THE ETHANOL EXTRACTS AND ESSENTIAL OILS FROM *PINUS SYLVESTRIS* AND *PINUS NIGRA*

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Abstract

The purpose of this study was to quantify the extraction of dry substances and essential oils from the needles of *P. sylvestris* and *P. nigra*, growing under the conditions of the Republic of Moldova. The moisture content in needle samples of both plant species *Pinus sylvestris* and *Pinus nigra* increased from March to September, while the concentration of solid compounds decreases. Nevertheless, the dry substances in the extracts obtained from needles collected in spring and autumn was not significantly different. The solids content in extracts of 96 % ethanol in ratio raw materials: solvent 1: 5 after their decantation and filtration was 4.4–4.6 % for *P. sylvestris* and 5.0–5.1 % for *P. nigra*. It was found that the optimal duration for extraction of essential oils from previous dried needles of both species *P. sylvestris* and *P. nigra* using the hydrodistillation method is 4.0 hours. The maximal yield of essential oil extracted by studied procedure was about 0.6 and 0.5% for *P. sylvestris* and *P. nigra,* respectively.

Key words: ethanol extracts, essential oils, *Pinus* spp.,hydrodistillation method, dry substances.

Scots pine (*Pinus sylvestris* L., Pinaceae) is a long-living, coniferous tree that is the most widely distributed pine, native to Eurasia. It is readily identified by its combination of fairly short, blue-green leaves and orange-red bark. The needles are blue-green, 3-5 cm long and occur in fascicles of two. The cones are pointed ovoid in shape and 3-7 cm long. The threes normally grow up to 25 m in height and in exceptional cases up till 40 m. Scots pine is, especially in the north of Europe, an economically important species. The wood is strong and easy to work with, making it excellent for general constructions, furniture-making and the pulp and paper industry. It is also used for stabilising sandy soils (Gardner, 2013; Daugavietis and Spalvis, 2014). Scots pine is an important timber tree. Earlier it use as mining props and for interior construction, for street paving blocks. At the moment most of the production goes to the paper industry. Other uses of scots pine wood are railway sleepers, fencing, crates, pallets, boxes, laminated wood, particleboard, fibreboard, and various wood-based materials.

Pinus nigra J.F.Arnold (syn. *Pinus austriaca* Höss), the Austrian pine or black pine occurs across southern Mediterranean Europe from Spain to the eastern Mediterranean on Anatolian peninsula of Turkey and on Corsica, Cyprus, including Crimea, and in the high mountains of the Maghreb in North Africa (Farjon, 2013). *Pinus nigra* use as condiment (vanillin flavouring), medicinal plant (antiseptic, diuretic, rubefacient and vermifuge) in the form of liniment plasters, poultices, herbal steam baths and inhalers. Also the plant use as dye, herbicide, shelterbelt and wood. It is very efficient for degraded soil colonisation and its adventitious roots are suitable to be exploited for deep reinforcement and soil strength enhancement (Allardice, 1993; Enescu *et al*, 2016).

Current population trends for both species *P. sylvestris* and *P. nigra* in the world are stable. Population of the *P. sylvestris* is locally abundant and dominant in many areas, but for the population of the *P. nigra* no declines have been recorded (Farjon, 2013; Gardner, 2013).

The secondary metabolites of pines have long attracted the attention of researchers as a raw material for the production of various biological preparations for organic agriculture. For this reason, our research purpose was to study the quantitative extraction of dry substances and

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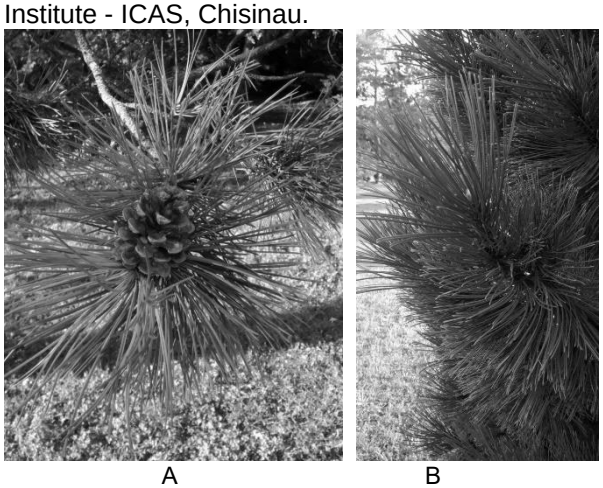
essential oils from needles of the *P. sylvestris* and *P. nigra*.

MATERIAL AND METHOD

The objects of research were plants of the species *Pinus sylvestris* and *P. nigra*, growing in the central zone of the Republic of Moldova (*figure 1a, b*). The plant materials (needles) were collected in February, March, April, May, September, by trimming 30–40 cm of regrown basal branches. The identification of the *P. sylvestris* and *P. nigra* plants was carried out by consultation with the specialists from the Botanical Garden (Institute) and the Forest Research and Management Institute - ICAS, Chisinau.



A B

Figure 1 ***Pinus sylvestris*** **(A) and** ***Pinus nigra*** **(B), growing in the Republic of Moldova, September 2018**

The collected plant material was dried at the temperature of 28–30° C in a well-ventilated area away from direct sunlight. The dried plant raw materials were crushed in an electric laboratory mill (Type MRP — 1, asynchronous motor) for 2–3 minutes and sifted on a sieve with a mesh size of 2 mm. The ground herbal raw materials were stored in a dark place at an air temperature of 20–22° C. The content of moisture and dry substances were determined both for fresh and dried herbal raw materials according to the standard methods using Moisture analyzer MAX series, RADWAG 26 – 600 Radom (Markova et al., 2003).

The moisture content (W) of the plant raw material was calculated as a percentage by the formula 1:

$$W = \frac{(m-m_1)\times 100}{m}, \quad (1)$$

where *W* – moisture content of plant raw materials, %;
m – mass before drying, g*;*
m_1 – mass after drying, g.

The dry content (D) of the plant raw material was calculated as a percentage by the formula 2:

$$D = 100 - W, \quad (2)$$

where *D* – dry content of plant raw materials, %;
W – moisture content of plant raw materials, %.

Preparation of the extract and essential oil for the laboratory testing.

As a solvent, 96 % ethyl alcohol was used, which allows extracting the maximum amount of biologically active substances from plant raw materials (Khanturgaev et al., 2003). The ground herbal raw material was mixed with a solvent in a ratio of 1:5 (raw material: solvent). The mixture was shaken in Erlenmeyer flasks on a laboratory shaker (LT 2, SKLO UNION, Czech Republic) for 4 hours and insisted 24 hours at the temperature +24 (±2) °C. Then the extract was filtered through a paper filter on a Buchner funnel under vacuum and evaporated on a rotary evaporator until completely removed the solvent. The residue was dissolved in a fourfold amount of 96 % ethyl alcohol, as a result was obtained 20 % (by dry residue) alcohol solution. The extracts were stored at the temperature of +4°C.

In addition to the alcohol extracts from the plants *P. sylvestris* and *P. nigra* the essential oils were obtained. The extraction of essential oils was carried out by hydrodistillation method (Hesham et al., 2016) by boiling of a sample of raw material (200 g for fresh and 100 g for a dry plants) with purified water (water was added in the ratio of raw materials: solvent 1:4 for fresh and 1:8 for a dry plants) in 1–2-liter flask with a reflux condenser. The essential oils were collected in a Ginsberg receiver suspended in a flask. After distillation, the receiver was removed, cooled in air to room temperature, the essential oils were separated with micropipettes and quantitatively transferred to a measuring tube with graduation 0.02 ml. The flask with contents was heated on a gas burner or electric stove with closed spiral and a power control, and boiled for 1.5; 2.5; 3.0; 4.0 and 4.5 hours (until the increase in the volume of essential oil determined visually).

The content of essential oil in mass-volume percent (X) per absolutely dry raw materials was calculated by the formula 3:

$$X = \frac{V\times 100\times 100}{\alpha\times(100-W)}, \quad (3)$$

where V – volume of essential oil, ml;
α – weight of the sample of raw materials, g;
W – moisture content, %.

Mathematical analysis of the obtained data was carried out according to the method of one-way ANOVA test and calculation of standard deviation using computer data processing methods: Microsoft Excel software package.

RESULTS AND DISCUSSIONS

We usually harvesting raw materials several times a year – from March to May, as well as in September. Comparative analysis showed that in needle samples of both plant species the moisture content increases from March to September, while the concentration of solid components decreases: in raw materials of the *Pinus sylvestris* by 8.68 %

and *Pinus nigra* – by 4.21 % (*table 1*). Mathematical processing of the results revealed that the difference between the collections is significant ($p \leq 0.05$). Despite the fact that the concentration of solids in the plants during vegetation period changes significantly, therefore, the yield of dry substances in the extracts of raw materials collected in spring and autumn, was not significantly different. On average, when the extraction was provided by 96 % ethanol in ratio raw materials: solvent 1: 5, the solids content in extracts after their decantation and filtration was 4.4–4.6 % for *P. sylvestris* and 5.0–5.1 % for *P. nigra*.

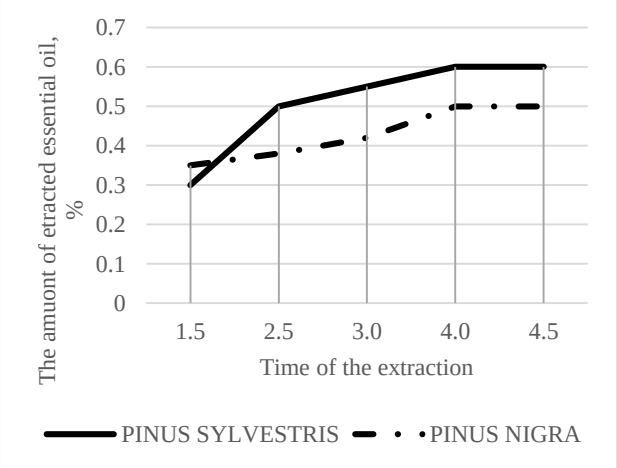
Table 1

The moisture and dry substances of raw material (needles) of *Pinus sylvestris* and *Pinus nigra*, depending on the vegetation period of plants

Plants species	The moisture (*W*) and dry substances (*D*), %					
	15.III.2018		09.V.2018		21.IX.2018	
	W	*D*	*W*	*D*	*W*	*D*
Pinus sylvestris	49.9	50.1	50.3	49.8	58.6	41.4
	$LSD_{0.05} = 1.31$ p ≤0.05					
Pinus nigra	46.7	53.3	48.6	51.4	50.9	49.1
	$LSD_{0.05} = 0.67$ p ≤0.05					

Note: differences are significant with p≤ 0,05.

We carried out the extraction of essential oils by the method of hydrodistillation for 1.5, 2.5, 3.0, 4.0 and 4.5 hours. The obtained data noted that the amount of essential oil extracted from dry plant material significantly grown ($LSD_{0.05} = 0.01$, $p \leq 0.05$) with the increasing of extraction time from 1.5 to 4.0 hours, However, a further increase in the extraction time was unreasonable because the extraction of the essential oil was practically stopped (*figure 2*).



Note: $LSD_{0.05} = 0.015$ p ≤0,05

Figure 3. **The amount of extracted essential oil from needles of *Pinus sylvestris* and *Pinus nigra*, growing in the conditions of the Republic of Moldova, in terms of the absolutely 100 g dry weight of the plant, depending on the duration of extractionб 2018**

At the same time, it was found that from the fresh raw material of *P. sylvestris* it is possible to obtain the same amount of essential oil significantly faster. So, for *P. sylvestris* from fresh material crushed with a pruner to a particle size of 2-3 mm long, 0.61 % of essential oil per 100 g of absolutely dry plant was obtained within 1.5 hours. The same amount of essential oil from a dry plant was obtained only after 4 hours of extraction. It is noted that with increasing extraction time (till 4.5 hours), the amount of extracted oil from fresh material increases insignificantly. At the same time, for *P. nigra*, the amount of extracted essential oil for 1.5 and 2.5 hours was almost the same for both fresh and dried plants (for 1.5 hours – 0.32 % for fresh and 0, 35 % for dry, for 2.5 hours – 0.34 % for fresh and 0.38 % for dry). It should be noted that despite the high yield of essential oil from a fresh plant of *P. sylvestris*, the last mentioned is rather difficult (and long) to grind without prior drying. The obtained data are of practical interest, since they vividly prove the need for an individual approach in choosing extraction methods, even for plants belonging to the same genus.

Thus, it was found that the moisture content of plant materials varies between 49.9–58.58 % for *P. sylvestris* and 46.70–50.91 % for *P. nigra*, depending on the vegetation period of the plants. In this case, from the total dry matter content only 9.0-9.1 % for *P. sylvestris* and 10.0–10.7 % for *P. nigra* were extracted with 96% ethanol. The maximum amount of essential oil (0.6 % in terms of the 100 g absolutely dry weight of the plant) was obtained by extraction of freshly grinded needles of *P. sylvestris* with method of hydrodistillation, the minimum – by extraction of fresh needles of *P. nigra* (0.32 %). It was also found that for the *P. nigra* plant the difference in the amount of extracted essential oil from fresh and dry raw materials is insignificant.

As a result, from *P. sylvestris* and *P. nigra*, growing in the Republic of Moldova, we managed to extract as much as possible up to 0.6 and 0.5 %, respectively, of the essential oil in terms of 100 g absolutely dry plant.

CONCLUSIONS

Thus it is established that the smallest recommended time for extraction essential oils from species *Pinus sylvestris* and *Pinus nigra* is 4.0 hours. In order to save working time, as well as to reduce the cost and automation of the material grinding process, it is advisable to dry the plant after harvesting; however, in the case of *P. sylvestris*, it is possible to use a fresh plant. The

yield of solids during extraction with 96% ethanol averages 4.5% for *P. sylvestris* and 5.1% for *P. nigra*. The maximal yield of essential oil from *P. sylvestris* and *P. nigra* plants growing under the conditions of the Republic of Moldova, using the hydrodistillation method when boiling the mixture for 4.0 hours is averages about 0.6 and 0.5%, respectively.

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