COMPARATIVE STUDY OF POLYPHENOLIC COMPOSITION AND REDUCING PROPERTIES OF EXTRACTS OF *CANNABIS SATIVA* L., *CANNABIS INDICA* AND *CANNABIS HYBRID*

Nikolay Vetskov[1], Nikolay Kaloyanov[1], Ivaylo Hinkov[1], Svetlomir Diankov[1], Katya Pashova[1], Vanya Gerasimova[2], Milena Funeva-Peycheva[3], Todorka Yankovska-Stefanova[3], Atanas Garbev[4]

[1]*University of Chemical Technology and Metallurgy*
8 Kliment Ohridski Blvd., Sofia 1797, Bulgaria
[2]*Institute of Organic Chemistry with Centre of Phytochemistry*
Bulgarian Academy of Sciences
Acad. G. Bonchev Str., bl. 9, Sofia 1113, Bulgaria
[3]*Central Laboratory of Veterinary Control and Ecology*
5 Iskarsko Shousse Blvd., Sofia, Bulgaria
[4]*Institute of Electrochemistry and Energy Systems*
"Academician Evgeni Budevski"
Acad. Georgi Bonchev Str., bl 10, Sofia 1113, Bulgaria
E-mail: nikolaykaloyanov@uctm.edu

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ABSTRACT

Cannabis Sativa L., Cannabis Indica and Cannabis Hybrid are widespread plant species that find legal use in many countries around the world. The first of them has found considerable application in medicine, cosmetics, building construction, textile and food industries. Cannabis Indica and Cannabis Hybrid are used as raw materials for the extraction of oils that are used as pain relievers as well as for recreation purposes.

The aim of the present study is to compare the polyphenolic composition of extracts from the three types of plants, by the maceration method of dry green leaf mass consisting of inflorescences and leaves. Ethanol (99.9 %) is used as an extractant, and the duration of the maceration process is seven days, at room temperature and stirring. The polyphenol composition is determined using HPLC-DAD method.

The following polyphenol composition is found in extracts of Cannabis Sativa L.: epigallocatechin, rutin, myricetin, quercetin and kaempferol. On the other hand, quercetin and kaempferol are detected in the Cannabis Indica samples, while epigallocatechin, quercetin and kaempferol are present in the hybrid sort.

In addition, green synthesis of silver nanoparticles by using sunlight or microwave irradiation was carried out to evaluate the reduction properties of three cannabis extracts. The least quantity of silver nanoparticles was obtained in the presence of Cannabis Sativa L. extracts. From the other two species of cannabis, microwave irradiation produces more silver nanoparticles, with smaller sizes, while exposure to direct sunlight produces fewer particles, but with larger sizes.

Keywords: Cannabis Sativa L., Cannabis Indica, Cannabis Hybrid, polyphenols, silver nanoparticles.

INTRODUCTION

Hemp, or so-called cannabis and marijuana, exists in the form of different plant species *Cannabis Sativa* L., *Cannabis Indic*a, *Cannabis Hybrid*, etc. Technical hemp (*Cannabis Sativa* L.), also known as medical or industrial hemp, is one of the oldest cultivated lyco-fibrous plants [1]. It is an annual plant, reaching a height from 1 m up to 5 m and is mainly grown for its fibers and seeds, which are used to extract hemp oil [2]. Due to the long length of its fibers, technical hemp is widely used in the textile, construction, fishing industry, etc.

[1]. For example, it is used to make various products: baskets, twine, ropes, fishing nets, bags, fabrics, belts, paper, bricks, concrete mixes, insulation materials and even the construction of entire houses [1].

Technical hemp oil is contained mainly in the seeds - from 30 to 37 %. It belongs to the group of easily drying oils and is therefore widely used in the production of varnishes and oil paints. Hemp oil is also used in medicine, cosmetics, chemical, pharmaceutical and food industries. It contains bactericidal substances, unsaturated fatty acids, glycerol, amino acids, vitamins (A, B_1, B_2, B_6, E, K, D), as well as various micro-elements. The seeds of this plant are also used for the preparation of painkillers, food additives, the production of soft soaps, raw materials for the agricultural industry, etc. Moreover, hemp oil is widely used in the fish canning industry as well as in confectionery production [1].

The two other types of cannabis - *Indica* and *Hybrid*, are used for several medicinal and recreational purposes. *Indica* helps reducing pain, relieving muscle tension, increasing relaxation, and helping patients rest and sleep. *Hybrid* strains are a cross between *Sativa* and *Indica* in different ratios [3].

Technical hemp differs from the other two plant species also by its low content of tetrahydrocannabinol (THC), which is less than 0.2 % and by its high percentage content of cannabidiol (CBD). Medical hemp has the same THC content but a low percentage of cannabidiol. It is an organic compound extracted from the inflorescences of the hemp's leaves. In this hemp, the percentage content of CBD reaches up to 10 %.

Cannabis Indica, the so-called marijuana is considered as a plant with psychotropic effects due to its high content of THC, which varies from 0.2 % to 18 %, while the percentage of CBD is low. *Cannabis Hybrid* strain is a crossbreed of the species *Cannabis Indica* and *Sativa*, which also represents a plant with psychotropic effects, as the THC content varies from 10 % to 27 % and at the same time it has a low percentage of CBD. In addition to the two most important cannabinoids mentioned above, the chemical composition of the three types of cannabis also includes other cannabinoids, terpenes, polyphenols, pigments, sugars, lipids, etc. [4].

It is also of interest that the biological approaches for the synthesis of silver nanoparticles (AgNPs) are proposed as simple, cost-effective and environmentally friendly methods. One promising method is the synthesis of AgNPs by using plant-based extracts, for example, extracts from *Tilia Cordata*, *Matricaria Chamomilla*, *Calendula Officinalis* and *Lavandula Angustifolia* flowers [5]. A large number of plant extracts are used as sources to produce AgNPs suitable for biomedical or pharmaceutical applications [6]. The most commonly used parts of plants are leaves, even roots, seeds, pods, and flowers. Biomolecules such as proteins, amino acids, enzymes, flavonoids, polysaccharides, tannins, terpenoids, polyphenols, etc., presented in plant extracts, act as a reducing, capping and stabilizing agents for the synthesis of silver nanoparticles. Nonetheless, the nucleation and growth mechanisms of AgNPs by these techniques are complex and remain not fully understood. On the other hand, photoinduced synthesis of silver nanoparticles via green methods is a more economic and eco-friendly technique compared to other sources of energy (conventional heating, microwave or ultrasonic irradiation, etc.), [7].

In literature, data on the polyphenol composition can only be found for *Cannabis Sativa* L, while for the other two species of hemp, there is no information on the type and content of these compounds [8]. Therefore, the aim of the present work is on one hand to obtain qualitative and quantitative data on the polyphenolic content of *Cannabis Sativa* L., *Cannabis Indica* and *Cannabis Hybrid*, as well as to make a comparative examination of the results obtained. On the other hand, it would be of both scientific and practical interest to investigate the formation of silver nanoparticles via green approach, using plant extracts of the three cannabis types described above.

EXPERIMENTAL

For determination of the content of tetrahydrocannabinol and polyphenols in *Cannabis Sativa* L., *Cannabis Indica* and *Cannabis Hybrid*, a dry ground mass of leaves and inflorescence (2 g) was used. It was subjected to maceration extraction method with 100 mL ethanol (99.9 %) for seven days at room temperature and stirring. After the end of extraction, the plant mass was separated from the extract by filtering through a filter paper and subsequently - through a MF-Millipore™ Membrane Filter, 0.45 μm pore size.

The THC content in extracts thus obtained was determined using gas chromatography (GC), on an

Agilent 7890A apparatus working at the 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.

Polyphenols content in the three cannabis samples, extracted by maceration method, was determined by high-performance liquid chromatography (HPLC), on an Agilent 1100 HPLC apparatus (Agilent Technologies, California, USA), ensured with diode-array detector (DAD) (G1315B, Agilent Technologies, California, USA). Purospher star, Hiber RT 125-4; RP18, with 125 mm length, 4 mm internal diameter and 5 µm particle size (Purospher star, Merck) was selected as most appropriate column, working at a temperature of 25°C. As an eluent system, 0.1 % trichloroacetic acid (A) and 100 % acetonitrile (B) were employed. The chromatographic system worked in a linear gradient that ensures maximum separation of the analytes: 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 [9, 10].

For the silver nanoparticles synthesis, first, a 5 mM solution of silver nitrate (> 99.8 %, Sigma-Aldrich) in distilled water was prepared by stirring at room temperature for at least half an hour. Afterward, 30 mL of this solution was mixed with 0.5 mL of the plant extract. The obtained mixture was exposed to natural sunlight irradiation for about 20 minutes. At this stage, the colorless solution of the mixture turned to an intense yellow-brown color, characteristic of silver nanoparticles formation. Another series of samples were prepared by heating in a microwave oven (Crown MGZ 2080 EG, 800W, 2450 MHz) using periodic irradiation of 6 seconds, with a time interval of 24 seconds. The mixture color change to yellow was observed after about 10 minutes. The synthesis of silver nanoparticles was proved by a UV-Vis spectrophotometer (T60, PG Instruments Ltd., U.K.). The adopted method can be easily implemented for any kind of scientific or industrial applications due to its cost-effective approach.

RESULTS AND DISCUSSION

Data derived from HPLC-DAD analysis of extracts from *Cannabis Sativa* L., *Cannabis Indica* and *Cannabis Hybrid* are described in Table 1. The type of analytes is determined according to the retention time of each analyte, which corresponds very accurately to the retention time of standard ones, described in the methodology paper [9]. From this table it can be seen that the highest number of polyphenols is contained in technical hemp and the lowest number - in *Cannabis Indica*. The identified polyphenols in *Cannabis Sativa* L. are epigallocatechin, rutin, myricetin, quercetin and kaempferol, of which epigallocatechin stands out with the highest content (44.58 µg mL^{-1}), which is about 10 times higher than most established analytes and about twice greater than rutin (Fig. 1). It is worth noting that there are data in the literature about some polyphenols, also found in *Cannabis Sativa* L. such as catechin,

Table 1. Polyphenol content of *Cannabis Sativa* L., *Cannabis Indica* and *Cannabis Hybrid* extracts.

	Analyte	Retention time (RT), min	Concentration, µg mL^{-1}
Cannabis Sativa L	epigallocatechin	17.65	44.58
	rutin	19.74	19.95
	myricetin	21.48	4.65
	quercetin	23.38	4.12
	kaempferol	24.84	3.15
Cannabis Indica	quercetin	23.39	2.49
	kaempferol	24.93	3.26
Cannabis Hybrid	epigallocatechin	17.68	24.65
	quercetin	23.44	2.22
	kaempferol	24.88	3.60

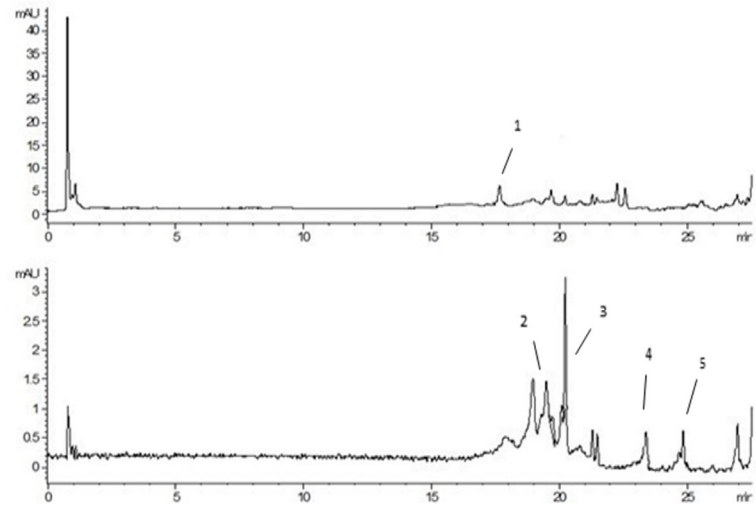

Fig. 1. HPLC chromatogram (upper chromatogram at 278 nm, bottom chromatogram at 368 nm) of the *Cannabis Sativa* L. ethanolic extract, where (1) (-) - epigallocatechin, (2) rutin, (3) myricetin, (4) quercetin, (5) kaempferol.

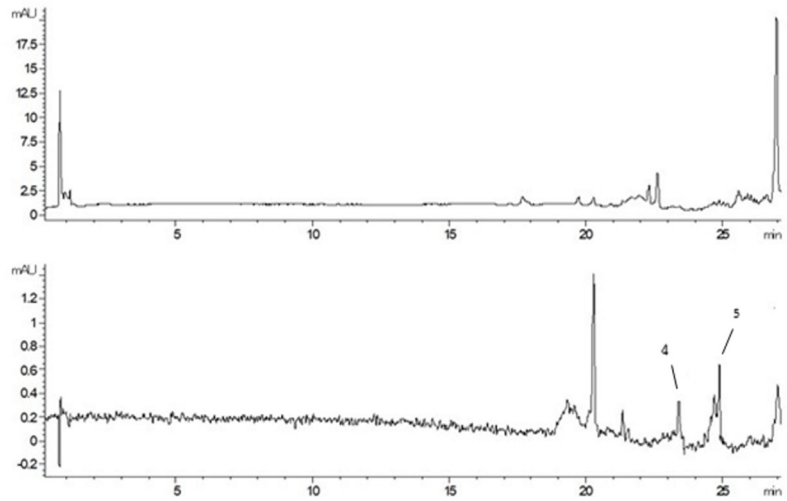

Fig. 2. HPLC chromatogram (upper chromatogram at 278 nm, bottom chromatogram at 368 nm) of the *Cannabis Indica* L. ethanolic extract, where (4) quercetin, (5) kaempferol.

epicatechin, rutin, quercetin and kaempferol [8].

Cannabis Hybrid ranks second in terms of established polyphenols (Table 1). It contains epigallocatechin, quercetin and kaempferol, of which epigallocatechin is again in the largest amount, compared to the other two - about 10 times (Fig. 3). In this case, quercetin has the lowest concentration - 2.22 μg mL^{-1}. Furthermore, as could be seen in Fig. 2, it is impressive that *Cannabis Indica* contains the least number of polyphenols - quercetin and kaempferol (2.49 and 3.26 μg mL^{-1}, respectively, Table 1).

It is worth noting that among five analytes identified, only quercetin and kaempferol are present in all three cannabis plant species investigated, as it is observable that the amount of kaempferol is comparable in all samples. Regarding quercetin, it can be said that its concentrations in *Cannabis Indica* and *Cannabis Hybrid* are comparable, while in technical hemp it is present at about a twice higher concentration (Table 1).

Considering THC quantity data derived from gas chromatographic (GC) measurements of extracts of *Cannabis Sativa* L., *Cannabis Indica* and *Cannabis Hybrid*, it is visible that this analyte is not present in all extracts of three cannabis species.

UV-Vis spectroscopy gives much insight about synthesized silver nanoparticles formed via green

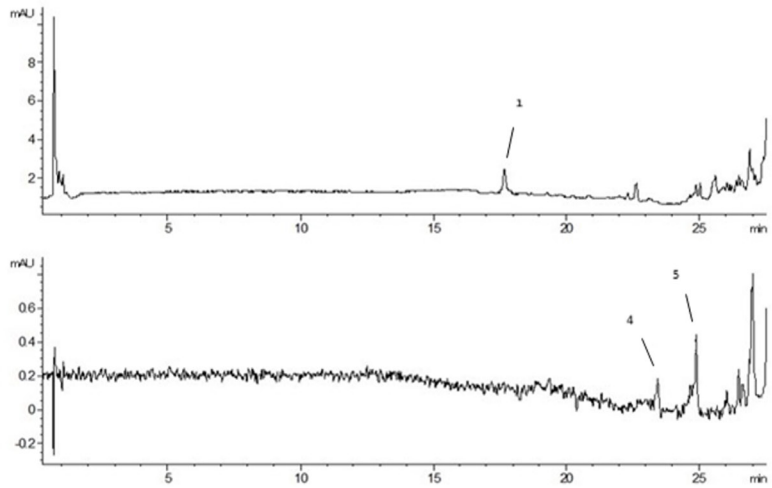

Fig. 3. HPLC chromatogram (upper chromatogram at 278 nm, bottom chromatogram at 368 nm) of the *Cannabis Hybrid* ethanolic extract, where (1) (-) - epigallocatechin, (4) quercetin, (5) kaempferol.

synthetic technique. Fig. 4 compares the results obtained for AgNPs synthesized with 5 mM silver nitrate aqueous solution by using *Cannabis Indica* or *Cannabis Hybrid* extracts, respectively, under 20 minutes of sunlight or 10 minutes of microwave irradiation. For all the samples, an absorption peak began to form at a wavelength of ~ 436 nm. A significant difference was found by comparing the spectra of the AgNPs prepared by microwave and sunlight irradiation. Relatively higher absorbance was obtained due to the higher concentration of silver nanoparticles when using microwave synthesis. Considering the experiments with *Cannabis Sativa* L. extracts, very weak absorption of AgNPs is registered both for microwave and sunlight irradiation, so its values are not given in Fig. 4.

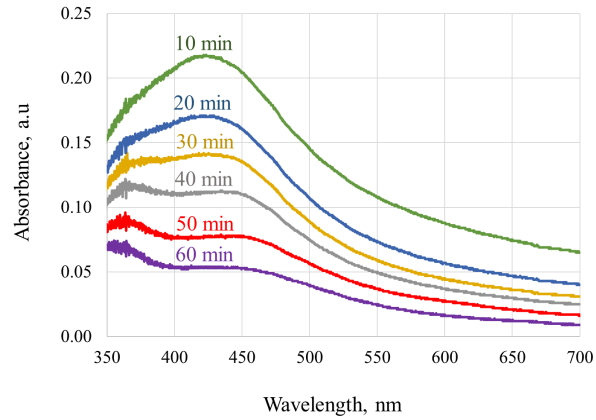

Fig. 4. Comparison of the UV-Vis spectra of AgNPs synthesized with 5 mM silver nitrate and *Cannabis Indica* or *Cannabis Hybrid* extracts.

The results derived verify that *Cannabis Indica* and *Cannabis Hybrid* extracts exhibit significantly better reducing properties than *Cannabis Sativa* L. extracts towards a strong oxidizing agent Ag^{+}. One probable explanation could be the fact that besides the polyphenols identified a large number of other organic compounds acting also as reducing agents are available in the cannabis extracts. Obviously, in *Cannabis Indica* and *Cannabis Hybrid* extracts, some substances having much better reducing power than in *Cannabis Sativa* L. extracts, which results in substantially larger amounts of AgNPs formed.

Furthermore, the evolution of the AgNPs colloidal solution with *Cannabis Sativa* L. seeds extracts (40:60 % vol. EtOH:H_2O, under 1 hour 37 kHz ultrasonication and 50°C heating), was studied by measuring UV-Vis spectra for 1 hour reaction time. The corresponding UV-Vis absorption spectra (Fig. 5) show a surface plasmon absorption band with maxima increasing from 10 minutes to 60 minutes of sunlight exposure. The sunlight irradiation of the reaction mixture containing 5 mM silver nitrate and 0.5 mL plant extract from *Cannabis Sativa* L. seeds results in color changes of the samples from transparent to yellow. The formation of silver nanoparticles increases with increasing irradiation time. The position of the absorption peak depends on the particle size and shape. Small, probably spherical nanoparticles formed after 60 minutes have their

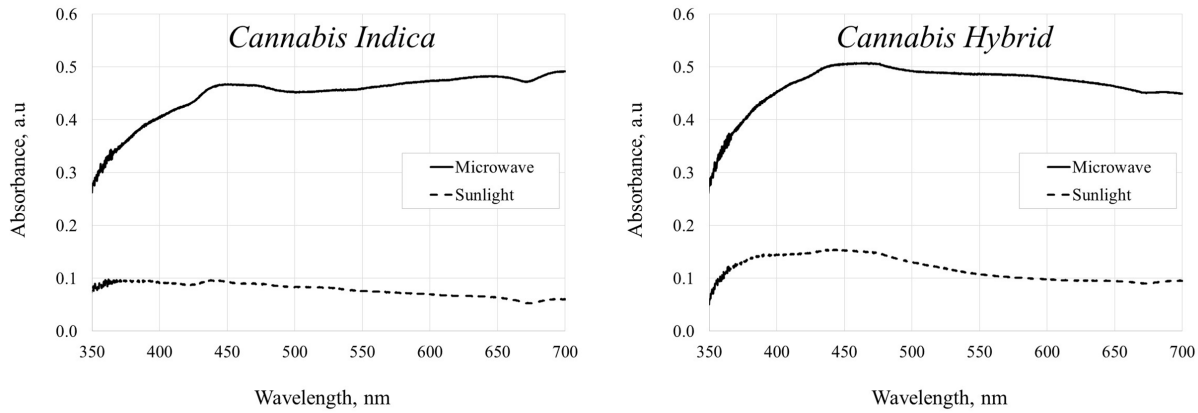

Fig. 5. Evolution of the UV-Vis spectra of AgNPs synthesized by using 5 mM silver nitrate and *Cannabis Sativa* L. seeds extract, for 1 hour of sunlight irradiation.

absorption maxima toward shorter wavelengths at 424 nm compared to bigger particles with absorption maxima at 447 nm after 10 minutes from the start of the experiment.

CONCLUSIONS

In this research, the composition and antioxidant potentials of plant extracts from *Cannabis Sativa* L, *Cannabis Indica* and *Cannabis Hybrid* were analyzed. Results show highest number of polyphenols contained in technical hemp and the lowest number - in *Cannabis Indica*. The extracts show also a reduction and stabilization ability for silver nanoparticles synthesis by using sunlight or microwave irradiation.

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