TOTAL LIPIDS, PROTEINS, MINERALS AND ESSENTIAL OILS OF *PINUS NIGRA* ARNOLD AND *PINUS SYLVESTRIS* GROWING WILD IN KOSOVO

Fatmir Faiku[a]*, Arben Haziri[a], Fatbardh Gashi[a], Naser Troni[a] and Haxhere Faiku[b]*

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The soil and climatic conditions are conducive for the growth of a lot of medical herbs in Kosovo. Lipids, proteins, minerals and essential oils were quantitatively determined from the *Pinus nigra* Arnold and *Pinus sylvestris* growing wild in Germia Park (located in the north-east of Pristina). Total proteins were analyzed, by Kjeldahl method. The total amount of proteins in *Pinus nigra* Arnold is 7.714 % and in *Pinus sylvestris* is 4.375 %. Lipids are analyzed by Soxhlet extraction. The total amount of lipids in *Pinus nigra* Arnold are 7.185 % and in *Pinus sylvestris* 6.670 %. Essential oils were isolated using steam distillation. The total amount of essential oils in *Pinus nigra* Arnold are 0.35 % and in *Pinus sylvestris* 0.32 %. The mineral content was studied and analyzed by flame atomic absorption spectrometry. Six elements, sodium, potassium, calcium, zinc, iron and copper were determined in *Pinus nigra* Arnold and *Pinus sylvestris*. The mean levels of sodium, potassium, calcium, zinc, iron and copper are 246 mg kg^{-1}, 1280 mg kg^{-1}, 4260 mg kg^{-1}, 17.2 mg kg^{-1}, 173 mg kg^{-1}, 3.83 mg kg^{-1} in *Pinus nigra* Arnold, respectively. The mean levels of sodium, potassium, calcium, zinc, iron and copper are 6.9 mg kg^{-1}, 420 mg kg^{-1}, 1180 mg kg^{-1}, 95 mg kg^{-1}, 840 mg kg^{-1}, 19 mg kg^{-1} in *Pinus sylvestris*, respectively. Calcium and potassium are present in large amounts in *Pinus nigra* Arnold while calcium and iron are present in large amounts in *Pinus sylvestris*.

*Corresponding Authors
E-Mail: f_faiku@hotmail.com; haxhere_f@hotmail.com
[a] Department of Chemistry, Faculty of Natural Sciences, University of Prishtina, Kosovo
[b] Association Loyola-Gymnasium, Prizren, Kosovo

Introduction

The cultivation and use of medicinal herbs dates from Roman times. Until the nineteenth century and beginning of the twentieth century, medicinal herbs were the raw material for making medicines. Today, even though the active medicinal materials are obtained from synthesised chemicals the use of medicinal herbs in medicine is widespread and is important.[1] There is an increasing demand for natural products as curative agent. The medicinal plants are the primary sources for natural products and are used not only in the poorer countries but also in developing countries as alternate source of medicines to treat different diseases.[2-4]

The genus *Pinus* of the family *Pinaceae* (division *Pinophyta*) is comprised of more than 100 species and is spread worldwide.[5]

Pinus nigra Arnold is a large coniferous evergreen tree, growing to 20–55 meters tall at maturity. The bark is grey to yellow-brown, and is widely split by flaking fissures into scaly plates, becoming increasingly fissured with age. The leaves ("needles") are thinner and more flexible in western populations.[6]

Pinus sylvestris is an evergreen tree of 20-45 m height living up to 500 years. Pine is a source of wood, it is also used to protect the erosion of soil and its buds, needles and bark are used in phototherapy. Pine needles are rich in vitamin C, tannins, alkaloids and essential oils. Moreover, pines as all coniferous are very sensitive to air pollution, and the analysis of secondary metabolites allow evaluating both the physiological state of a plant and the environmental conditions under which it is growing.[5]

Our research group is interested to analyze the chemical profile of different medicinal plants which are growing wild in the region of Kosova and Albania.[7-13] The aim of this research was to determine the quantity of proteins, lipids, minerals and essential oil in the *Pinus nigra* Arnold and *Pinus sylvestris* in growing in Germia Park. Germia is a regional Park located in the north-east of Pristina, capital city of Kosovo, and it covers an area of 62 km^2.

Materials and methods

The needles of *Pinus nigra* Arnold and *Pinus sylvestris*, growing wild in Germia Park (located in the north-east of Pristina), was collected in July 2013. We took three samples from *Pinus sylvestris* and three from *Pinus nigra* Arnold. Voucher specimens were deposited in the Herbarium of the Department of Veterinary, University of Prishtina. The plants were dried at room temperature (22 °C). Proteins were determined according to the Kjeldahl method,[14] whereas lipids were determined according to the Soxhlet method.[15,16] As solvent was used diethyl ether. The essential oil was extracted with steam distillation for 4 h of 100 g of air dried needles.

The analysis, of the dry needles samples collected, resulted in the finding of minerals as sodium, potassium, calcium, zinc, iron and copper by using the method described by Taleisnik et al.[17] Therefore, the needles were

washed with destillated water to remove any dust and were dried in an oven at 105 °C for 48 h. The dried samples were pounded in a porcelain mortar until they formed a powder. Then, a 2 g sample, was calcified in an oven at 300-400 °C. The ashes were placed into 100 cm^3 of normal flask. Next, 10 cm^3 of 1 mol dm^{-3} nitric acid (HNO_3) was added to each flask, homogenized, and then shaken for 20 minutes in a shaker. The homogenized samples were filtered and filled till 100 cm^3 with 1 mol dm^{-3} HNO_3. Minerals like sodium, potassium, calcium, zinc, iron and copper were analyzed Atomic Absorption Spectrometry (Buc Scientific Model 200A).

Results and Discussion

Pinus nigra Arnold and *Pinus sylvestris* are analyzed in chemical aspect with the goal to research the chemical nature. We have analyzed the primary and secondary metabolites in quantitative manner. The amount of lipids, proteins and essential oil of *Pinus nigra* Arnold and *Pinus sylvestris* is given in Tables 1 and 2.

From experimental data (Table 1) we can see that the amounts of lipids were 7.185 %, proteins 7.714 % and essential oil 0.35 % in the *Pinus nigra* Arnold. From experimental data (Table 2) we can see that the amounts of lipids were 6.670 %, proteins 4.375 % and essential oil 0.32 % in the *Pinus sylvestris*.

Figures 1 and 2 shows the diagrams for the lipids, proteins and essential oil amounts to the *Pinus nigra* Arnold and *Pinus sylvestris* giving in percentage.

On Figures 1 and 2 we can see that the amounts of proteins, lipids and essential oils in *Pinus nigra* Arnold and in *Pinus sylvestris* are as follows: proteins > lipids > essential oil. The amount of essential oil is same in both the plants, but if we compare the amounts of proteins and lipids we can see that *Pinus nigra* Arnold contains higher amount of proteins and lipids (7.714 %, 7.185 % respectively) compared with the amounts of proteins and lipids in *Pinus sylvestris* (4.375 %, 6.670 % respectively).

The amount of essential oil in *Pinus nigra* Arnold (0.35 %) is almost same if we compare it with the amount of essential oil from different cities of Turkey[6] (Bursa 0.33-0.65 %, Antalya 0.38-0.55 %, Içel 0.38-0.65 %). The amount of essential oil of *Pinus sylvestris* (0.32 %) is same with the amount of essential oil of *Pinus sylvestris* growing wild in Lithuania (0.25-0.47 %).[5]

The amount of proteins in *Pinus nigra* Arnold (7.714 %) is lower than the amount of proteins from Turkey[18] (25.75 %). The amount of proteins in *Pinus sylvestris* (4.375 %) is lower than the amount of proteins from Mongolia[19] (45.1 %). The amount of lipids from *Pinus nigra* Arnold (7.185 %) are greater than to the *Pinus sylvestris* (6.670 %) growing wild in Kosovo. Also we did the research in the quantity of the minerals sodium, potassium, calcium, zinc, iron and copper. The amount of minerals of *Pinus nigra* Arnold and *Pinus sylvestris* are given in Tables 3 and 4

Table 1. The amounts of lipids, proteins and essential oil of the *Pinus nigra* Arnold

Components (%)	Mean	Standard deviation
Lipids	7.185	0.313
Proteins	7.714	0.169
Essential oil	0.35	0.112

Table 3 shows that the average values in mg kg^{-1} of sodium, potassium, calcium, zinc, iron and copper from *Pinus nigra* Arnold are 246 mg kg^{-1}, 1280 mg kg^{-1}, 4260 mg kg^{-1}, 17.2 mg kg^{-1}, 173 mg kg^{-1} and 3.83 mg kg^{-1}. From Table 4 we can see that the average values in mg kg^{-1} of sodium, potassium, calcium, zinc, iron and copper from *Pinus sylvestris* are 6.9 mg kg^{-1}, 420 mg kg^{-1}, 1180 mg kg^{-1}, 95 mg kg^{-1}, 840 mg kg^{-1} and 19 mg kg^{-1}.

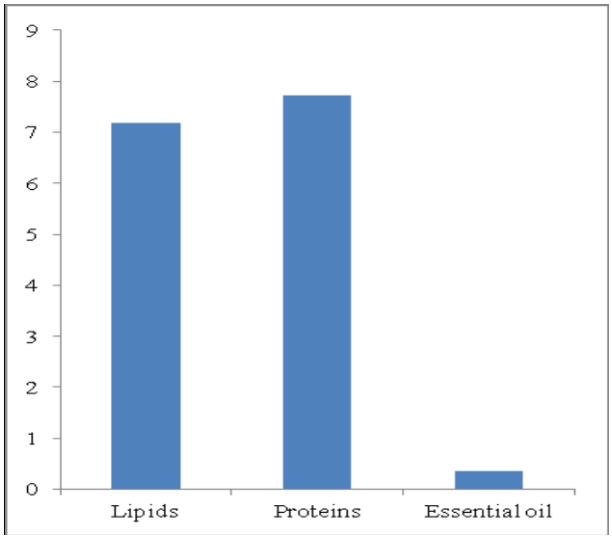

Figure 1. The diagram of the quantity of the lipids proteins and essential oil of the *Pinus nigra* Arnold

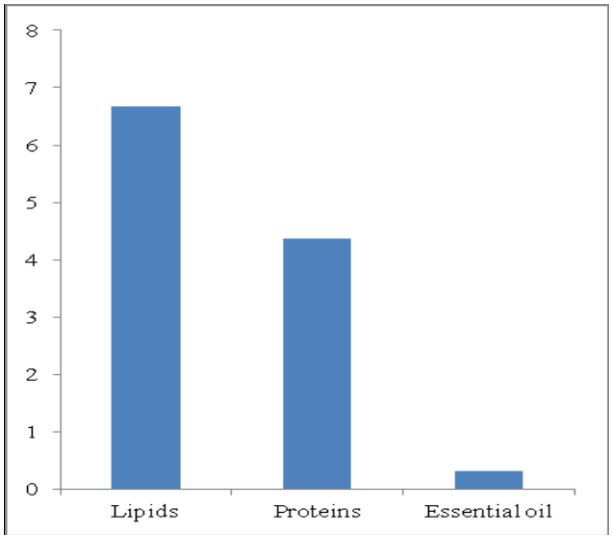

Figure 2. The diagram of the quantity of the lipids, proteins and essential oil of the *Pinus sylvestris*

Figures 3 and 4 shows the average values of elements in mg kg^{-1} from *Pinus nigra* Arnold and *Pinus sylvestris*.

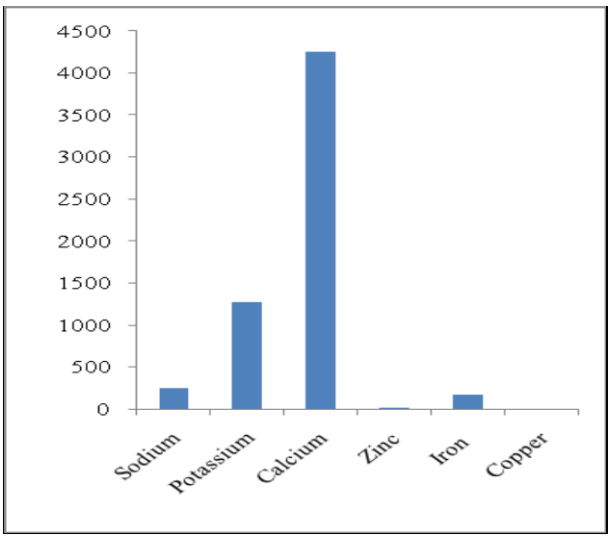

Figure 3. Diagram of the quantities of the minerals in the *Pinus nigra* Arnold

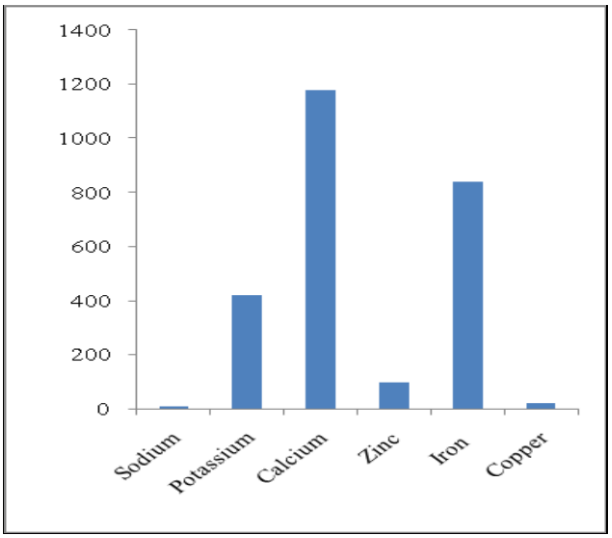

Figure 4. Diagram of the quantities of the minerals in the *Pinus sylvestris*

Pinus nigra Arnold contains large amounts of calcium (4260 mg kg^{-1}) and potassium (1280 mg kg^{-1}) (Figure 3), while *Pinus sylvestris* contains large amounts of calcium (1180 mg kg^{-1}) and iron (840 mg kg^{-1}) (Figure 4). The amount of sodium, potassium calcium from *Pinus nigra* Arnold are greater than that present in the *Pinus sylvestris*, while the amount of zinc, iron and copper in *Pinus sylvestris* are greater than to the *Pinus nigra* Arnold (Table 3 and 4).

The amounts of zinc (95 mg kg^{-1}), iron (840 mg kg^{-1}) and copper (19 mg kg^{-1}) of *Pinus sylvestris* growing wild in Kosovo are lower than to the *Pinus sylvestris* growing wild in Mongolia[19] (zinc 300 mg kg^{-1}, iron 2000 mg kg^{-1} and copper 500 mg kg^{-1}).

Pinus sylvestris contains large amounts of calcium (1180 mg kg^{-1}) compared to the amounts of calcium (500 mg kg^{-1}) in *Pinus sylvestris* growing wild in Mongolia.[19]

Table 2. The amounts of lipids, proteins and essential oil of the *Pinus sylvestris*

Components (%)	Mean	Standard deviation
Lipids	6.670	0.52638
Proteins	4.375	0.35865
Essential oil	0.320	0.05292

Table 3. Quantity of minerals of the *Pinus nigra* Arnold giving in mg kg^{-1}

Elements	Mean	Standard deviation
Sodium	246.0	30.000
Potassium	1283	20.167
Calcium	4260	16.252
Zinc	17.20	2.843
Iron	173.0	25.658
Copper	3.830	1.756

Table 4. Quantity of minerals in the *Pinus sylvestris* giving in mg kg^{-1}

Elements	Mean	Standard deviation
Sodium	6.90	0.56862
Potassium	420	88.88194
Calcium	1180	75.05553
Zinc	95.0	4.50925
Iron	840	87.17798
Copper	19.0	2.08167

Conclusion

The aim of this research was to determine the quantity of primary metabolites (lipids and proteins) and to analyze minerals as sodium, potassium, calcium, zinc, iron and copper.

From the results we can conclude that needles of *Pinus nigra* Arnold contain more proteins 7.714 % than *Pinus sylvestris* 4.375 %. Also, lipids in *Pinus nigra* Arnold are 7.185 % and in *Pinus sylvestris* 6.670 %, which means that they contain equal amounts of them. The total amount of essential oils in *Pinus nigra* Arnold are 0.35 % and in *Pinus sylvestris* 0.32 %.

In this research we have analyzed the amounts of minerals and from them we can order these elements in terms of their quantity. In *Pinus nigra* Arnold they are ordered as follows: Ca > K > Na > Fe > Zn > Cu. Order of the elements of *Pinus sylvestris* given mg kg^{-1} is as follows: Ca> Fe> K> Zn> Cu > Na. *Pinus nigra* Arnold contains large amounts of calcium and potassium, while *Pinus sylvestris* contains large amounts of calcium and iron.

As conclusion these two trees contain large amounts of essential oil and proteins. In the future our country should has attention to grow them in terms to get essential oil for different purposes. Let's hope that in the future programs of agricultural policies of our country, through developmental programmers in different private enterprises and municipalities, this field will get the attention it deserves.

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