

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S t r e s z c z e n i e

Stosowanie biedrzeńca mniejszego – *Pimpinella saxifraga* L. w fitoterapii oraz jego duża zmienność spowodowały podjęcie interdyscyplinarnych badań dotyczących taksonomicznej sfery tego polimorfizmu. Morfologiczna analiza specjalnie do tego celu zgromadzonej kolekcji zielnikowej wykazała występowanie na obszarze Wielkopolski dwóch gatunków: *P. nigra* Mill. i *P. saxifraga* L. s.str. Opracowany zestaw cech morfologicz-nych o znaczeniu diagnostycznym posłużył do zebrania w okolicach Poznania surowca (korzeni) wykorzystanego następnie w badaniach fitochemicznych. W analizie chromatograficznej olejku eterycznego otrzymanego z korzeni stwierdzono w badanym kompleksie występowanie 8 składników, wśród których często dominuje myrcen i stale obecny limonen. Obecność cymenu oraz α- i β-pinenu wskazuje na *P. saxifraga* s.str., natomiast ich brak określa *P. nigra*. Zarówno w analizie morfologicznej jak i fitochemicznej znajdują się formy o pośrednim charakterze.

Słowa kluczowe: *Apiaceae, Umbelliferae, Pimpinella saxifraga* L. s.l., *taksonomia, zmienność, cechy morfologiczne, analiza fitochemiczna, korzenie, olejek eteryczny*