INFLUENCE OF VARIOUS SOLVENTS ON THE POLYPHENOLIC COMPOSITION OF EXTRACTS FROM *CANNABIS SATIVA* L. AND *CANNABIS INDICA* PLANTS

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ABSTRACT

The extraction ability of different solvents and their mixtures with water is investigated towards the qualitative and quantitative polyphenolic composition extracted from the plants Cannabis Sativa L. and Cannabis Indica and it is determined by HPLC - DAD technique. Extraction is carried out by the maceration method, and the effect of following solvents is compared: 96 % ethanol, 80 % ethanol (aq.), 50 % ethanol (aq.), dichloromethane, acetone, 80 % acetone (aq.) and 50 % acetone (aq.). The results derived reveal that acetone/water mixture (1:1) can extract the largest number of polyphenols from Cannabis Sativa L. - epigallocatechin, rutin, myricetin and kaempferol, as first three of them present in the largest amounts, compared to the other solvents. It is worth noting that ethanol/water mixtures (80:20 and 50:50) both extract three of the studied polyphenols from Cannabis Sativa L. Considering the polyphenolic content in Cannabis Indica extracts, it is found out only ethanol/water (1:1) extracts the largest number of polyphenols - epigallocatechin, rutin, myricetin and quercetin, as the amount of all these is the largest, compared to the other solvents. Another ethanol/water mixture (80:20) is also quite effective in extracting the traced compounds, since three polyphenols - rutin, myricetin and quercetin are identified.

Keywords: Cannabis Sativa L, Cannabis Indica, polyphenols, HPLC - DAD.

INTRODUCTION

The cannabis plant, according to Clarke and Merlin, has been known to mankind since before the dawn of civilization [1]. *Cannabis* seeds have been found in fossils as early as 10.000 years ago in Japan, and fibers of this plant have been found in the Czech Republic as early as 27.000 years ago, while written records for this date back more than 4000 BC [2]. Ancient people found the hemp seeds were a rich source of nutrients, and its lyco-fibrous stems could be used to make strong ropes and fabrics. In addition to these benefits, it has been found that the resin around the seeds of female bracts has a psychoactive effect, which is expressed in a state of altered consciousness - from mild euphoria, a feeling of complete ecstasy and even falling into a trance [2]. For this reason, *Cannabis* has been used as a very important component of rituals of the shamans of almost all known cultures, being a means of communication with the gods. Finally, this plant has been used for thousands of years as a medicinal remedy for a number of diseases.

Cannabis Sativa L. was first described in 1753 by Carolus Linnaeus, who classified only this species. Later, Jean-Baptiste Lamarck described another species of this plant, which he called *Cannabis Indica*. It is known that one of the significant differences between both species is the different content of cannabinoids, which are the active substances in these plants [2]. The most important

of these substances are tetrahydrocannabinol (THC) and cannabidiol (CBD), as *Cannabis Indica* contains about a hundred times more THC than *Cannabis Sativa* L., which is responsible for *Indica*'s strong psychotropic effect. However, *Cannabis Indica* is characterized by a significantly lower cannabidiol content [3].

Cannabis Sativa L., also known as technical or medical hemp, is an annual plant, having a height up to 5 m [4] and is cultivated for using its long length fibers, necessary for textile production (baskets, twine, mats, bags, fabrics, belts and so on), for construction (bricks, concrete mixes, insulation materials), etc. [5]. On the other hand, hemp seeds are very useful for obtaining oil that is utilized for production of varnishes, oil paints as well as for products in medicine, cosmetics, chemical, pharmaceutical, fish canning industry as well as in confectionery production, agricultural and food industries, etc. [3]. The reason for such widespread applications is that this oil contains a lot of very useful components - unsaturated fatty acids, bactericidal substances, vitamins, amino acids, polyphenols, terpenes, pigments, sugars, lipids as well as various micro-elements [5]. On the other hand, *Cannabis Indica* is used mainly for various medicinal and recreational purposes, thanks to its ability to reduce pain, muscle tension and to promote relaxation and better sleep [6].

There is scarce data in literature about the content of individual polyphenols in *Cannabis Sativa* L., as mainly total polyphenol content (TPC) is determined by the method of Folin-Ciocalteu [7 - 10]. In most of these studies, methanol, ethanol, acetone and their aqueous mixtures are used for the extraction processes.

Studies focused on the qualitative and quantitative determination of some individual polyphenols in *Cannabis Sativa* L. are quite few [3, 8]. This gives a reason to study and compare the influence of a larger number of different solvents on the degree of extraction of some of the most important polyphenols contained in *Cannabis Sativa* L. and *Cannabis Indica* species. It is worth noting that a similar study for *Cannabis Indica* is not found at all in the literature.

EXPERIMENTAL

To determine the content of polyphenols in *Cannabis Sativa* L. and *Cannabis Indica*, 1 g dry ground mass of leaves and inflorescence of both species was used. Extraction was carried out by the maceration method, for seven days, at room temperature and shaking. For this purpose, the following solvents were used, each one with a total volume of 100 mL: 96 % ethanol, 80 % ethanol (aq.), 50 % ethanol (aq.), dichloromethane, acetone, 80 % acetone (aq.) and 50 % acetone (aq.). All solvents were of analytical grade and were purchased from Merck (Darmstadt, Germany).

The THC content in plant extracts of both species *Cannabis Sativa* L. and *Cannabis Indica* was ascertained by means of gas chromatographic technique (GC), on an Agilent 7890A apparatus working at following column temperature regime: 160ºC for 5 min and a subsequent increase in temperature, with a heating rate of 15ºC min^{-1} to 300ºC and hold for 8 min. As a carrying gas nitrogen with a flow rate of 1.5 mL min^{-1} was used.

Polyphenol content extracted from both *Cannabis* species was determined applying high-performance liquid chromatography (HPLC) technique, performed on an Agilent 1100 HPLC apparatus (Agilent Technologies, California, USA), equipped with diode-array detector (DAD) (G1315B, Agilent Technologies, California, USA). The column selected for analysis was Purospher star, Hiber RT 125-4; RP18, with 125 mm length, 4 mm internal diameter as well as 5 μm particle size (Purospher star, Merck) and working temperature of 25°C. Trichloroacetic acid with a concentration 0.1 % (A) and 100 % acetonitrile (B) formed the eluent system. Maximum separation of the analytes was ensured using the chromatographic system that works in a linear gradient: the process began with 5 % B, 15 % B at 16.5 min, 33 % B at 22.5 min, 100 % B at 30.5 min, 5 % B at 35 min until 40 min for re-equilibration. The volume injected for each sample as well as for standards was 30 μL, 1.6 $mL.min^{-1}$ flow rate was in operation and 200 - 400 nm absorbance range of DAD for data acquisition was set up [11, 12].

RESULTS AND DISCUSSION

Table 1 presents the results for type and concentration of some of the most important polyphenols extracted from *Cannabis Sativa* L., by the maceration method, with different solvents described. The qualitative and quantitative analyses of extracts are performed using the HPLC - DAD technique, according to the method of Dimcheva et al. [11]. From the data in Table 1, it is worth noting that acetone/water mixture

Table. 1. Concentration of the phenolic compounds, identified by HPLC - DAD in *Cannabis Sativa* L. extracts, obtained with different solvents.

Cannabis Sativa L. extracts	Solvents	epigallocatechin, μg mL^{-1}	rutin, μg mL^{-1}	myricetin, μg mL^{-1}	kaempferol, μg mL^{-1}
1	Ethanol 96 %	-	-	2.326	-
2	Ethanol/Water, 80/20	32.89	-	61.43	2.778
3	Ethanol/Water, 50/50	-	16.17	8.045	0.739
4	Dichloromethane	-	-	-	-
5	Acetone	-	-	-	0.882
6	Acetone/Water, 80/20	-	-	4.247	0.741
7	Acetone/Water, 50/50	42.65	22.55	102.9	1.578

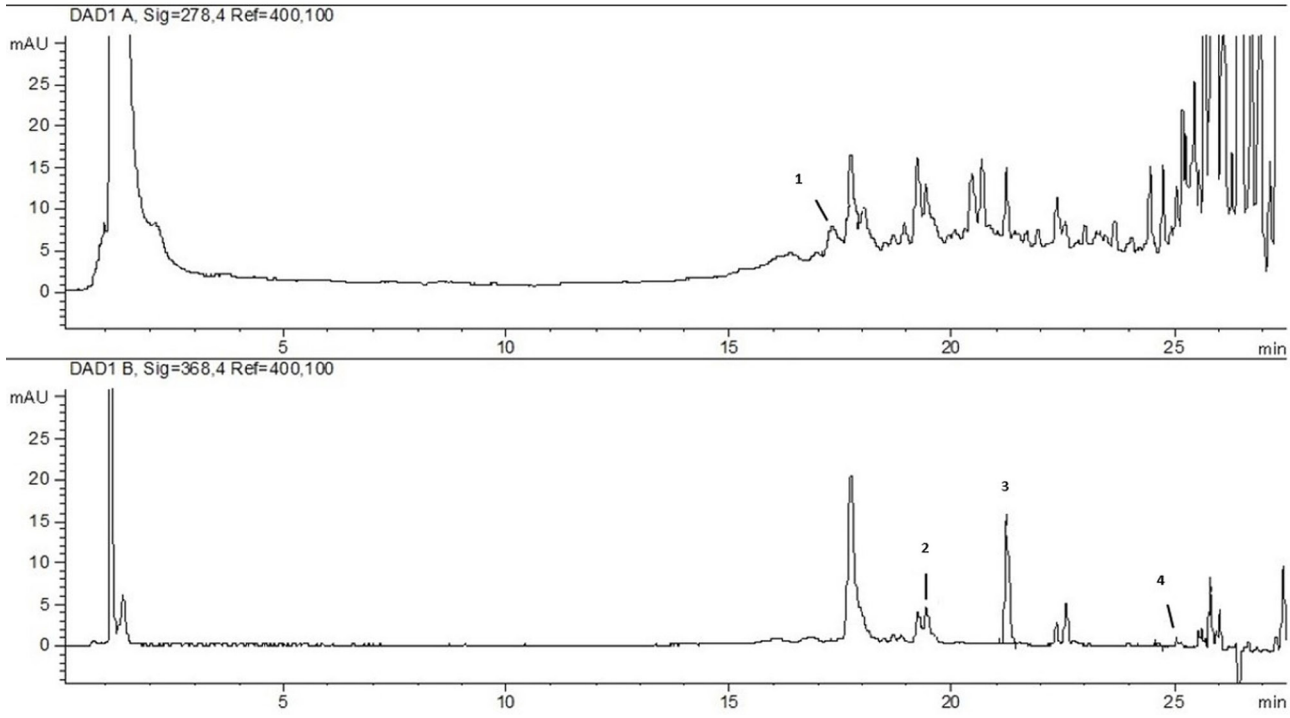

Fig. 1. HPLC chromatogram (upper chromatogram at 278 nm, bottom chromatogram at 368 nm) of *Cannabis Sativa* L. extract in acetone/water mixture (50:50). 1 - epigallocatechin, 2 - rutin, 3 - myricetin and 4 - kaempferol.

in a 1:1 ratio (Fig. 1) extracts the largest number of polyphenols - epigallocatechin, rutin, myricetin and kaempferol, with first three of them being registered in the largest amounts, compared to the other solvents. The amount of myricetin - 102.9 μg mL^{-1} is quite impressive. On the other hand, ethanol/water mixtures, in ratios of 80:20 (Fig. 2) and 50:50 (Fig. 3), both extract three of the studied substances. Regarding first ratio, epigallocatechin, myricetin and kaempferol are identified, as the amount of the latter is the highest, compared to other solvents - 2.778 μg mL^{-1} (Table 1). Mixture ethanol/water (50:50) also extracts three of the analysed polyphenols: rutin, myricetin and kaempferol, as it could be seen that amount of the latter is the lowest - 0.739 μg mL^{-1}. The acetone/water mixture, in a ratio of 80:20, extracts two of the analysed compounds - myricetin and kaempferol, while pure acetone and 96 % ethanol extract only one of the studied polyphenols, kaempferol and myricetin, respectively, with registered amount of the latter being the smallest one, compared

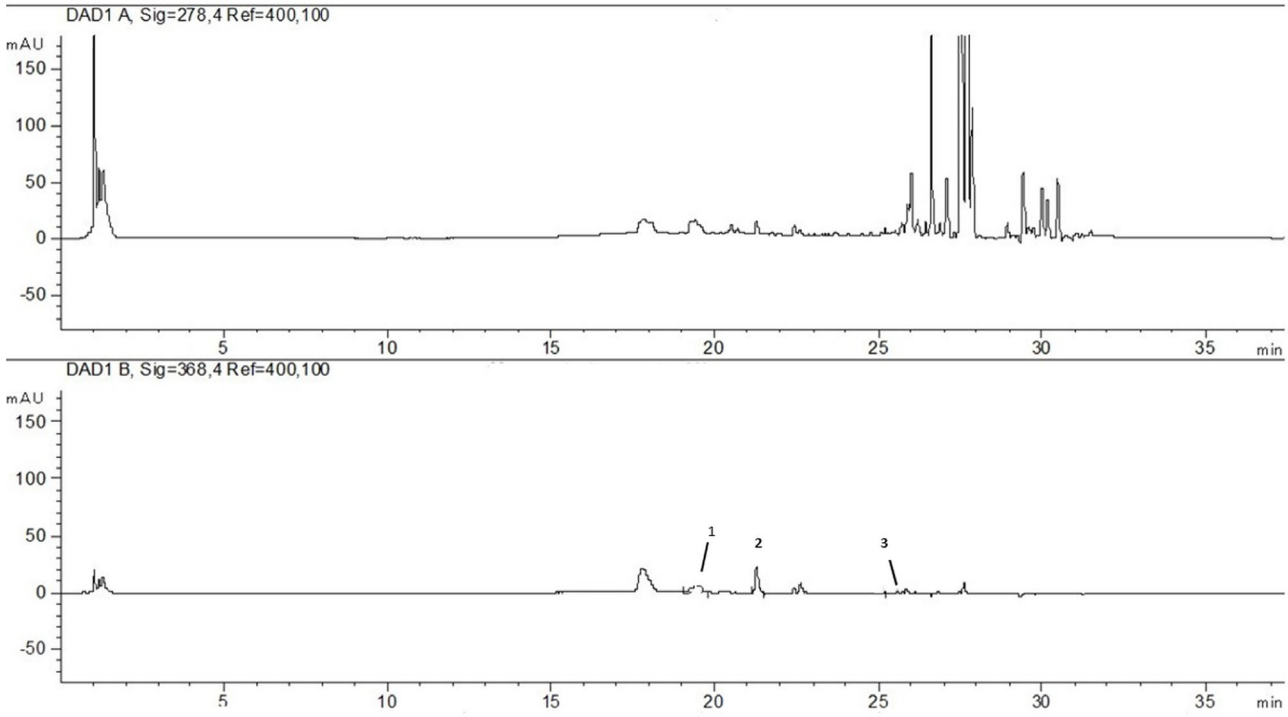

Fig. 2. HPLC chromatogram (upper chromatogram at 278 nm, bottom chromatogram at 368 nm) of *Cannabis Sativa* L. extract in ethanol/water mixture (80:20). 1 - epigallocatechin, 2 - myricetin and 3 - kaempferol.

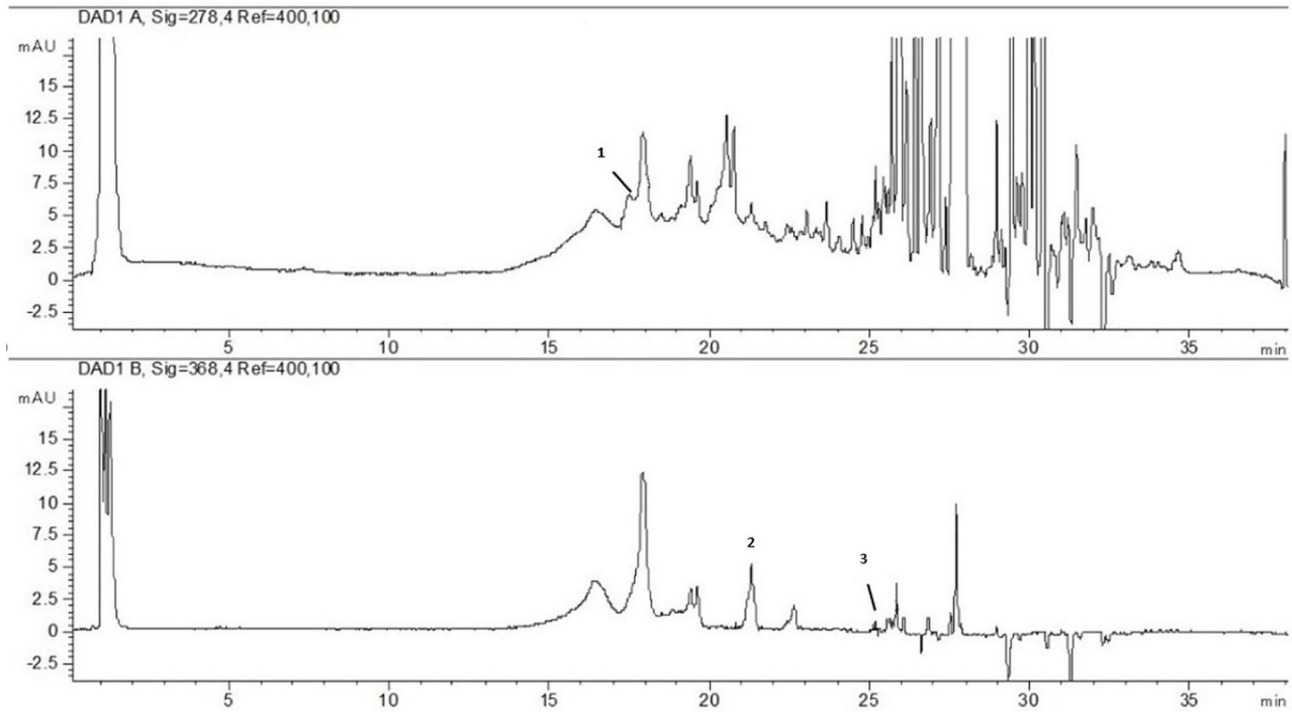

Fig. 3. HPLC chromatogram (upper chromatogram at 278 nm, bottom chromatogram at 368 nm) of *Cannabis Sativa* L. extract in ethanol/water mixture (50:50). 1 - rutin, 2 - myricetin and 3 - kaempferol.

to the other solvents (2.326 μg mL^{-1}). As could be seen from Table 1, only dichloromethane does not extract any of polyphenols traced.

Table 2 presents data about the qualitative and quantitative composition of analysed polyphenols extracted by maceration from *Cannabis Indica*, using the same solvents described in Table 1. Again, the identical analytical method is used, based on the HPLC - DAD technique [11]. It is worth noting that only ethanol/water mixture in the ratio of 1:1 (Fig. 4) extracts the largest number of polyphenols - epigallocatechin, rutin, myricetin and quercetin, with the amount of all of them being the largest, compared to the other solvents (Table 2). It is of interest that ethanol/water mixture (80:20) is also quite effective in extracting the monitored compounds, since three polyphenols - rutin, myricetin and quercetin are detected (Fig. 5). Furthermore, two of the substances studied, rutin and myricetin, are extracted with both

Table. 2. Concentration of the phenolic compounds, identified by HPLC - DAD in *Cannabis Indica* extracts, obtained with different solvents.

Cannabis Indica extracts	Solvents	epigallocatechin, μg mL^{-1}	rutin, μg mL^{-1}	myricetin, μg mL^{-1}	quercetin, μg mL^{-1}	kaempferol, μg mL^{-1}
1	Ethanol, 96 %	-	7.86	2.45	-	-
2	Ethanol/Water, 80/20	-	16.56	3.77	0.442	-
3	Ethanol/Water, 50/50	31.08	80.37	8.965	1.297	-
4	Dichloromethane	-	-	-	-	-
5	Acetone	-	-	-	-	0.749
6	Acetone/Water, 80/20	-	-	-	-	-
7	Acetone/Water, 50/50	-	0.695	6.202	-	-

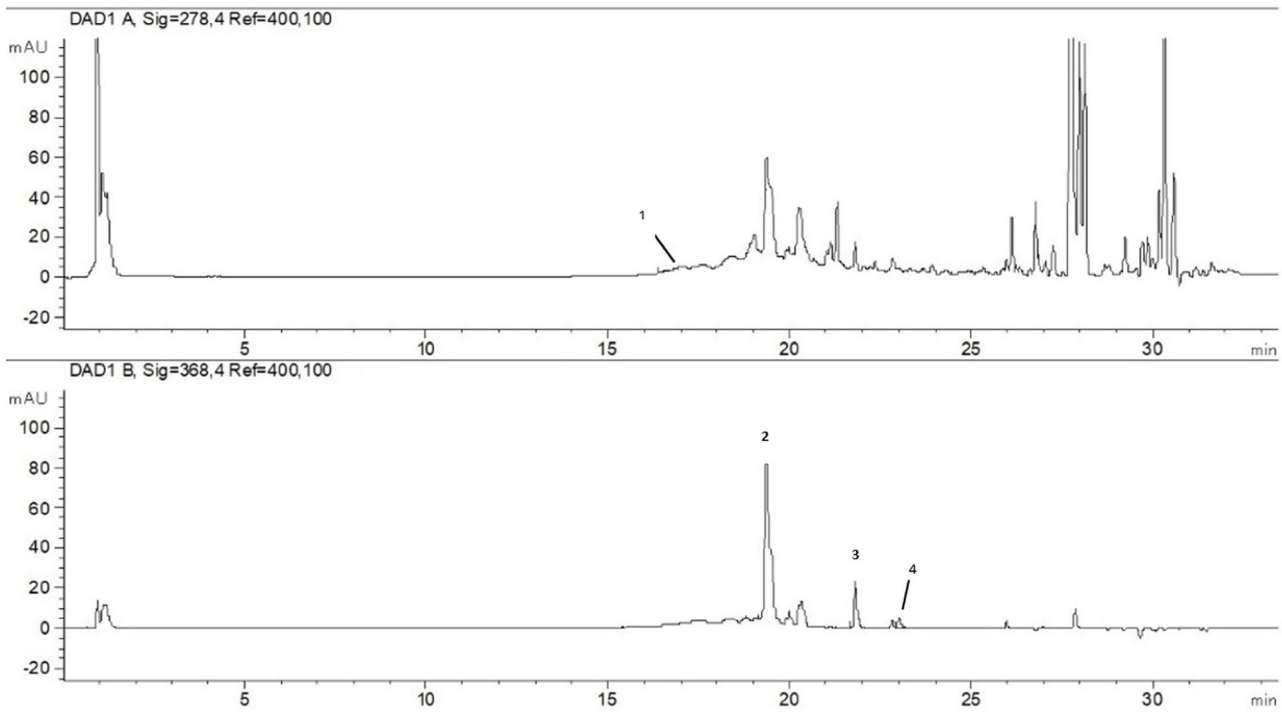

Fig. 4. HPLC chromatogram (upper chromatogram at 278 nm, bottom chromatogram at 368 nm) of *Cannabis Indica* extract in ethanol/water mixture (50:50). 1 - epigallocatechin, 2 - rutin, 3 - myricetin and 4 - quercetin.

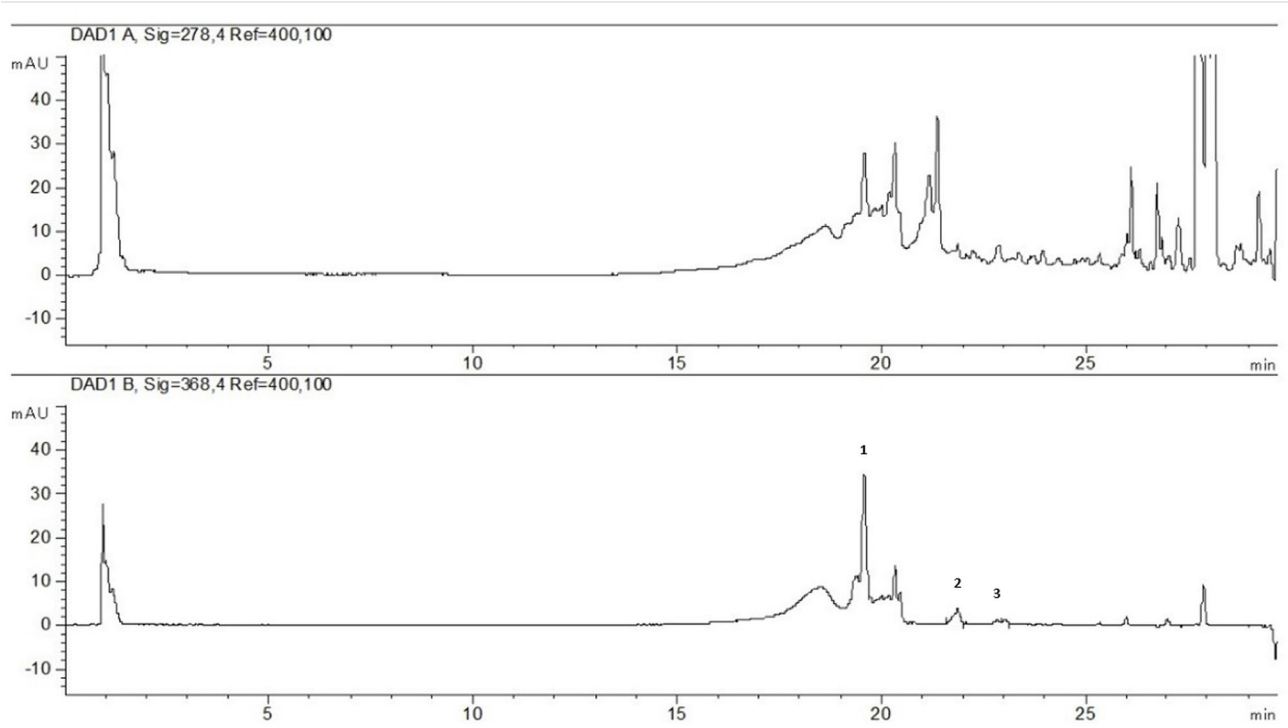

Fig. 5. HPLC chromatogram (upper chromatogram at 278 nm, bottom chromatogram at 368 nm) of *Cannabis Indica* extract in ethanol/water mixture (80:20). 1 - rutin, 2 - myricetin and 3 - quercetin.

ethanol 96 % and acetone/water mixture (1:1), with the latter comprising the lowest rutin content compared to the other solvents - 0.695 μg mL^{-1}. When pure acetone was used as an extractant from leaves and inflorescence of *Cannabis Indica*, only kaempferol is detected, while when dichloromethane as well as acetone/water (80:20) are used, none of the polyphenols under investigation are detected (Table 2).

When comparing the effect of different solvents used (Tables 1 and 2), it is noteworthy that ethanol/water mixtures (at both ratios of the components), show overall the best extraction ability in terms of the number of polyphenols studied, both for *Cannabis Sativa* L. and for *Cannabis Indica*. In addition, only when using ethanol/water mixtures, another polyphenol - quercetin is recorded from *Cannabis Indica*. It is of interest, however, that concerning *Cannabis Sativa* L. the maximum number of extracted polyphenols and generally maximum relative amounts of these substances, is achieved when using acetone/water mixture 1:1. On the other hand, acetone/water mixture (80:20) does not extract any of the expected polyphenols from *Cannabis Indica* plant.

As for the individual solvents used, 96 % ethanol extracts myricetin in comparable amounts from leaves and inflorescence of both *Cannabis* species, and in addition it extracts rutin from *Cannabis Indica*. Pure acetone extracts only kaempferol from both plants in similar concentrations. It is worth noting that when using pure dichloromethane as an extracting agent from *Cannabis Sativa L.* and *Cannabis Indica* samples, none of the traced polyphenols are detected.

The THC content in extracts of *Cannabis Sativa* L. and *Cannabis Indica* is identified by means of gas chromatographic technique, as the results reveal that *Cannabis Sativa* L. contains 0.18 %, while the THC content in *Cannabis Indica* amounts to 15.48 %. Both values lie within the acceptable range of this substance.

CONCLUSIONS

Extraction experiments are performed on plant material from *Cannabis Sativa* L. and *Cannabis Indica* using different solvents, to identify some of the most important polyphenols contained. Concerning *Cannabis Sativa* L., the largest number of extracted polyphenols and generally maximum relative amounts of these

substances, are observed for acetone/water mixture 1:1. It is found that the ethanol/water mixtures in both studied ratios (80:20 and 50:50) are characterized for both plants with the most diverse polyphenol composition. This gives reason for considering mixtures of ethanol/water and acetone/water in different ratios as promising extractants of polyphenols, in the extraction of other *Cannabis* plant species.

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