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The effect of sowing rate on the bioaccumulation of biologically active compounds and their radical scavenging activity in *Cannabis sativa* L.

Ieva Čalkaitė[1],

Algimanta Kundrotaitė[1, 2],

Vita Tilvikienė[1],

Karolina Barčauskaitė[1*]

[1] *Institute of Agriculture, Lithuanian Research Centre for Agriculture and Forestry, 1 Instituto Avenue, Akademija, 58344 Kėdainiai District, Lithuania*

[2] *Faculty of Natural Science, Vytautas Magnus University, 8 Vileikos Street, 44404 Kaunas, Lithuania*

Industrial hemp (*Cannabis sativa* L.) is a very beneficial plant because it is characterised as easy adaptable and advantageous raw material for many products which are used in daily life. Hempseeds are investigated most of all, but there is no a lot of information about other parts of this plant (stem, leaves and inflorescences). In this study, polyphenolic compounds, flavonoids and the radical scavenging activity of *Cannabis sativa* L. were investigated. The influence of two different sowing rates (15 and 80 plants per square meter) and two solvents on the recovery of biologically active compounds was determined. Spectrophotometric tests were used for the identification of phenolic compounds and flavonoids content and their radical scavenging activity. It was found that industrial hemp which grew in the sparse area accumulated more biologically active compounds and was a better radical scavenger than the other one, which grew under denser conditions. Moreover, it was determined that polyphenolic compounds dissolved better in methanol and thus showed better radical scavenging properties while flavonoids dissolved better in ethanol.

Keywords: sowing rate, industrial hemp, polyphenolic compounds, flavonoids content, radical scavenging activity

INTRODUCTION

Industrial hemp (*Cannabis sativa* L.) is one of the oldest crops cultivated all over the world to produce fibre, food products and biomass which is rich in biologically active compounds [1] and particularly as a wood alternative. Since ancient times, hemp has been used in folk medicine as a drug for various diseases [2]. Industrial hemp became widely used because it could adapt to various climates and broad soil. Due to its deep and large root system hemp has a high potential for removing nutrients and water from the soil [3]. Phenolic compounds present around 10,000 various structures, including flavonoids (flavones and flavonols), phenolic acids, stilbenes and lignans [4]. Flavonoids and polyphenolic compounds are called plants secondary metabolites which protect plant tissues from harmful effects of the environment [5]. Several studies have presented the results that enhanced abiotic and biotic stress could increase the synthesis of plants secondary metabolites [6–9]. These biologically active compounds consist of more than one hydroxyl groups that are methylated or glycosylated. Phenols may be divided into five subgroups: phenolic acids, flavonoids, coumarins, lignins and tannins [9, 10]. Abiotic stresses are one of the common ways which affect the metabolic pathway of secondary metabolites production [11]. To decrease the hazard caused by abiotic stress plants enhance the synthesis of biologically active compounds such as flavonoids and polyphenolic compounds. Due to the ability to scavenge free radicals, flavonoids and phenolic

* Corresponding author: Email: karolina.barcauskaite@lammc.lt

acids are important contributing factors to antioxidant activity [12]. They are produced in the cytoplasm through the phenylpropanoid pathway and later transported in the vacuole or deposited in the cell wall [2]. Free radical DPPH• can form a stable DPPH-H molecule when it is scavenged by an antioxidant through the donation of H• (hydrogen atom) [13]. Therefore, scavenging of this free radical directly correlates with the number of hydroxyl groups [14]. Consequently, it can be assumed that the more phenolic compounds are found in extract the higher radical scavenging activity should be. The same can be stated about some of the flavonoids as flavones (amentoflavone, alpinumisoflavone), flavonols (quercetin, morin) and flavanones (6, 8-diprenyleriodictyol, 7-O-methyleriodictyol) [15]. About 20 flavonoids, which belong to the flavone and flavonol subclasses, have been identified in fibre hemp [16]. Plants have evolved to produce flavonoids to protect against herbivores, pathogens, fungal parasites and oxidative cell injury [17]. There are many studies about hempseed composition and nutritiousness in various databases, but other parts of this plant (stem, leaves and inflorescences) were not investigated as much. Scientists from Italy used the non-THC-producing species of *Cannabis sativa* L. (the ones that have less than 0.2%) called fibre hemp or industrial hemp in their research [18, 19]. The obtained results showed that the total amount of phenolics accounted for approximately 20% of flavonoids and phenolic acids [19]. However, they did not explore differences in all parts of the plant and how the quantity of these compounds changes in various vegetation stages. The experiments performed in our study show how different cultivation technology (sowing density) influences the accumulation of biologically active compounds in various morphological parts of the plant. It is known that the biological activity and accumulation of bioactive compounds in the plant depend on its phenotype and more precisely on the geographic origin, climatic conditions or genotype [20, 21], but there is the lack of results on the sowing rate effect on the synthesis of biologically active compounds and their radical scavenging activity in industrial hemp. This study aimed to investigate the seasonal variation of the total amount of polyphenolic compounds, flavonoids and their radical scavenging activity in different morphological parts of industrial hemp grown in the Central part of Lithuania at Lithuanian Research Centre for Agriculture and Forestry.

EXPERIMENTAL

Chemicals

Methanol was obtained from Sigma-Aldrich, France. Aluminium chloride anhydrous (pure for analysis) and sodium nitrite (pure for analysis) were purchased from Chempur, Poland. Rutin ≥97% was obtained from Acros Organics, Germany. Folin–Ciocalteu reagent and sodium carbonate were obtained from Sigma-Aldrich, Germany. Ethyl alcohol was obtained from JSC MV GROUP Production, Lithuania. Sodium hydroxide was purchased from Carl Roth, Germany. DPPH (1,1-Diphenyl-2-picrylhydrazyl Free Radical) was obtained from TCI EUROPE, Belgium.

Preparation of extracts

The objects of this research were three morphological parts (stem, leaves and inflorescences) of *Cannabis sativa* L. variety *Felina 32*. Plants were collected from the Lithuania Research Centre for Agriculture and Forestry test fields grown under different densities: 15 and 80 plants per square meter. During the vegetation season, any pesticides and fertilisers were not applied. Six times during vegetation plants were harvested and divided into different parts (stem, leaves and inflorescences), dried at ambient temperature, packed in paper bags and stored at ambient temperature until use. Before the analysis, leaves and inflorescence samples were ground using a coffee bean mill (Bosch, Germany), and to shredded stems a Retsch ZM 200 ultra centrifugal mill was used. Not in all cases, inflorescences were formed. The detailed sample list and vegetation stage are presented in the Table.

To determine the content of total phenolic compounds and flavonoids two different solvents for extraction were used (70% methanol and 70% ethanol solution in water). 1 g of powdered material soaked with 10 ml 70% methanol was treated in an ultrasonic bath (VWR International, Malaysia) for 60 min. After that, the extract was filtered through a paper filter (weight 90 g/m^2) into a test tube. The same extraction was made using a 10 ml 70% ethanol solution.

Table. **Samples and industrial hemp vegetation phases at harvesting time [42]**

	1st harvest	2nd harvest	3rd harvest	4th harvest	5th harvest	6th harvest
	4th leaf pair	GV point, change of phyllotaxis on the main stem from opposite to alternate	Female flower formation	Beginning of seed maturity	Leaf desiccation	Stem desiccation
Stem	+	+	+	+	+	+
Leaves	+	+	+	+	+	+
Inflorescences	–	–	–	+	+	+

Determination of total phenolic compounds, total flavonoids content and radical scavenging activity

To determine the content of total flavonoids (TFC) 1 ml of the prepared extract was stirred with 0.3 ml 5% $NaNO_2$. After 5 min keeping them in the dark, a 0.5 ml 2% $AlCl_3$ aqueous solution was added, vortexed (IKA, USA) for 30 s and left for 6 min in the dark again. Then 0.5 ml 1 M NaOH was mixed and the final reaction was incubated for 10 min in the dark. To determine the total amount of phenolic compounds (TPC), 0.1 ml of the prepared extract was mixed with 2.5 ml bidistilled water, 0.1 ml Folin–Ciocalteu reagent and a 0.5 ml 20% Na_2CO_3 aqueous solution. The reaction solutions were incubated for 30 min in the dark [22].

To measure the radical scavenging activity (RSA) of extracts a slightly modified Brand-Williams, Cuvelier and Berset method [23] was used. 0.077 ml of the prepared extract was mixed with 3 ml freshly prepared DPPH solution (6×10^{-5} M) in methanol [14]. The change of reaction absorbance was measured after 30 min. All reaction solutions were kept in the dark. The extracts capability to bleach DPPH free radical solution was estimated using the following formula

$$\text{Inhibition (\%)} = ((A_B - A_E)/A_B)) \times 100, \quad (1)$$

where A_B is the absorption of a blank sample, $t = 0$ min, A_E is the absorption of tested extract, and $t = 30$ min.

Absorptions were measured by UV–VIS spectrophotometer (Shimadzu Corp., Japan) at 510 nm for flavonoids, 760 nm for phenolic compounds and 515 nm for radical scavenging activity. Rutin was used as a standard to perform the calibration curve (0.1–1 mg mL^{-1}) for the content of total flavonoids and polyphenolic compounds. The obtained results were expressed as mg g^{-1} of rutin equivalent (RUE).

Statistical analysis

The results were presented as a mean of three replicates ± standard deviation. Correlation coefficients were estimated to determine the significance of correlations between radical scavenging activity and the content of biologically active compounds at $P < 0.05$ and $P < 0.01$.

RESULTS AND DISCUSSION

The amount of biologically active compounds and their radical scavenging activity were determined in various morphological parts (stem, leaves and inflorescences) of industrial hemp six times per vegetation season. Plants were grown in different densities of 15 and 80 plants per square meter. Results were obtained by means of spectrophotometric methods and samples were extracted using different solvents.

Sowing density effect on the amount of total phenolic compounds and total flavonoids

The amount of TPC extracted using ethanol is presented in Fig. 1a, b. It can be noticed that in the first two vegetation phases the amount of TPC was quite similar, from 37.57 to 42.24 mg RUE g^{-1} in both densities. In the third vegetation phase, a higher difference was recorded, about 47.79 mg RUE g^{-1} TPC obtained from plants grown under denser conditions and 36.11 mg RUE g^{-1} in the sparse area. The results obtained in this study correlated with the previous research when the higher TPC content was determined in young industrial hemp compared to that of mature plants [24]. After the forth vegetation phase, where hemp grew up inflorescences, the quantity of TPC was higher in the plants collected from the sparse area (from 76.05 to 78.53 mg RUE g^{-1}) than in those which were grown in the denser area (from 67.55 to 69.70 mg RUE g^{-1}). In the last sixth vegetation phase, the TPC concentration started to

decrease but remained resembling in both densities around 44.29 mg RUE g^{-1} in the plants grown under sparse conditions and 46.17 mg RUE g^{-1} in the plants collected from the denser area. The obtained results indicated that the industrial hemp grown in the sparse area during the flowering stage accumulated slightly more polyphenolic compounds compared to the plants grown under denser conditions, but it should be noted that the determined amount strongly correlates with the solvent used for extraction. The solubility of phenolic compounds is affected by polarity of the solvent and interaction with other compounds such as carbohydrates and proteins in plant tisues [25].

The quantity of TPC in methanolic extracts is summarised in Fig. 1c, d. The results do not show the difference between the sowing rates when comparing 42.41 mg RUE g^{-1} with 39.74 mg RUE g^{-1} in the first vegetation phase, 47.41 mg RUE g^{-1} with 41.61 mg RUE g^{-1} in the second vegetation phase and 39.36 mg RUE g^{-1} with 37.00 mg RUE g^{-1} in the third vegetation phase. In the fourth and fifth vegetation phases, the obtained TPC content was higher around 81.46 mg RUE g^{-1} and 78.99 mg RUE g^{-1} in the plants collected from the sparse area and 81.73 mg RUE g^{-1} and 77.91 mg RUE g^{-1} in the plants grown under denser conditions. Extracts of hemp leaves obtained using 70% methanol as a solvent (Fig. 1c) distinguished an extremely low TPC content (7.05 mg RUE g^{-1}) and was ~60% lower compared to the amount determined in ethanolic extract. These results indicate that hemp grown in the denser area was more superior in the last stage for 48.15 mg RUE g^{-1} compared to cultivated at lower density (36.96 mg RUE g^{-1}). Barčauskaitė et al. determined that sowing density does not show a significant effect on TPC content in hemp seeds [26]. It is hard to compare TPC and TFC content in plant materials with other research due to different extraction solvents and analysis techniques used. The previous study reported that the sowing rate is directly related to the ratio of crop growth rate [27].

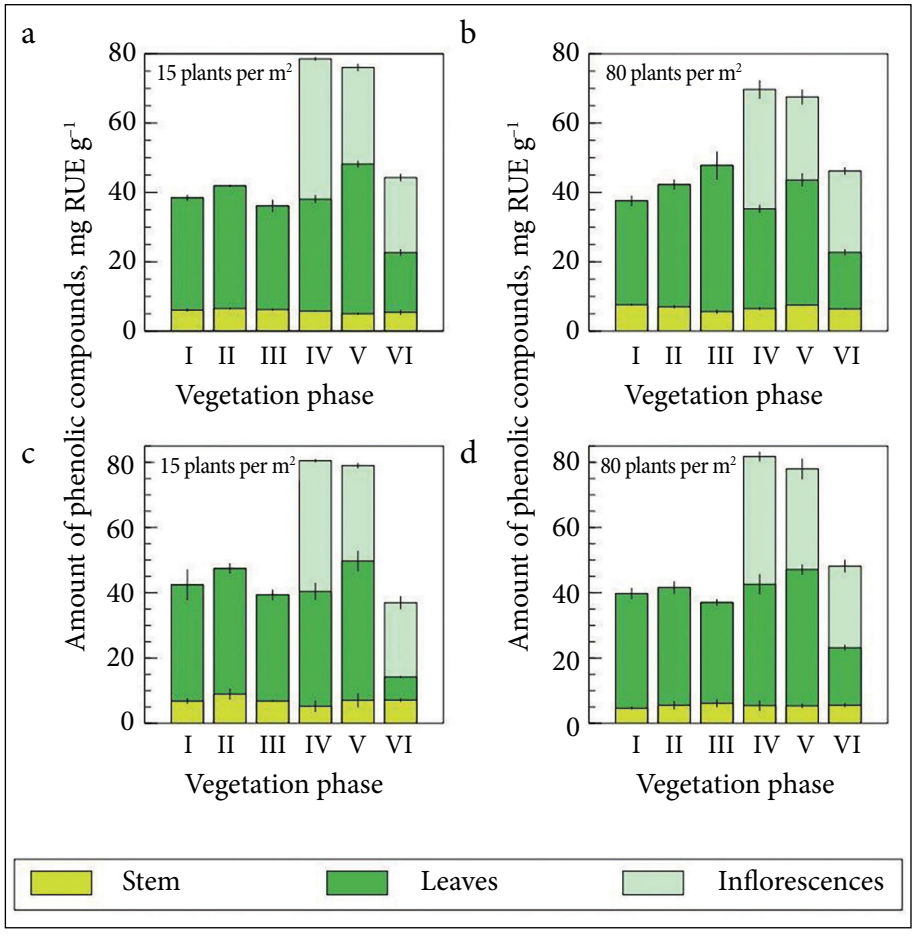

Fig. 1. Amount of total polyphenolic compounds in the morphological parts of fibre hemp during vegetation; (a) and (b) extraction solvent is ethanol; (c) and (d) extraction solvent is methanol

The total amount of flavonoids which were extracted using ethanol is presented in Fig. 2a, b. The tendency was quite similar to that of phenolic compounds, in the first two vegetation stages, the amount of TFC was found from 31.95 to 32.73 mg RUE g^{-1} in the plants grown in the sparse area and from 29.04 to 32.52 mg RUE g^{-1} in others which were grown in the denser area. The lowest amount of TFC content was determined at the flowering formation stage and varied from 21.83 to 23.96 mg RUE g^{-1} in different plant densities. The quantity of TFC after inflorescences reached the peak 56.32 mg RUE g^{-1} in the plants collected from the sparse area and 49.26 mg RUE g^{-1} in the plants from the denser area. The amount of TFC drastically decreased in the sixth vegetation phase. As well as in TPC, it can be noticed that very little but still more TFC was determined in the industrial hemp plants grown 15 plants per square meter.

The amount of TFC in the extracts of methyl alcohol is shown in Fig. 2c, d. In the first three vegetation phases, where inflorescences were not formed, the trend remains the same: 23.28, 25.97 and 17.06 mg RUE g^{-1} in the field where 15 plants were planted per square meter, and 22.08, 25.47 and 16.83 mg RUE g^{-1} in the area with 80 plants per square meter. After inflorescences that appeared in the fourth vegetation phase, hemp collected from the sparse area still accumulated more 44.65 mg RUE g^{-1} TFC compared to the one grown in denser area, 39.42 mg RUE g^{-1}. However, later when plants reached leaf and stem desiccation phases (V and VI vegetation phases), denser conditions outrun the sparse area with 40.71 and 12.67 mg RUE g^{-1} against, respectively, 40.55 and 10.55 mg RUE g^{-1}. It can be concluded that hemp in its young stage accumulates more flavonoids in less competition, but after it gets mature, hemp can be more useful in conditions of increased competition. Previous studies demonstrated that the amount of secondary metabolites such as flavonoids and anthocyanins increased with increasing the sowing

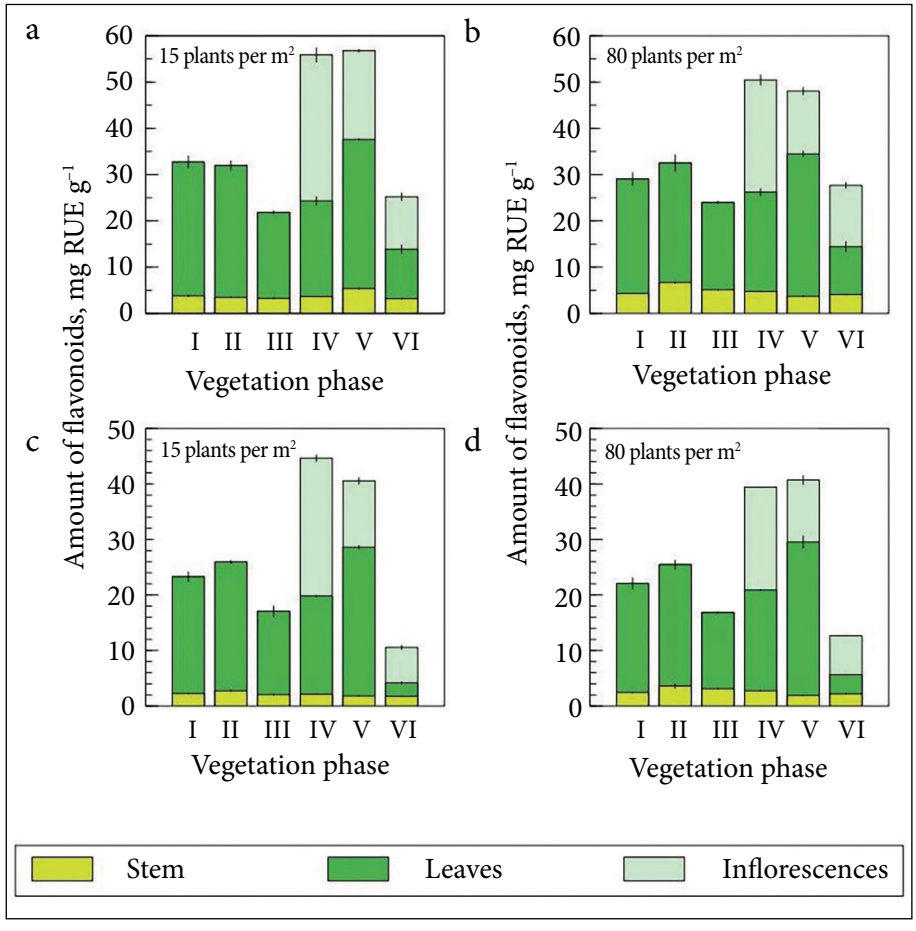

Fig. 2. Amount of total flavonoids in the morphological parts of fibre hemp during vegetation; (a) and (b) extraction solvent is ethanol; (c) and (d) extraction solvent is methanol

rate in experiments of perilla sprouts [28]. It was determined that the flavonoid concentration in hemp aromatic water was 4.04 mg RUE g^{-1} [19]. The results obtained in this study demonstrate the importance of harvest time which depends on the properties of the final product. Researchers from Italy reported that the harvest time of hemp significantly affects the yield and composition of the essential oil [29].

Solvent effect on the recovery of biologically active compounds

Two different solvents to evaluate the recovery of TPC and TFC from different morphological parts of industrial hemp were chosen. The amount of TPC in extracts with methanol was slightly higher than in those where ethanol was used (Fig. 1). The opposite observation was noticed in the sixth vegetation phase when ~20% more TPC was determined in ethanolic extracts compared to methanolic ones. However, the trend was similar independently of the growing density of investigated plants. In the third vegetation phase, the extracts of leaves obtained using 70% ethanol as a solvent showed an unusually high amount of phenolic compounds, 42.17 mg RUE g^{-1}. That changed the system which was valid in other figures, therefore, it was not taken into account. Methanol influence on flavonoids was opposite than on phenolic compounds. Flavonoids dissolved worse in it and, using spectrophotometry measurements, it was obtained that in both densities and all vegetation phases methanol extracts showed a lower amount of TFC than ethanol ones (Fig. 2). The first three vegetation phases in methanol left behind approximately from 18.72 to 29.78% compared with ethanol extracts. The results of the fourth and fifth vegetation phases were lower from 15.3 to 28.57% using methanol as a solvent. Ultimately, the highest difference was noticed in the sixth vegetation phase where the estimated data showed 54.22–58.17% reduction using methanol. In this research, ethyl alcohol and methyl alcohol were compared as solvents

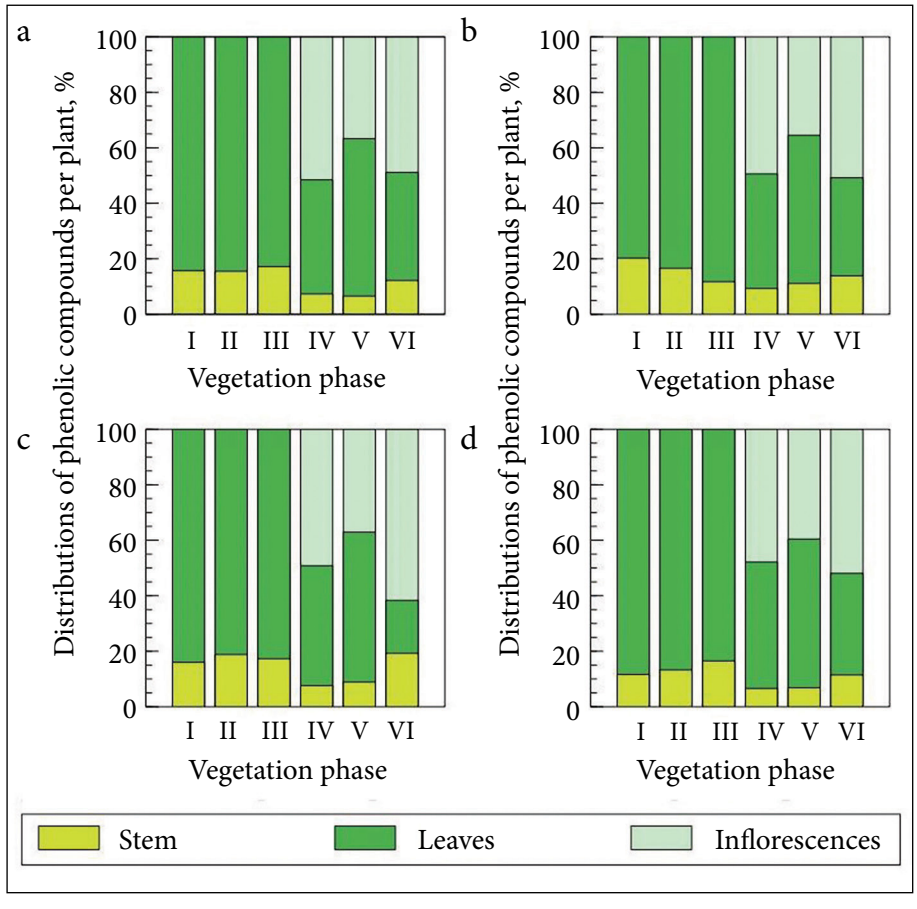

Fig. 3. Distribution of total phenolic compounds in morphological parts of fibre hemp during vegetation; (a) and (b) extraction solvent is ethanol, sowing rate 15 and 80 plants per square meter, respectively; (c) and (d) extraction solvent is methanol, sowing rate 15 and 80 plants per square meter

to influence the amount of flavonoid solubility and as a result, it was learned that using ethanol better recovery of flavonoids can be obtained than using methanol. Hence, 70% methanol was a better solvent for more polar phenolic compounds and a bit less polar 70% ethanol for flavonoids. This finding agreed with the previous research which shows the importance of solvent and target compounds solubility interaction [30]. Moreover, scientists who carried out the investigations with ethanol, acetone and ethyl acetate find out that acetone was the best one [31]. However, it is very important to pay attention that different extraction techniques were used.

Distribution of biologically active compounds per different morphological parts of *Cannabis sativa* L.

After the performed experiments, it was relevant to estimate how the total quantity of phenolic compounds or flavonoids distributes per different plant morphological parts during each vegetation phase. At the beginning of industrial hemp growing (I–III vegetation phases), approximately from 10 to 20% of phenolic compounds were determined in the stem and other from 80 to 90% were found in leaves (Fig. 3). When inflorescences appeared (starting with the fourth vegetation phase), the amount of TPC in the stem and leaves decreased almost two times, which means that the highest part of plant secondary metabolites accumulates in inflorescences: in the fourth vegetation phase from 50 to 55%, in the fifth phase from 35 to 40% and in the sixth one from 50 to 60%. The percentage reduction in TPC content in the fifth vegetation phase was related to the formation of seeds. These results were quite similar in both solvents (Fig. 3). The diversity of sowing density did not make any important differences either.

The stems in the fourth and fifth vegetation phases accumulated twice less – from 5 to 10% – flavonoids compared to stems in residue vegetative phases – from 10 to 20% (Fig. 4). Moreover, in

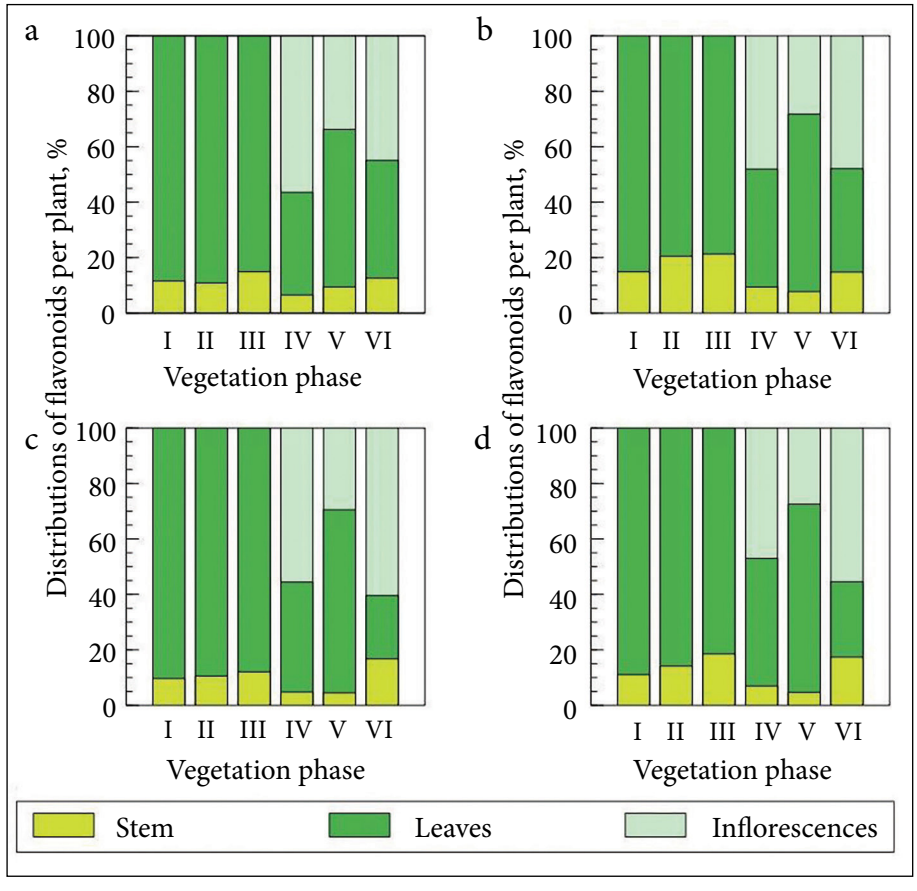

Fig. 4. Distribution of total flavonoids in morphological parts of fibre hemp during vegetation; (a) and (b) extraction solvent is ethanol, sowing rate 15 and 80 plants per square meter, respectively; (c) and (d) extraction solvent is methanol, sowing rate 15 and 80 plants per square meter

the first three growing stages percentage amount of TFC in leaves progressively decreased – from 90 to 80%, while an increase was recorded in stems. It was determined that around half of TFC accumulated in inflorescences except less was obtained in the fifth vegetation phase. The obtained data do not show specific differences based on the solvent, only in the sixth vegetation phase the results of inflorescences were more scattered from 45 to 47% when the solvent was ethanol and 55–60% when the solvent was methanol. In this case, the distribution of leaves in the sixth vegetation phase was unequal between the solvents either 37–42% in ethanol or 23–27% in methanol. The sowing rate did not make any huge differences in TFC percentage distribution per separate industrial hemp morphological parts. Generally, it is accepted that compositions and concentrations of various secondary metabolites in plants can vary between different organs and locations within a plant [32, 33]. This is an important issue considering hemp use as a botanical therapeutic, ideally with a fixed dosage of active compounds [32].

Radical scavenging activity

It can be noticed that inhibition was higher in the extracts of methanol than ethanol (Fig. 5). This investigation once again proves the previous studies, which were performed at Indian Veterinary Research Institute [14] and at Kaunas University of Technology [22]. Methanol extracts showed stronger radical scavenger properties in all morphological parts of industrial hemp. It is also visible that in leaves (Fig. 5b) and inflorescences (Fig. 5c) fibre hemp which grew up in the sparse area were more effective radical scavengers. However, in stems (Fig. 5a) this tendency has changed and made hemp from the field with 80 plants per square meter slightly superior. Different parts of industrial hemp had its tendency during all harvests as well. For example, stems remain similar during all six vegetation phases.

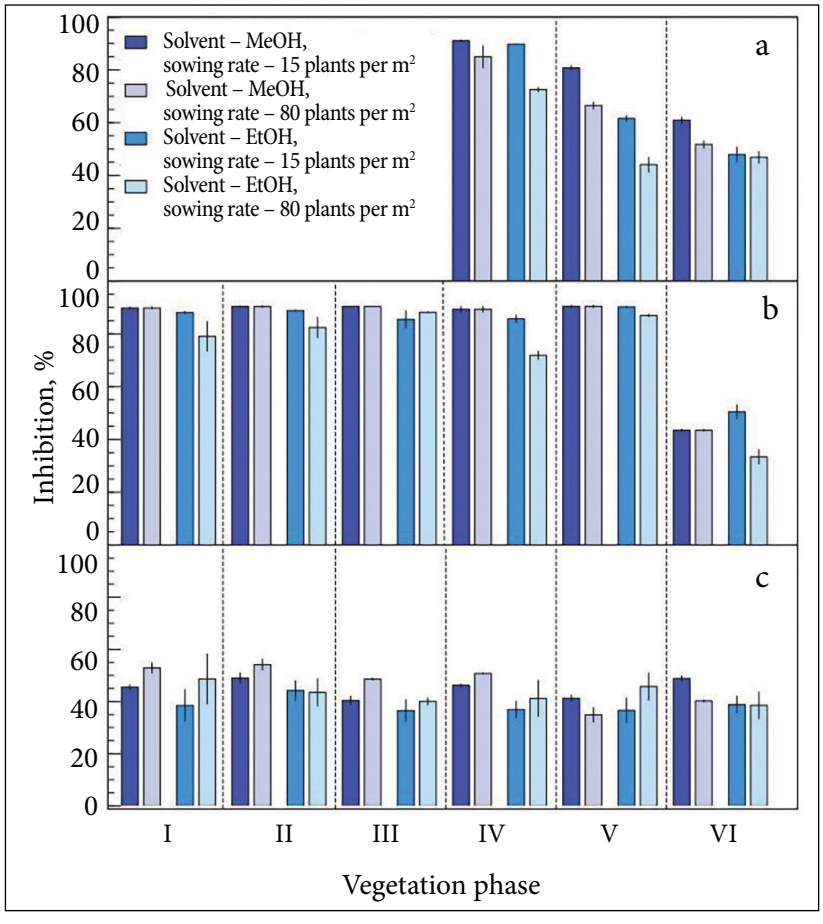

Fig. 5. Changes of the radical scavenging activity of industrial hemp morphological parts during vegetation; (a) inflorescences, (b) leaves and (c) stem

Leaves showed stability in the first five harvests (~87%), but the sixth one decreased more than twice (~41%). Inflorescences present an equivalent decrease in the last vegetation phases of hemp existence. These findings could be explained by natural plants life cycle when plants reached the senescence vegetation phase. In summary, RSA of leaves (I, II, III, IV and V vegetation stages) and inflorescences collected from the sixth vegetation phase can be considered as a full absorption inhibition, especially in the extracts with methanol, of DPPH free radical. Whereas, after the incubation of reaction, a yellowish solution was obtained which comparing with a colourless methanol (or ethanol) solution cannot reach 100% [22]. Other scientists who tested different methods for RSA determination obtained distinctions between the same variety cultivated in different locations (north, south, east) areas and used various determination methods as well showed differences [34].

The direct correlation between the biologically active compounds and antioxidant activity might be related to the presence of various active compounds in the plant, which gave different trends of radical scavenging activity [35]. TPC significantly correlated with the determined RSA in industrial hemp leaves at both densities (r = 0.964 and r = 0.920) and inflorescence collected from the denser area (r = 0.998) at $P \leq 0.01$ (Fig. 6). No significant correlation was obtained between TPC and RSA in industrial hemp stem in all treatments. Other authors reported no relation between TPC and RSA, on the other hand, their research object was wheat [36]. A strong positive correlation was observed between the determined TFC content and RSA of industrial hemp stems grown in the denser area (r = 0.786) and leaves (r = 0.850) at $P \leq 0.05$ probability level. The determined TFC content significantly positively correlated with RSA in industrial hemp inflorescence (r = 0.998) grown under denser conditions and leaves (r = 0.880) collected from the sparse area. The synergistic effects of different compounds, experimental conditions and mechanisms of antioxidant reactions method used may affect this association [35]. Moreover, most

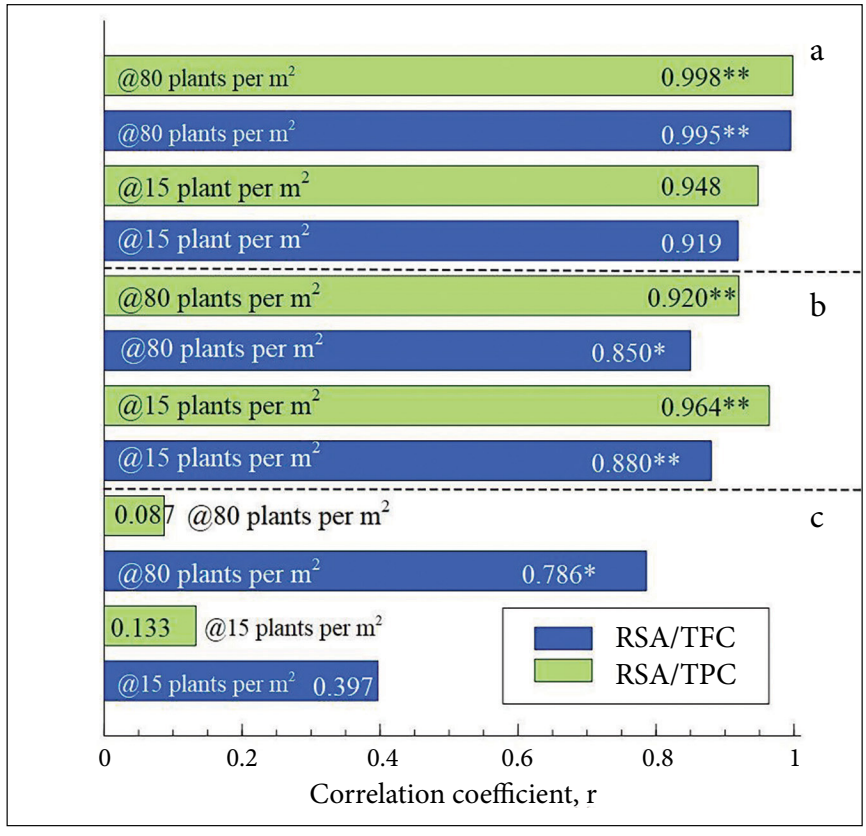

Fig. 6. Correlation between radical scavenging activity (RSA), total phenolic compounds (TPC) and total flavonoids content (TFC); * shows statistically significant correlations at $p \leq 0.05$ probability and ** at $p \leq 0.01$; (a) inflorescences, $n = 3$; (b) leaves, $n = 6$; (c) stem, $n = 6$

of the determination methods have their limitations to measure antioxidant activity [37, 38]. Nevertheless, most of the studies demonstrated a strong correlation between biologically active compounds and RSA investigated in selected herbs, fruits, grain products and vegetables [35, 39–41].

CONCLUSIONS

This study was conducted to determine the sowing density effect on the industrial hemp accumulation of total phenolic compounds and total flavonoids and their radical scavenging activity during different vegetation phases. Many important morphological-related chemical trends were presented. The obtained results indicate that hemp which was grown in the sparse area (15 plants per square meter) accumulates around 13% more phenolic compounds compared to the plants grown in the denser area (80 plants per square meter). The determined flavonoid content varied between 21.83 and 56.87 mg RUE g^{-1} and the highest total flavonoid content as the content of total phenolic compounds was determined in the plants collected from the sparse area. During this study, it was found that to recover more phenolic compounds methanol can be used, but ethanol for flavonoids. The sowing rate and solvent did not affect the results of the distribution of biologically active compounds in industrial hemp stems, leaves and inflorescence. This study indicates that inconsiderably more effective radical scavengers were industrial hemp inflorescence and leaves compared to the investigated stems. No significant correlation was demonstrated between the radical scavenging activity and the amount of total phenolic compounds in industrial hemp stems grown under both densities as well as inflorescence collected from the sparse area. Significant and positive linear correlations were found between the radical scavenging activity and the amount of the content of total phenolic compounds and between the radical scavenging activity and the content of total flavonoids, indicating that phenolic compounds, and flavonoids were the dominant antioxidant constituents in the tested plant parts. The correlation coefficient r varied from 0.998 to 0.880 at $P \leq 0.01$ and from 0.786 to 0.850 at $P \leq 0.05$.

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Ieva Čalkaitė, Algimanta Kundrotaitė, Vita Tilvikienė, Karolina Barčauskaitė

SĖJOS TANKIO ĮTAKA BIOLOGIŠKAI AKTYVIŲ JUNGINIŲ BIOAKUMULIACIJAI IR JŲ RADIKALŲ SURIŠIMO AKTYVUMUI *CANNABIS SATIVA* L.

S a n t r a u k a

Pluoštinės kanapės (*Cannabis sativa* L.) yra nuo seno naudojamas vertingas augalas. Jis yra lengvai ir plačiai pritaikomas, kuriant kasdienėje veikloje naudojamus produktus. Labiausiai ištirtos pluoštinių kanapių sėklos, tačiau apie kitas šio augalo dalis (stiebą, lapus ir žiedynus) informacijos nėra daug. Atliktame tyrime analizuota pluoštinių kanapių morfologinėse dalyse bendras polifenolinių junginių, bendras flavonoidų kiekis ir antiradikalinis poveikis. Įvertinta dviejų skirtingų sėjos normų (15 ir 80 augalų m^2) ir dviejų tirpiklių įtaka biologiškai aktyvių junginių kiekiui. Bendras polifenolinių junginių, flavonoidų kiekis bei jų radikalų surišimo aktyvumas nustatyti spektrofotometriniu metodu. Ištirta, kad pramoninės kanapės mažesnio tankio variante sukaupė daugiau biologiškai aktyvių junginių ir pasižymėjo geresniu antiradikaliniu aktyvumu palyginti su augintomis tankiau. Be to, nustatyta, kad polifenoliniai junginiai geriau išekstrahuojami naudojant metanolį kaip tirpiklį. Tai lėmė, kad šie ekstraktai išsiskyrė geresniu radikalų surišimo aktyvumu, o flavonoidų didesnė koncentracija nustatyta pluoštinių kanapių mėginiuose, ekstrahuotuose etanoliu.