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PINUS SYLVESTRIS L. PLANT POLYPHENOLS

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ABSTRACT

In this paper, promising methods for the extraction of polyphenols from the plant Pinus Sylvestris L., physicochemical methods of their identification are introduced, extracts are obtained from ordinary pine bark and cones, from which the sum of polyphenols is extracted and the bark and dome polyphenols were compared comparatively.

Results. Pinus silvetris L. polyphenols contain gallic acid, (+) - catechins, campherol, 1-O-galloyl glucose, myrecitin, epicatechin, isoramnetin, luteolin, which belong to the class of phenolic compounds.

Methods. Technological (extraction, precipitation, drying), physicochemical (UV, IR spectroscopy) and analytical (paper and thin layer chromatography) methods were used in the work.

Conclusion. A total of polyphenols were isolated from Pinus sylvestris L., with a content of 1.5% in the bark and 4.5% in the dome.

Introduction. In medicine today, it is important to extract medicinal compounds from plants. Among the natural compounds that exhibit this property, flavonoids also play an important role. Due to the wide range of biological effects and low toxicity of such compounds, they are included in the list of promising compounds in the development of new drugs. Although a number of new effective drugs based on the above class of flavonoids have been developed and introduced into medical practice in recent years, their potential in this regard has not yet been fully explored.

In modern medicine, polyphenols belonging to the group of flavonoids have played a role in the development of drugs, their powerful antioxidant, antihypoxant and effective properties against viruses and inflammatory conditions have been studied. Therefore, the identification of plants rich in local polyphenols and in-depth analysis of their chemical composition, the study of the relationship between their chemical structure and biological activity is still ongoing.

Methods. In order to isolate and study the phenolic compounds and polyphenols in

the plant Pinus sylvestris L., we conducted the following studies.

We removed the bark and dome of an ordinary pine plant and dried and crushed it. We took 100 g of crushed dome and bark separately and extracted 3 times with chloroform at 50-550C to get rid of lipophilic compounds. Each extraction lasted 2 hours. The raw materials were then dried and extracted 3 times with 70% aqueous acetone (6: 1 ratio of acetone: water) after the solvent odor disappeared. Combining the extracts, we drove the acetone under vacuum (35-400C, 30 mm Hg column pressure) at low temperature. We treated the aqueous part with chloroform 3 times (to get rid of oily compounds). The aqueous particles were then mixed with ethyl acetate (1: 6) in a separation funnel, processed several times, and ethyl acetate was obtained. We added anhydrous Na2S04 to the ethyl acetate extract and left to dry for 24 hours. We then filtered the extract and pumped it under vacuum using an evaporator rotor to increase its concentration. The concentrate was precipitated using hexane to obtain an amorphous precipitate, a mixture of polyphenols. Then we filtered it and dried it in the oven. We found that the mixture of polyphenols from the bark was light brown, and the polyphenols from the domes were yellow.

Qualitative reactions revealed that the total polyphenols contained compounds belonging to the class of flavonols, phenolic acids and tannins. Also, when we compared the polyphenols of the individual organs of the plant on a paper chromatogram, it became clear that they are almost indistinguishable in composition, only quantitatively, some components predominate.

Paper chromatography of polyphenols in a solvent system n-butanol: acetic acid: water 4: 1: 5 (system 1) and n-butanol: acetic acid: water 40:12:28 (system 2) $FeCl_3$ 1% aqueous and alcoholic solution and 1% $FeCl_3$ + 1% $K_3[Fe(CN)_6]$ (1: 1) When tested using openers, it was observed that the plant dome and bark contained compounds belonging to 8 classes of phenolic substances.

Systems used to divide the total amount of polyphenols into individual compounds:

1. Butanol - acetic acid - water (4: 1: 2)

2. Butanol - acetic acid - water (40: 12: 28)

3. 6% aqueous solution of acetic acid

4. 15% aqueous solution of acetic acid

Reagents for opening chromatograms:

For phenolic compounds

1. $FeCl_3$ 1% aqueous and alcoholic solution of;

2. 1% $FeCl_3$ + 1% $K_3[Fe(CN)_6]$ (1: 1)

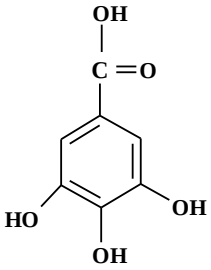
3. 1% solution of vanillin in HCl;

4. 3% aqueous solution of $KClO_3$.

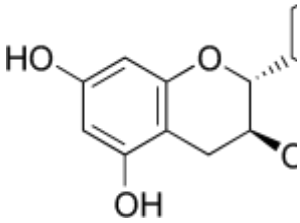
Using paper chromatography, n-butanol-acetic acid-water 4: 1: 5 (system 1, high phase), n-butanol-acetic acid-water 10: 3: 7 (system 2) and 15% vinegar the composition of polyphenols isolated in the presence of an acid (system 3) solvent system was analyzed. To open the chromatograms, a 1% solution of vanillin in

concentrated HCl, a 1% aqueous and alcoholic solution of $FeCl_3$, a 1: 1 solution of 1% $FeCl_3$ and 1% K_3 [Fe (CN) $_6$] were used.

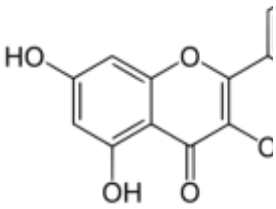
Results. Qualitative reactions (ammonia vapor, 5% Na2CO3 solution) and analysis of physicochemical quantities of Pinus silvetris L. polyphenols include gallic acid, which belongs to the class of phenolic compounds, (+) - catechins, campherol, 1-O-galloyl glucose, mirecitin , epicatexin, isoramnetin, luteolin were found:



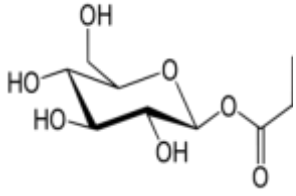
Gall acid



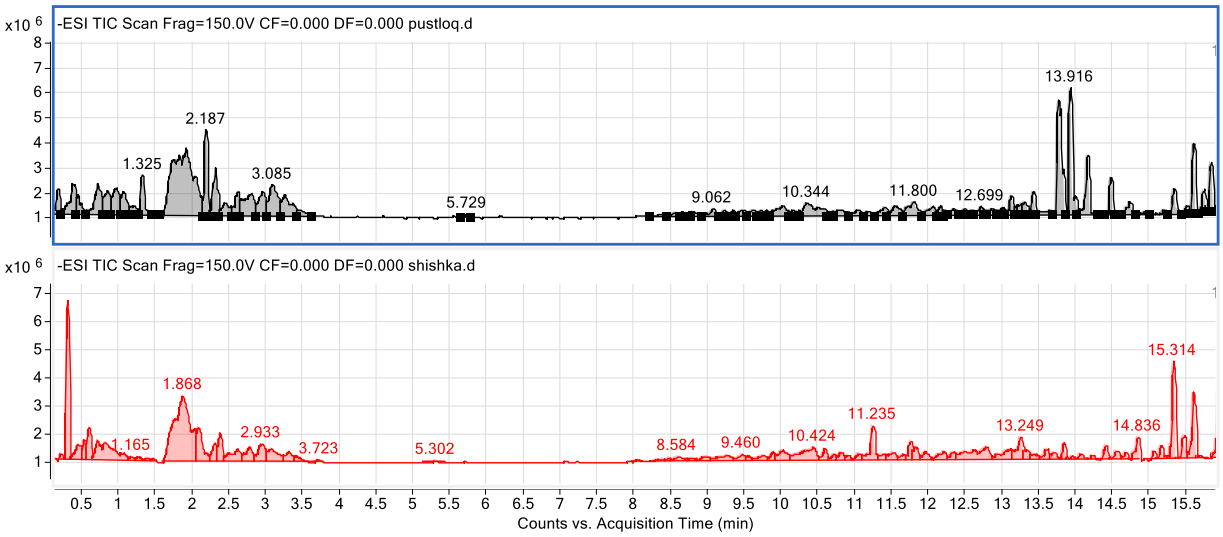
(+)-catechins



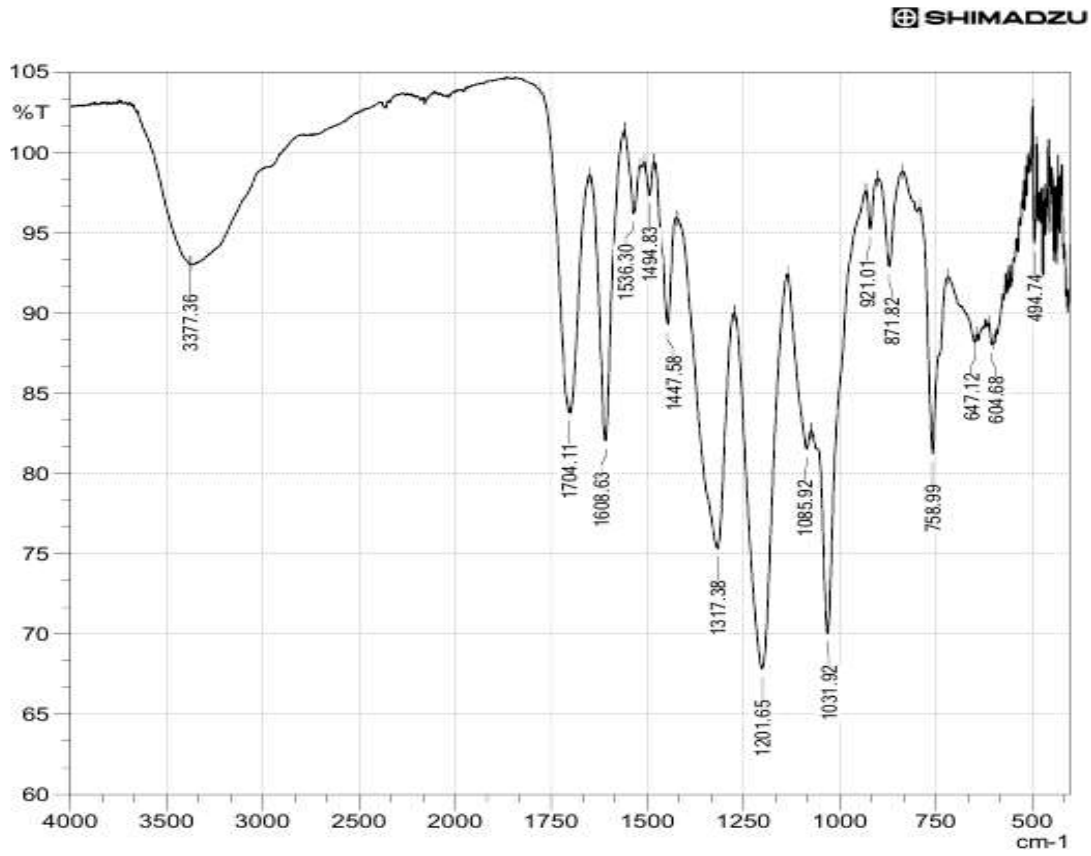
Kempferol



1-O- galloil glucose



Fractionation of polyphenols isolated from the bark and dome of the plant Pinus sylvestris L. by the method of YUSSX (HPLC)



IR spectrum of polyphenols in the dome of the plant Pinus sylvestris L.

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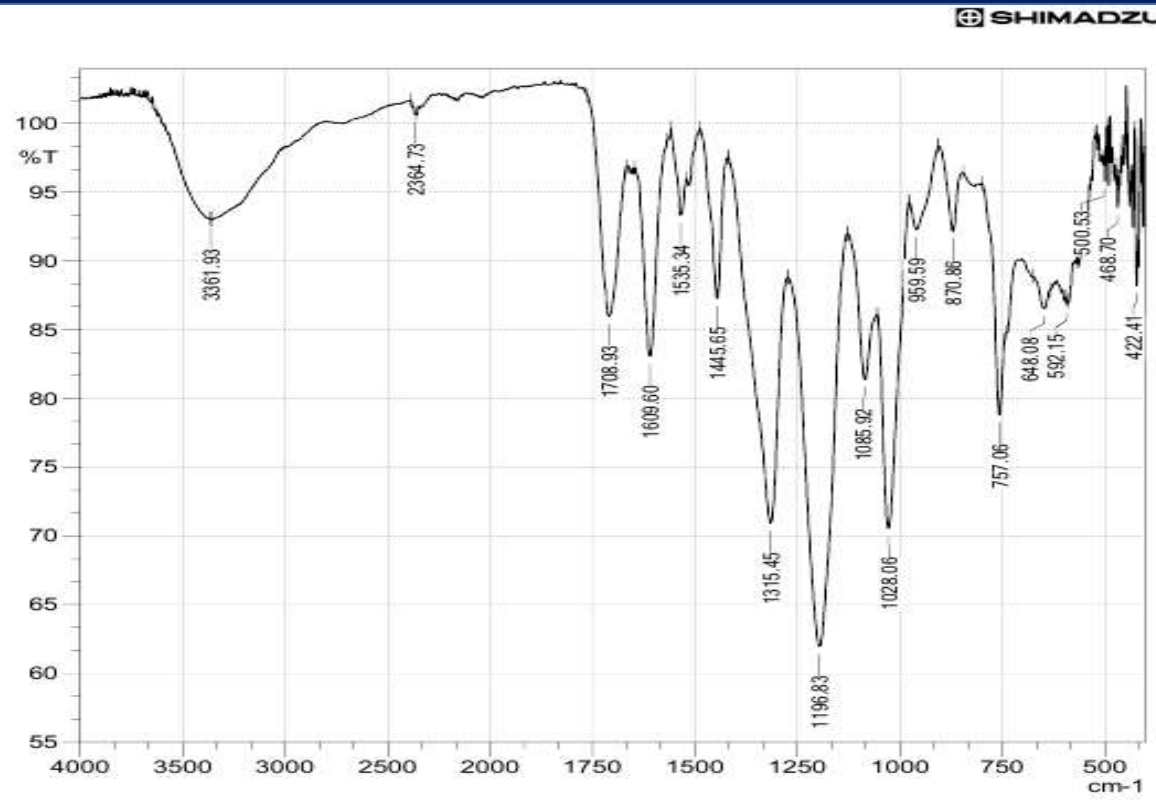

IR spectrum of polyphenols in the bark of Pinus sylvestris L.

Gallic acid is a white crystalline substance. T^0 суюq. 221-223°C , R_f 0.51 (1- system, high phase).

Kempferol- $C_{15}H_{10}O_6$ light yellow tiny crystals, R_f 0.79 (1- system), T_{liquid} 279-280 ^{0}C , UB-spektr (λ_{max}, lg ε, нм, EtOH): 266 (4.41), 366 (3.80).

Alkaline hydrolysis produces fluoroglucin (Rf 0.64, system 1) and p-oxybenzoic acid (Rf 0.80, system 1).

1-O-galloyl glucose is a dark brown amorphous powder, , R_f 0.25, 0.38, (1, 2-system). UB-spektr (EtOH, λ_{max}, нм): 217, 276. ИQ (KBr, ν, $см^{-1}$) spektr 3300-3400 sm^{-1} (OH), 1620-1610,1450 sm^{-1} (aromatic ring), 1320 $см^{-1}$ (C-OH), 1250, 1045 sm^{-1} (C-O-C), 1080-1070 sm^{-1} (C-O), 1040, 1010 sm^{-1} (sugar section). Acid hydrolysis resulted in the formation of gallic acid and glucose in a 1: 1 ratio.

(+)-katexin - 5,7,3', 4'-tetraoksiflavan-3-ол. M= 290, $T_{суюq.}$ 172-173^{0}C, R_f 0.64 (1-system), $[\alpha]^{22}_D$+18^0 (c=1.0, acetone-water 1:1). UB-spektr (λ_{max}, нм, EtOH): 280 (lg ε 3.94). **Miritsetin** $C_{15}H_{10}O_8$, bright yellow crystals, R_f 0.82, 0.44 (1, 2-system), $T_{liquid.}$ 358-360 ^{0}C, UB-spektr (λ_{max}, lg ε, нм, EtOH): 255 (5.73), 305 (5.32), 370 (3.32)

Isoramnetin is a yellow crystal, R_f0.53 (3-system), liquefaction h. 306-307 ^{0}C found to have UV spectrum (EtOH, λ_{max} нм): 254, 265, 371, (CH_3COONa, λ_{max}, нм) 380, 274, ($CH_3COONa+H_3BO_3$, λ_{max}, нм) 372, 254, ($AlCl_3$, λ_{max}нм) 430, 267, ($AlCl_3$·HCl, λ_{max}, нм) 428, 267, (C_2H_5ONa, λ_{max}, нм) 420, 270. $[\alpha]^{20}_D$=–56° (c0,1, ДМФА). ИQ-spektr (KBr, υ,$см^{-1}$) 3455 (OH), 2895 (–OCH_3), 1658 (>C=O), 1605, 1565, 1505 (Ar) found to be a compound with values of Alkaline decomposition in the presence of KOH revealed the presence of fluoroglucin (Rf 0.64, system 2) and protokatex acid (Rf 0.73, system 2).

The biological activity of the isolated polyphenols was studied. Experiments have shown that catechins, which are abundant in polyphenols, including Pinus sylvestris L., exhibit high antioxidant and membrane activity, which is directly related to their chemical structure.

Conclusion. In summary, a total of polyphenols were isolated from Pinus sylvestris L. by extraction, with a content of 1.5% in the bark and 4.5% in the dome. In the future, this research will result in the production of a number of effective natural remedies based on local plant raw materials.

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