Estimation of the total phenolic compounds in juniper sprouts (*Juniperus communis* L., *Cupressaceae*) from different places at the kujawsko-pomorskie province

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Summary

Common juniper (*Juniperus communis* L.) is the well-known and valuable medicinal plant. Juniper berry is used as a herbal medicine mainly due to its essential oil with diuretic, anti-inflammatory, choleretic and cholagogue activities. Some other substances, as polyphenols, are present in juniper berries and other parts of this plant. The aim of this study was to estimate total polyphenols content in common juniper sprouts (leaves) originating from different places at the kujawsko-pomorskie province. The total polyphenols content was carried out by means of spectroscopic method with Folin-Ciocalteu reagent and shown as pyrogallol equivalents. The obtained results ranged in limits from 2.40±0.23% to 3.43±0.17%.

Key words: *Common juniper, Juniperus communis, polyphenols, Folin-Ciocalteu reagent, kujawsko-pomorskie province*

INTRODUCTION

Common juniper (*Juniperus communis L.*) is a shrub or little tree widespread in many continents. This plant is very often noticed at lowlands and lower parts of Polish mountains [1]. *Juniperus communis* as all *Cupressaceae* family representatives have characteristic needle-like leaves. Juniperus berries (strictly – pseudo-fruits) are a highly valued, essential oil-rich plant material. Juniper has been presented in

many pharmacopoeias over the world (also in Polish) for long time. No less than 1% of essentail oil in plant material is required according to Polish Pharmacopoeia. *Juniperi pseudofructus* is known mainly as diuretic, anti-inflammatory, cholagogic and choleretic agent due to its essentail oil composition. This mixture of mainly monoterpenes and its derivatives is the best known chemical fraction in this material. The most important components of juniper essential oil are: α- and β-pinenes, terpinene-4-ol, sabinene, limonene and also sesquiterpenes: cadinene, α- and β-caryophyllene. Other compounds found in juniperi pseudofructus are: flavonoids, leucoanthocyanides, catechine-derived tannins and inverted sugar [2-5].

According to literature, essential oil is the most investigated compound in *Juniperus* genus. There are also a polyphenols (eg. flavonols and its dimers, neolignans) coumarins, and long-chain polyprenoids present in some other species [6-8]. These compounds are often located in leaves and other parts of plant also [4, 5]. Many of this substances have a well-documented or confirmed pharmacological activity. There are some report that extract from leaves of chinese juniper have antitumor-promoting activity [9]. The aim of this study was estimation of total polyphenols content in leaves of juniper populations growing in different parts of Kujawy area.

MATERIAL AND METHODS

Plant material

The estimation of the total phenolic compounds content in five samples derived from different areas near Bydgoszcz and Toruń were carried out. A specification of collected samples is described in table 1.

Table 1.

Specification of plant material

sample number	collecting place	place description	comments
1	Chomiąża Szlachecka (Żnin district)	pinewood with oaks, near lake and forest path, at sandy soil	
2	Łysinin (Żnin district)	pinewood with oaks, near forest path, at sandy soil	
3	Oćwieka (Żnin district)	pinewood, single plant	
4	Czarnowo (Toruń district)	pinewood, near busy road, at sandy soil	
5	Brzoza/Białe Błota (Bydgoszcz district)	pinewood with oaks, near busy road, at sandy soil	in the forest placed between this two locations

The fresh sprouts were dried in room temperature protected from light. The crumbling and extraction of plant material were performed. Extraction procedure and estimation of total polyphenols were carried out according to reduced method [10] described in Polish Pharmacopoeia [11] (modified method for tannins

determination, without reaction with skin powder). The amounts of polyphenols were calculated as pyrogallol (PG) equivalents. A different variants of this method were used by many authors [12, 13].

Reagents and equipment

Folin-Ciocalteu reagent (Sigma-Aldrich, US) and sodium carbonate (Poch, Pl) were used for total phenolic contents estimation. Pyrogallol (Sigma-Aldrich) was used as a reference.

The WAS 100/X analytical balance (Radwag, Pl), ML 147 water bath (AJL Electronic, Pl) were used to prepare extracts. The U1800 UV spectrophotometer (Hitachi, JP) was used for measuring absorbance. The MS Excel calculating sheet (Microsoft, US) was used for calculation.

Extraction

A sample of 0.5 g (approximately) of leaves was weighed accurately on analytical balance and extracted with 150 mL of distilled water for 30 min at boiling temperature in water bath. The flask with water extract was cooled down by stream of running water. A whole content of flask was quantitatively replaced into a calibrated flask and filled up to 250 mL by distilled water. After complete sedimentation of plant material, water extract was percolated through paper filter into another flask. First 50 mL of filtered liquid were rejected. The same procedure was applied for extraction of each samples.

Measuring

The extract in a volume of 5.0 mL was placed in calibrated flask and filled up to 25 mL with distilled water, then 1.0 mL of Folin-Ciocalteu reagent, 10.0 mL of distilled water were added to 2.0 mL of diluted extract (from 25 mL calibrated flask) and filled up to 25 mL with sodium carbonate solution (290 g/L). An absorbance of prepared sample was measured by means of UV spectrophotometer at 760 nm after 30 min of incubation in darkness. The same liquid (with pure water instead of plant extract) was used as a blind test. All determinations were performed in triplicate.

The total phenolic contents of each plant extract was calculated as shown:

$$X = \frac{62{,}5 \cdot A_1 \cdot m_2}{A_2 \cdot m_1}$$

where

X – total phenolic compounds calculated as pyrogallol equivalents (%);
A_1 – absorbance of investigated extract;
A_2– absorbance of pyrogallol solution;
m_1 – mass of investigated sample [g];
m_2 – mass of pyrogallol [g].

Reference substance (Pyrogallol)

50 mg of pyrogallol was dissolved in distilled water and filled up to 100 mL in calibrated flask. 5.0 mL of obtained solution was diluted in another 100 mL calibrated flask. Absorbance of 2.0 mL of pyrogallol solution (with the adequate reagents) was measured by the same method as described for leaves samples.

RESULT AND DISCUSSION

The total amounts of phenolic compounds in all investigated samples vary in limits from 2.40±0.23% to 3.43±017% (see tab. 2). The highest amount of polyphenols was found in one of samples collected in Oćwieka (Żnin district), the lowest were observed for material from the forest near Białe Błota and Brzoza (Bydgoszcz district). The results obtained for samples derived from Chomiąża, Łysinin and Czarnowo are very similar and ranged from 2,71±0.06 to 3.00±0.03%. The narrow range of presented results is probably connected with similar environmental conditions and plant requirements. The highest content of phenolic compounds noticed in plant material from Oćwieka suggest presence of the factors, which were important for phenolic compounds biosynthesis. Determination of this factors and its significance require further research with more samples collected from different areas. The relatively high amounts of polyphenolic compounds in juniper sprouts give reasons for continuation of next planned researches, eg. estimation of the antioxidant activity.

Table 2.

Total polyphenols contents in investigated samples (as pyrogallol eq./dry mass)

sample number	sample description	total percentage amount of phenolic compounds [%]
1	Chomiąża Szlachecka (Żnin district)	3.00±0.0289
2	Łysinin (Żnin district)	2.78±0.1344
3	Oćwieka (Żnin district)	3.43±0.1697
4	Czarnowo (Toruń district)	2.71±0.0611
5	Brzoza /Białe Błota (Bydgoszcz district)	2.40±0.2300

CONCLUSION

Juniperi pseudofructus is an important and well-known substance in herbal medicine. The essential oil is a main group of compounds of this material but phenolic substances are present, too. Collecting procedure of *Juniperi pseudofructus* may be difficult due to the fact that many populations of juniper do not create any "berries" or create pseudofruits in a very low amount. It is probably related with general plant conditions. It seems that *Juniperi pseudofructus* acquired from nature

is not a rich source of phenolic substances, because of it's low availability. The results of this work suggest that leaves of common juniper, which is easy accesible plant material may be a good source of polyphenols. Further research of phenolic profile in Juniperus communis leaves are needed.

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OZNACZANIE OGÓLNEJ ZAWARTOŚCI POLIFENOLI W PĘDACH JAŁOWCA (*JUNIPERUS COMMUNIS* L., *CUPRESSACEAE*) POZYSKIWANYCH Z RÓŻNYCH STANOWISK NA OBSZARZE WOJEWÓDZTWA KUJAWSKO-POMORSKIEGO

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Streszczenie:

Jałowiec pospolity (*Juniperus communis* L.) jest znaną i cenioną rośliną leczniczą. Jako surowiec znajduje zastosowanie szyszkojagoda jałowca (*Juniperi pseudofructus*) zawierająca głównie olejek eteryczny o działaniu moczopędnym, przeciwzapalnym, żółciopędnym i żółciotwórczym. W szyszkojagodach występują także inne substancje, w tym związki polifenolowe. Ta ważna grupa składników naturalnych obecna jest także w innych częściach rośliny. Celem pracy było określenie ogólnej zawartości polifenoli w pędach (liściach) jałowca pospolitego z różnych stanowisk na terenie województwa kujawsko-pomorskiego. Zawartość polifenoli oznaczono metodą spektrofotometryczną z zastosowaniem odczynnika Folina-Ciocalteu i przedstawiono w przeliczeniu na pirogalol. Uzyskane wyniki ogólnej zawartości polifenoli w liściach jałowca pospolitego wahały się od 2,40±0,23% do 3,43±0,17%.

Słowa kluczowe: *Jałowiec pospolity, Juniperus communis, polifenole, odczynnik Folina-Ciocalteu, województwo kujawsko-pomorskie*