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Isolation of shikimic acid from *Picea abies* needles. The future prospects

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Abstract. The composition and concentration of hydrophilic extractives in *Picea abies* needles were studied. The optimal time for harvesting of spruce needles for shikimic acid production was evaluated. The concentration of shikimic acid was ranging from 4.7 mg/g of spruce needles harvested in August to 94.7 mg/g of spruce needles harvested in November.

1. Introduction

Shikimic acid or 3,4,5-trihydroxycyclohex-1-ene-1-carboxylic acid is an important chiral metabolite in plants and microorganisms. First shikimic acid was isolated and from Japanese plant *Illicium religiosum* in 1885, but its structure was elucidated only 50 years after that [1, 2]. Nowadays shikimic acid is successfully used as a precursor for various high-value chemicals.

Shikimic acid is also used as a precursor in pharmaceutical industry. In the last decades, studies of shikimic acid have been carried out especially actively, since it is used as a base material for the synthesis of a neuraminidase inhibitor medicine. This medicine is used to treat and prevent Influenza A and Influenza B. Shikimic acid derivatives have many pharmacological effects such as anti-coagulant, anti-inflammatory, antibacterial, and antioxidant [3, 4]. Therefore, shikimic acid as an important precursor for the chemical and pharmacological industries is needed in a relatively large quantities. In the global industry, there are three main approaches to production shikimic acid: isolation from lignocellulosic biomass, microbiological, and chemical synthesis [1, 3, 4].

The first successful synthesis of shikimic acid in the laboratory was performed in 1960. But all methods of chemical synthesis have several disadvantages - high cost, low yield of the target product, and high probability of formation of biologically inactive isomers [2]. Another option for production shikimic acid is microbiological synthesis. Many microorganisms are able to synthesize shikimic acid, but its yield is extremely low [5]. Nowadays the main source of shikimic acid is biomass. Currently, shikimic acid is successfully isolated from Chinese star anise (*Illicium verum*). The yield of the target product is 3-7wt% of dry seeds. Unfortunately star anise is cultivated only in specific climate zones [2-4].

Several studies showed that shikimic acid can be readily isolated from the needles of several species of pine trees. According to research estimates, about 35.5 million m^3 of wood waste is generated annually in Russian Federation. The waste differs from bark and knots, to sawdust. Needles from coniferous trees are considered to be waste as well [6]. However, the chemical composition of needles of coniferous trees allows their use of in the production of various medicines, perfumes,

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fertilizers, and animal feed [7]. Coniferous plants of the *Pinaceae* family are capable of producing many valuable chemicals as mono- and sesquiterpens, phenols, acids, and other compounds [8, 9].

However, efficient industrial processing of needles has certain challenges, for example, seasonal changes in content of certain chemical compounds. Hence, the study of seasonal changes in the chemical composition of the needles is required. The results of this study will help to develop the effective ways of needles processing with the aim of valuable compounds isolation [10].

To isolate shikimic acid from needles, both polar organic solvents and water can be used as extraction solvents. However, taking into account economic criteria and environmental regulations for industrial processes, the use of large volumes of organic solvents is often unprofitable. The key to future wood biorefineries is the continued development of green techniques in order to obtain more efficient separation processes with a minimal environmental impact. Comparing to other industrially used solvents, water is inexpensive, easy to handle and non-toxic and thus making it a very good solvent for the chemical industry.

The aim of this study was to assess the possibility to use *Picea abies* needles as a source of shikimic acid as well as to evaluate the effect of harvest time on concentration of shikimic acid in spruce needles.

2. Materials and Methods

The spruce needles were harvested monthly during one year from the same *Picea abies* tree growing in the ecologically clean zone of the Kalininsky district, Tver region. Raw materials were collected in dry weather and only from healthy, well-developed, undamaged tree growing far from large industrial sites and roadsides with heavy traffic. Initial processing of raw materials was carried out immediately after collection and consisted of the sorting of collected needles. Damaged dry needles and other impurities were removed.

The needles were air dried and ground to 2-3 mm particle size. After that needles were extracted with distilled water at 23±1°C for 24 hours. The solid to liquid ratio was 1:20. The extract was separated from the residue on the filter. The total dissolved solids (TDS) of extracts were determined.

The extractives were analyzed quantitatively by gas chromatography using the internal standard method. Aliquots of extracts were evaporated, after that the internal standarts mix was added to the samples, evaporated in nitrogen flow, and further dried in vacuum desiccator. Silylation of the dried samples was performed with pyridin, N,O-Bis(trimethylsilyl)trifluoroacetamide (BSTFA), and chlorotrimethylsilane (TMCS) mixture in the ratio 1:4:1 v/v at 70°C for 45 minutes. After that the silulated samples were transferred to GC vials using Pasteur pipettes and analysed by GC-FIDequipped with a long 25 m × 0.20-mm i.d. column coated with cross-linked methyl polysiloxane (HP-1, 0.11-µm film thickness).

GC-eluting individual compounds were identified by GC-MS analysis (an HP 6890-5973 GC-MSD instrument). The compounds were identified as silylated derivatives.

3. Results and discussion

The results of spruce needle extracts analysis confirm the complex composition of hydrophilic extractives in the needles (Table 1). Various groups of compounds were found in the extracts: organic acids (malic, quinic and others), carbohydrates, sugar alcohols (xylitol, sorbitol) etc. Significant changes in composition of extractives were found throughout a year.

Table 1. Chemical composition of *Picea abies* needles hydrophilic extractives.

Compound	Concentration of extractives in needles, mg/g											
	Jan	Feb	Mar	Apr	May	June	July	Aug	Sept	Oct	Nov	Dec
Oxalic acid	0.32	0.34	0.31	0.20	0.28	0.36	0.81	0.63	0.29	0.33	0.78	0.36
Benzoic acid	0.64	0.82	0.58	0.42	0.98	3.09	0.82	0.25	1.23	0.98	3.41	0.64
Phosphoric acid	8.83	9.09	6.40	5.24	5.63	4.30	14.18	3.13	5.04	5.41	8.49	6.42
Glycerol	3.33	3.41	2.54	2.92	2.43	1.65	-	-	1.57	1.53	5.64	2.39
Proline	2.57	2.30	3.01	3.41	6.91	2.22	0.29	0.54	1.85	2.08	6.62	3.28
2,3-dihydroxy-propane acid	0.31	0.31	0.27	0.19	0.29	0.65	2.40	1.06	0.46	0.55	0.97	0.30
4- hydroxyl-benzoic acid	0.71	0.76	0.69	0.91	0.94	1.24	0.60	0.14	0.98	1.15	1.54	0.66
Malic acid	1.64	1.71	1.74	2.43	1.92	1.50	0.67	0.49	1.54	1.80	3.44	1.61
4- hydroxy-acetophenone	15.98	15.83	17.55	17.49	12.22	16.14	2.09	2.37	15.19	15.92	21.51	18.27
Shikimic acid	80.69	81.96	79.94	88.69	53.86	29.69	6.79	4.71	27.16	29.21	94.72	79.25
Citric acid	1.84	2.57	1.86	3.77	3.03	2.95	16.83	5.25	2.40	2.43	2.68	2.05
Quinic acid	45.6	45.50	42.51	44.64	38.7	36.65	22.9	18.57	30.55	37.11	48.21	41.26
Pinitol	38.72	39.81	35.27	37.38	31.07	27.86	10.52	6.48	27.38	27.24	41.55	36.43
1-guaiacyl-glycerol	0.72	0.74	0.60	0.68	0.69	0.84	0.21	0.20	0.74	0.79	1.08	0.64
Gluconic acid	0.37	0.51	0.27	0.34	0.54	4.11	2.29	2.01	1.05	0.98	2.14	0.30
Myo-inositol	2.25	2.26	2.83	4.73	3.64	3.08	0.44	0.53	2.73	2.86	7.84	2.79
(+)-catechin	1.76	1.29	2.72	2.63	4.87	7.47	-	0.11	4.81	5.00	3.95	2.67
Mono/ disaccharides	201.09	189.22	203.98	246.48	248.71	147.0	69.81	21.16	151.18	160.33	354.39	210.35

The difference in TDS of extracts obtained from needles harvested throughout a year was found. It can be seen in figure 1 that the maximum TDS was determined in extracts from November and April/May needles, and the lowest – in extracts from August needles. Summer (June-August) is a period of intensive growth of needles and new shoots and concentration of extractives in needles is the lowest in the year. Relatively high concentration of extractives in the late autumn and winter can be possibly explained by plant adaptative mechanisms and decrease of growth processes in this period.

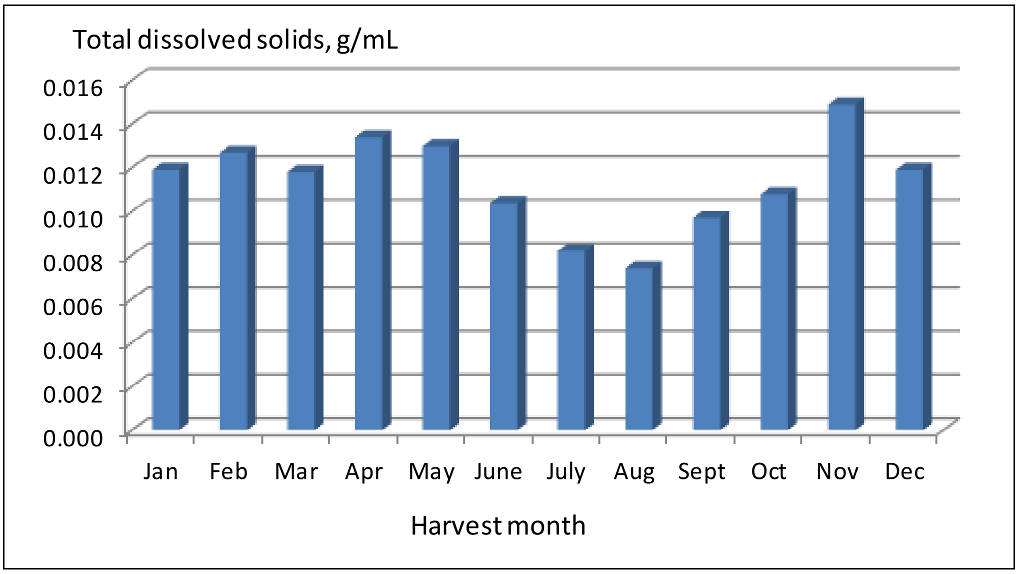

Figure 1. Total dissolved solids in spruce needle extracts.

Concentration of shikimic acid in spruce needles also changes during a year (figure 2). The maximum concentration of shikimic acid (94.7 mg/g) was found in needles harvested in November. Based on the results of this study, the time period from November to April is recommended as a harvesting time for sprue needles for shikimic acid production.

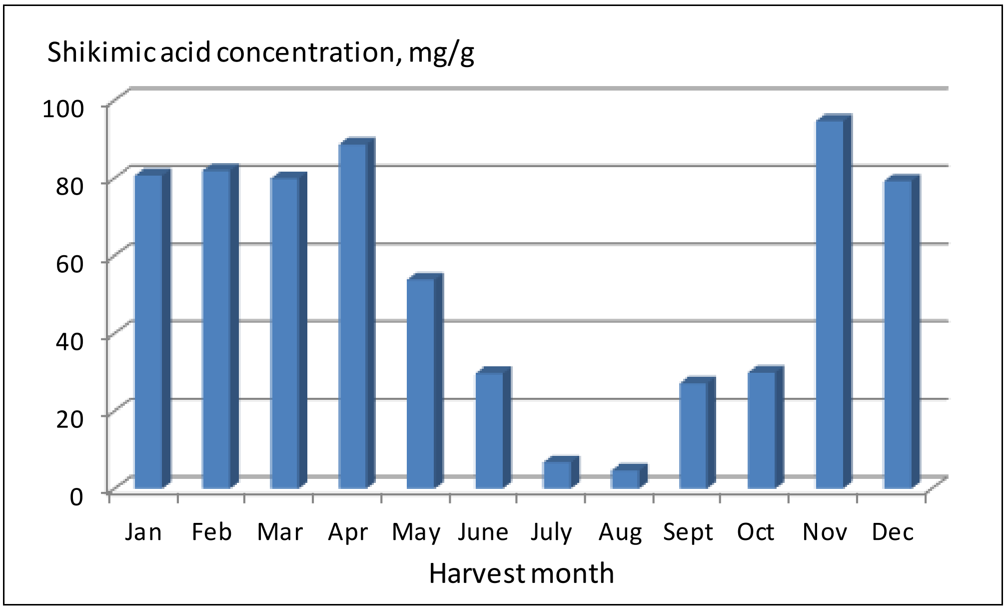

Figure 2. Concentration of shikimic acid in spruce needles.

4. Conclusions

The results of this work confirm that *Picea abies* needles can be used as a promising source of shikimic acid. For each cubic meter of logged spruce wood approximately 36 kg of needles are going to waste. From this needles 3.4 kg of shikimic acid can be isolated with plain water as an extraction solvent.

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