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Sambucus nigra and *Sambucus racemosa* fruit: a schematic review on chemical characterization

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ABSTRACT

Elderberry is a plant which parts are used for healing purposes. It is rich in polyphenolic compounds (anthocyanins, flavonols, phenolic acids, proanthocyanidins). Black elderberry is the most characterized of all elderberry types. In this paper, the emphasis is on published results about the fruit of black elderberry (*Sambucus nigra*) and red elderberry (*Sambucus racemosa*), as well as different cultivars within these species. The first step in chemical analysis of a plant material is the extraction. It is important to choose the appropriate extraction technique and solvent(s) for the extraction. Spectrophotometric methods enable the determination of total phenol content, total monomeric anthocyanin content, antioxidant activity (ABTS $^{\cdot+}$, DPPH$^{\cdot}$, TEAC, β-carotene / linoleic acid assays). High performance liquid chromatography technique combined with appropriate detectors (for carbohydrates and organic acids: HPLC-PDA; for individual phenolic compounds: HPLC-DAD-MS, HPLC-DAD-ESI-MS-MS; for individual anthocyanins: HPLC-DAD–, HPLC-MS-MS, HPLC-UV-MS-MS, HPLC-DAD-ESI-MS, HPLC-DAD-ESI-MS-MS; for proanthocyanins: HPLC-ESI-MS-MS) provides the results about chemical composition, which were determined. Differences in chemical composition are evident between black and red elderberry, and less within different cultivars of the same species. Values for the total anthocyanin content obtained by using the HPLC method are two or more times higher than those obtained spectrophotometrically. The same can be said for the results for phenolic compounds. Elderberry fruit should be more commercialized since the chemical composition makes it a source of a cosmetically active substances.

Keywords: elderberry, chemical composition, Sambucus nigra, Sambucus racemosa

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Introduction

The history of using herbs for healing purposes is as old as humanity. There are many written documents which confirm this statement. Egyptian papyrus (Vinatoru, 2001), recipes written by Hippocrates, Paracelsus, Dioscorides and others showed the use of plants for medicinal purposes (Paulsen, 2010). One of the oldest and very often used species for medical purpose is elderberry. Since the prehistoric times, all parts of elderberry (root, herb, cortex, leaves, flowers, berries) were used as a healing plant material (Akbulut et al., 2009). In that time, the mankind didn't know about bioactive molecules.

Black elderberry (*Sambucus nigra* L.) and red elderberry (*Sambucus racemosa* L.) belong to the Adoxaceae family (Christensen et al., 2008). Both of these species grow on sunlight-exposed locations. It is interesting that the red elderberry (*S. racemosa*) is the most common and reliable shrub indicator of O_3 phytotoxic effects (Manning, 2005). By using the chemical analyses and appropriate method, it is possible to identify and quantify bioactive compounds. Elderberries are rich in phytochemicals (Thole et al., 2006). Both of mentioned elderberry fruits contain phenolic compounds. Phenolic compounds belong to the class of the bioactive compounds (Mikulic-Petkovsek et al., 2015). Black elderberries contain organic acids, flavonols glycosides (Veberic et al., 2009) and anthocyanins (Wu et al., 2004).

Extraction is a very important step in analyzing of plant species. Before starting an extraction, there are lot of choices to be made – about extraction technique, solvent(s), temperature, extraction time and other extraction parameters. Bioactive compounds can be identified and characterized from various plant parts such as leaves, stem, flower and fruits (Hernandez et al., 2009). For the identification and quantification of chemical compounds spectrophotometric and HPLC methods are the options. The HPLC analyses provide a simultaneous determination of each chemical compound (Zhang et al., 2010).

There are numerous scientific papers about *Sambucus* species. They all contribute to better chemical characterization and understanding of use for healing purposes. *S. nigra* was investigated by scientists more than other *Sambucus* species. The aim of this work was *de facto* to give in one place as much as possible information about chemical composition and characteristics of *S. nigra* and *S. racemosa* fruit, and also different cultivars of these two species, to make easier a future research by scientists interested in this plant material.

Experimental

A guide for the analysis of *S. nigra* and *S. racemosa* fruit is shown in Figure 1. It is formed on the bases of the published results of authors who studied mentioned plant material. The scheme shows an integrated approach for elderberry study. It starts with a literature review. All sequences of the schematic representation will be discussed in more details further in this paper.

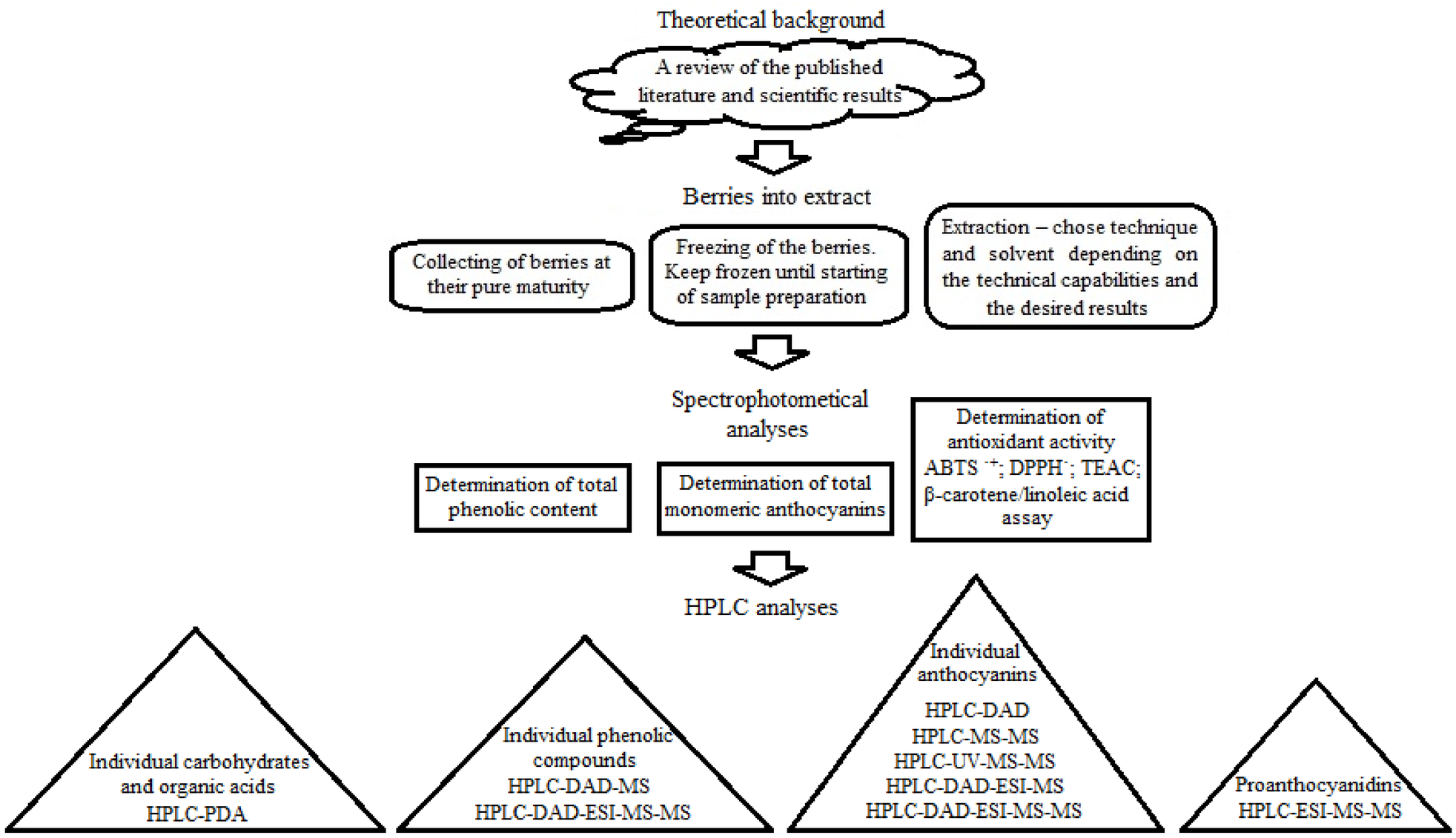

Figure 1. The flow chart of *Sambucus* fruit study

1. Theoretical background

Firstly, published results for *Sambucus* fruits should be studied. Based on the numbers of published papers, *S. racemosa* fruit was less interesting for scientists than *S. nigra*. Generally, there are results of several studies which can characterize *S. nigra* plant material well, but there is not enough published papers on the characterization of *S. racemosa*. In this mini-review paper, several papers with enviable citation were considered and their data for *S. nigra* and *S. racemosa* fruits for different cultivars within the same species are processed.

2. Plant material

The fruits of two *Sambucus* species – *Sambucus nigra* and *Sambucus racemosa* are the focus of this paper. These two species have different genotypes, and the difference between them will be mentioned through the results obtained. All berries have been collected at their full maturity. After that, they were air-dried to constant weight (Dawidowicz et al., 2006; Duymus et al., 2014), frozen at -20 °C (Lee and Finn, 2007; Wu et al., 2004) or -80 °C (Mikulic-Petkovsek et al., 2015). Wu et al. (2004) grinded frozen berries into powder and kept it at -70 °C. Until the beginning of analyses of plant material, it was kept in plastic bags under mentioned conditions.

3. Extraction

The selection of proper extraction method is the basis for further qualitative and quantitative studies of bioactive compounds from plant material (Sasidharan et al., 2011; Smith, 2003). Extraction is the first step of any medicinal plant study; it plays a significant and crucial role on the final result. Extraction methods are sometimes referred as ‘‘sample preparation techniques’’. In the study conducted by Majors (1999), it was confirmed the importance of the sample preparation for analysis.

In order to extract chemical components of interest from the plant material, extraction may play a crucial role in further analyses. Specific nature of the targeted bioactive compounds dictates the selection of solvent system for the extraction. There are many different options for the selection of solvent systems for the extraction from plant material. It is possible to use a single solvent for the extraction, or a mixture. The extraction of hydrophilic compounds is performed using polar solvents such as methanol, ethanol or ethyl-acetate. Target compounds may be non-polar to polar and thermally labile, so the suitability of the methods of extraction must be considered (Sisidharan et al., 2011). The polarity of the targeted compound is the most important factor for the solvent selection. Molecular affinity between the solvent and the solute, mass transfer, use of cosolvent, environmental safety, human toxicity and financial feasibility should also be considered during the selection of the solvent for bioactive compound extraction (Azmir et al., 2013). Since plant extracts usually occur as a combination of various types of bioactive compounds or phytochemicals which have different polarity, the question about their identification, separation and characterization needs to be answered. Extraction efficiency of any conventional method mainly depends on the selection of solvents (Cowan, 1999).

Various methods, such as sonification, heating under reflux, Soxhlet extraction are commonly used (Pharmacopoeia of the People’s Republic of China, 2000; The Japanese Pharmacopeia, 2001; United States Pharmacopeia and National Formulary, 2002) for the plant samples extraction. All these techniques have some common objectives: (a) to extract targeted bioactive compounds from complex plant sample, (b) to increase selectivity of analytical methods (Smith, 2003). Some of these techniques are considered as ‘‘green techniques’’ as they comply with the standards set by Environmental Protection Agency, USA (https://www.epa.gov/greenchemistry/basics-green-chemistry#definition). These techniques include less hazardous chemical synthesis, designing safer chemicals and safe solvents auxiliaries, design for energy efficiency, use of renewable feedstock, reducing of derivatives, catalysis, design to prevent degradation, atom economy, and time analysis for pollution prevention and inherently safer chemistry for the prevention of accidents (Azmir et al., 2013). Deep eutectic solvents are considered as an ideal substitute for conventional solvents and they are used for the extraction of plant material (Paiva et al., 2014).

There are conventional methods and numerous other methods established for the extraction of bioactive compounds from plant material. However, there is no standard method. Factors, such as understanding of the nature of plant matrix and chemistry of bioactive compounds have influence on the efficiency of the extraction method used, regardless of the method applied (Azmir et al., 2013). Table 1 provides an overview of the extraction techniques and parameters used by the researchers who analyzed *Sambucus* fruit.

Table 1. Extraction parameters for *Sambucus* fruit

Extraction method	Solvent	Temperature	Extraction time (in total)	Reference	Applied in the paper
Maceration	Water 70% Ethanol 70% Acetone	Room temperature	5 days (in total)	Duymus et al., 2014; Vlachojannis et al., 2009	Duymus et al., 2014
Solid/liquid extraction	Acidified methanol (0.3% HCl, v/v)	Not mentioned	Until extraction solvent becomes colorless	Anton et al., 2013.	Anton et al., 2013
Pressured liquid extraction	Ethanol-water (80:20 v/v)	20 °C 100 °C	10 minutes	ASE 200 accelerated solvent extractor operator's manual, Document No. 031149, 1995.	Dawidowicz et al., 2006
Ultrasonic extraction	Acidified methanol (0.1% v/v formic acid)	Not mentioned	30 minutes	Rodriguez-Saona & Wrolstad, 2005.	Lee & Finn, 2007
Accelerated solvent extraction	1st hexane: dichloromethane (1:1, Hex, Dc) 2nd acetone:water: acetic acid (70:29.5:0.5, AWA)	70 °C 80 °C	20 minutes	Wu et al., 2004.	Wu et al., 2004
Ultrasonic extraction	MeOH acidified with 3% (v/v) formic acid, containing 1% 2,6-di-*tert*-butyl-4-methylphenol BHT	Cooled water bath	120 minutes	Escarpa & Gonzalez, 2000.	Petkovsek et al., 2007

4. Chemical analyses of berry extracts

Presence of the berry fruits in the human diet as a food and dietary supplement (Haminiuk et al., 2012; Seeram, 2006), also creates a need for the chemical analyses of berry extracts.

4.1. Spectrophotometric analysis

Spectrophotometric analysis is very important as a preliminary step for profiling and quantification of chemical compounds (Escarpa and Gonzalez, 2001). An important analytical disadvantage of direct spectrophotometric measurements could be attributed to the lack of selectivity, mainly because they overestimate the phenolic content (Robards and Antolovich, 1997); nonetheless, the spectrophotometric methods are still widely used in the analytical chemistry labs (Kanner et al., 1994; Sato et al., 1996).

4.1.1. Determination of total phenolic content (TPC)

The polyphenols are chemical compounds which possess the majority of the health benefits of berry fruits (Sidor and Gramza-Michalowska, 2014). They are target of many developed analytical procedures for studying the polyphenolic compounds. The methods used for total polyphenols determination are generally based on Folin-Ciocalteau's phenol reagent and spectrophotometric determination (Milivojevic et al., 2011; Rutz et al., 2012). One of the most cited papers for the determination of total phenolic content is published by Singleton et al. (1999). However, the most successful approaches for phenolic composition have been based on both, spectrophotometric and chromatographic (section 4.2.2.) methods.

Tables 2a and 2b provide an overview of the results for the TPC in *S. nigra* fruit. Results for different cultivars of *S. nigra* fruit are also available. There is no published result for TPC of *S. racemosa* fruit. Results in Table 2a and 2b are expressed as gallic acid equivalent – milligrams of gallic acid (g. a.) per 100 grams of berries.

Table 2a. Total phenolic content data for *S. nigra* fruit spectrophotometrically determined

Plant material	Extraction solvent	TPC in mg g. a./100 g	Reference
S. nigra fruit	Water	8974	Duymus et al., 2014
	70% ethanol	7594	
	Methanol	4917	
	70% acetone	8206	
	Acidified methanol	6399	
	Infusion	6715	

Table 2b. Total phenolic content data spectrophotometrically determined for different cultivars of *S. nigra* fruit in two growing seasons

Plant material	Extraction solvent	TPC in mg g.a./100 g	Reference
S. nigra fruit (cultivar Korsor 2004)	Acidified methanol	387	Lee and Finn, 2007
S. *nigra* fruit (cultivar	Acidified methanol	582	

Korsor 2005)			
S. nigra fruit (cultivar Haschberg 2004)	Acidified methanol	364	
S. nigra fruit (cultivar Haschberg 2005)	Acidified methanol	510	
S. nigra fruit	Acidified methanol	683.1	Mikulic-Petkovsek et al., 2016
S. nigra fruit (cultivar viridis)	Acidified methanol	268.8	

From the results in Table 2a, it is obvious that the extraction solvent has a key impact on the total phenolic content. To achieve the highest total phenolic content in the extract, maceration with water as an extraction solvent is recommended. Results in Table 2b show that the phenolic content in berries is affected by genetic differences (Zadernowski et al., 2005). Environmental conditions, soil composition, pollutions, light, temperature, stress conditions of plant during cultivation, also have an impact on the chemical composition (Tomas-Barberan and Espin, 2001). Identification and quantification of individual phenolic compounds is a topic in the section 4.2.2.

4.1.2. Determination of total monomeric anthocyanin content

Measurements of anthocyanin content, which contributes as the major colorant in berries and berry products, is an indicator of the quality of fresh and processed berry products (Wrolstad et al., 2005). Lee et al. (2005) validated and demonstrated the pH differential method as a simple, quick and accurate for measuring the total monomeric anthocyanin content of a sample. For the total anthocyanin determination there are simple spectrophotometric methods in use. One of the most cited papers on the determination of total monomeric anthocyanins spectrophotometrically is published by Giusti and Wrolstad (2000).

Table 3a shows results for total anthocyanins published by Duymus et al. (2014). Results in Table 3a are expressed as cyanidin-3-glucoside equivalent milligrams of cyanidin-3-glucoside per 100 grams (dry weight) of the extract. Table 3b shows results for total anthocyanins in two different cultivars of *S. nigra* expressed as mg cyanidin-3-glucoside (c-3-g) equivalent per 100 g of berries.

Table 3a. Total monomeric anthocyanins content data for *S. nigra* fruit spectrophotometrically determined

Plant material	**Extraction Solvent**	**TPC in mg c-3-g/100 g**	**Reference**
S. nigra fruit	Water	878.5	Duymus et al., 2014
	70% ethanol	1066.6	
	Methanol	408.6	
	70% acetone	651.1	
	Acidified methanol	600	
	Infusion	734.2	

Table 3b. Total monomeric anthocyanins content data spectrophotometrically determined for different cultivars of *S. nigra* fruit

Plant material	Extraction solvent	TPC in mg c-3-g/100 g	Reference
S. nigra fruit (cultivar Korsor 2004)	Acidified methanol	176	Lee and Finn, 2007
S. *nigra* fruit (cultivar Korsor 2005)	Acidified methanol	343	
S. nigra fruit (cultivar Haschberg 2004)	Acidified methanol	170	
S. nigra fruit (cultivar Haschberg 2005)	Acidified methanol	268	

The highest total monomeric anthocyanins content in extract is obtained when 70% ethanol is used as the extraction solvent for maceration of berries. As well as for the TPC, total monomeric anthocyanins content in extracts is affected by genetic differences (Zadernowski et al., 2005). Black elderberry fruit is rich in biologically active components, primarily polyphenols where anthocyanins belong (Sidor and Gramza-Michalowska, 2014). Cultivar Korsor of *S. nigra* is the richest in total monomeric anthocyanins content in fruit. The identification and quantification of them is of high importance. For that purpose, HPLC analyses are described in section 4.2.3.

4.1.3. Determination of antioxidant activity

Phytochemicals such as phenolic acids, flavonols and anthocyanins are responsible for the antioxidant activity of the fruits (Anton et al., 2013). The antioxidant characteristics of *S. nigra* fruit extracts are in correlation with phytochemicals (Pietta et al., 1992; Rice-Evans et al., 1996).

a) Determination of $ABTS^{\cdot+}$ radical cation scavenging activity

This assay determines the capacity of elderberry extracts to scavenge the $ABTS^{\cdot+}$. Relatively stable blue/green $ABTS^{\cdot+}$ is converting into a colorless product. Discoloration directly reflects the amount of $ABTS^{\cdot+}$ that has been scavenged, and can be measured spectrophotometrically. Trolox equivalent antioxidant capacity (TEAC) is calculated by comparing the scavenging capacity of the tested antioxidant to that of Trolox (Badarinath et al., 2010; Duymus et al., 2014) (Table 4a).

Table 4a. Antioxidant activity of *S. nigra* fruit extracts determined using $ABTS^{\cdot+}$ assay

Plant material	Extraction solvent	mM TroloxL^{-1}	Reference
S. nigra fruit	Water	1.85	Duymus et al., 2014
	70% ethanol	1.52	

Methanol	1.0
70% acetone	1.96
Acidified methanol	0.89
Infusion	1.23

Determination of $ABTS^{\cdot+}$ radical cation scavenging activity was also done (Mikulic-Petkovsek et al., 2016). The results are provided according to the published method of (Re et al., 1999). Results for antioxidant activity of two different black elderberry fruits are shown in Table 4b and expressed as mM Trolox equivalents per kilogram of berries (Mikulic-Petkovsek et al., 2016).

Table 4b. Antioxidant activity of *S. nigra* fruit extracts determined using $ABTS^{\cdot+}$ assay (mM Trolox/kg)

Plant material	**$ABTS^{\cdot+}$ assay**
S. nigra fruit	36.5
S. nigra fruit (cultivar viridis)	3.2

b) DPPH˙ radical scavenging activity

The 1,1-diphenyl-2-picrylhydrazyl (DPPH˙) radical is a stable radical with absorption maximum at 517 nm. Method using DPPH˙ radical for determining of scavenging activity is well known (Brand-Williams et al., 1995). The ability of polyphenolic compounds to act as free radical scavengers against DPPH˙ radical can be expressed as IC_{50} value or % of the residual DPPH˙. IC_{50} values are defined as the concentration required to scavenge 50% of the available free radicals; lower values are the indicators of higher radical scavenging activity.

Duymus et al. expressed their results for DPPH˙ radical scavenging activity as IC_{50} values (µg mL^{-1}). They found that the 70% acetone extract of *S. nigra* fruit was the most active with IC_{50}=117 µgmL^{-1}. The second most active extract was the water extract with IC_{50}=123 µgmL^{-1}. They investigated only these two extracts of *S. nigra* fruit with full details, and the others put results in the following order: 70% acetone > water > 70% ethanol > infusion > acidified methanol > methanol (Duymus et al., 2014). Positive control was ascorbic acid with IC_{50}=8 µg mL^{-1} (94% inhibition of DPPH˙).

The results in Table 2a may be related to the results for antioxidant activity determined by DPPH˙ method. Namely, the antioxidant activity of phenolic compounds shows the neutralization of free radicals initiating oxidation process or the termination of radical chain reaction. It can be concluded that higher total phenol content of extracts contributes to its higher antioxidant activity. However, these two properties are not directly correlated (Dawidowicz et al., 2006). This statement can be confirmed for the pure compounds, but not for plant extracts (Moure et al., 2001).

Table 5 shows results published by Anton et al. (2013) and Dawidowicz et al. (2006) which are arranged to show percentage of inhibition of DPPH˙. Also, Dawidowicz et al. (2006) consider the influence of the temperature at which the extraction of *S. nigra* fruit was carried out.

Table 5. Percentage of inhibition estimated by means of DPPH˙ method

Plant material	Extraction Temperature, °C	IC_{50}, $\mu g ml^{-1}$	Reference
S. nigra fruit	Not mentioned	63.26	Anton et al., 2013
S. nigra fruit	20 °C	50.25	Dawidowicz et al., 2006
S. nigra fruit	100 °C	67.69	

The difference in the antioxidant activity of the same extract as a function of temperature is due to the different content of phytochemicals in it. Precisely, concentration of rutin, isoquercitrin and astragalin in the extracts increases with the increase of the extraction temperature (Dawidowicz et al., 2006).

c) Inhibition of β-carotene/linoleic acid co-oxidation

This spectrophotometric method for the determination of antioxidant activity is based on the ability of extracts to decrease oxidative losses of β-carotene in a β-carotene/linoleic acid emulsion (Tag et al., 1984; Velioglu et al., 1998). Results for this assay obtained by Dawidowicz et al. (2006) are presented in Table 6.

Table 6. Percentage of inhibition estimated by β-carotene/linoleic acid method

Plant material	Extraction temperature °C	Percentage of inhibition	Reference
S. nigra fruit	20 °C	3.87	Dawidowicz et al., 2006
	100 °C	6.63	

Similar to DPPH˙ assay results, results for inhibition of β-carotene/linoleic acid co-oxidation assay showed dependence on temperature. The explanation is the same - higher concentrations of different phytochemicals in the extracts at higher extraction temperature (Dawidowicz et al., 2006).

4.2. HPLC analyses

Identification and quantification of individual chemical components in *Sambucus* fruit is of great importance. There are many analytical procedures with this topic described in the literature. Almost all of them are based on high performance liquid chromatography (HPLC). High performance liquid chromatography (HPLC) is a versatile, robust, and widely used technique for the isolation of natural products (Cannell, 1998). This technique gained popularity as the main choice for identification and quantification of chemical components from plant material, among other analytical techniques (Fan et al., 2006). Usually, a reversed-phase C_{18} column, an UV-VIS detector and a binary solvent system containing acidified water (solvent A) and a polar organic solvent (solvent B), are the chromatographic conditions for the HPLC methods (Gonzalez-Molina et al., 2012; Jakobek and Seruga, 2012; Sellappan et al., 2002;

Veberic et al., 2009). For accurate peak identification, procedures based on LC-MS, HPLC-PDA and HPLC-ESI-MS are developed (Osorio et al., 2012; Tamer 2012; Veberic et al., 2009).

4.2.1. Individual carbohydrates and organic acids – HPLC-PDA

Sugars and organic acids are primary metabolites of *S. nigra* fruit. Their concentrations are important in processing because sugars can be added into final product and organic acids cannot be added afterwards (Veberic et al., 2009).

Sample preparation and detailed description of method is published by Sturm et al. (2003). Veberic et al. (2009) used this method for the analysis of individual carbohydrates and organic acids in *S. nigra* fruit. The column used was Gemini C_{18} (150 × 4.6 mm, 3µm; Phenomenex) operated at 25 °C. As elution solvents 1% formic acid in twice distilled water (A) and 100% acetonitrile (B) were used. The concentrations of individual sugars and organic acids for two cultivars and three selections of *S. nigra* fruit are shown in Table 7 (Veberic et al., 2009).

Table 7. Concentrations of individual sugars and organic acids in *S. nigra* fruit of two cultivars and three selections (g kg^{-1} of fruit weight)

***S. nigra* cultivar/selection**	**Sucrose**	**Fructose**	**Glucose**	**Citric acid**	**Malic acid**	**Shikimic acid**	**Fumaric acid**
Haschberg	1.21	33.99	33.33	4.81	1.10	0.18	0.29
Selection 13	0.47	44.14	42.42	3.11	1.10	0.16	0.14
Selection 14	0.48	45.30	45.17	3.39	1.31	0.14	0.10
Selection 25	1.68	52.25	50.23	3.09	0.97	0.93	0.18
Rubini	1.38	44.12	41.93	3.08	1.02	0.24	0.13

These results were compared with those of other plant species. Black elderberry fruit contains moderate amounts of sugars compared to apple (115-183 g kg^{-1} fruit weight (FW)), and significantly lower amounts of total sugars than sweet cherry (150-230 g kg^{-1} FW). The content level of sugars in sour cherry (90 g kg^{-1} FW) is similar to *S. nigra* fruit (Veberic et al., 2009).

When it comes to organic acids, the total organic acids concentration of all cultivars/selections of *S. nigra* fruit is lower than in apple (6-14 g kg^{-1} FW) and sweet cherry (3.5-8.2 g kg^{-1} FW) (Veberic et al., 2009).

4.2.2. Individual phenolic compounds

Profiling the phenolic content is necessary to examine process-related variability of phenolic composition. The reason why HPLC has been the method of choice is because of its versatility, precision, and relatively low cost. Most frequently, the method is used on the reversed-phase C_{18} or C_8 columns in

conjunction with aqueous mobile phases and methanol, acetonitrile buffers as modifiers (Escarpa and Gonzalez, 2001).

a) HPLC-DAD-MS

Samples were prepared according to the protocol described by Mikulic-Petkovsek et al. (2013) with some modifications. Mikulic-Petkovsek et al. (2015) used this method for the determination of *Sambucus* fruit phenolic composition. The column was a Gemini C_{18} (150 × 4.6 mm, 3 μm; Phenomenex) set on 25 °C. The mobile phase A was aqueous 0.1% formic acid and B 0.1% formic acid in acetonitrile. Samples were eluted according to a gradient described by Wang et al. (2002). The results for qualitative and quantitative analysis of phenolic composition from *S. nigra* (2 different cultivars) and *S. racemosa* fruit are presented in Table 8 (Mikulic-Petkovsek et al., 2015).

Table 8. Phenolic compounds of two different varieties of *S. nigra* and *S. racemosa* fruit. The quantified compounds are expressed as mg kg^{-1} FW

Chemical compound	Fruit of *Sambucus* species			Reference
Cinnamic acid derivatives	*S. nigra* (nigra)	*S. nigra* (viridis)	*S. racemosa* (miquelli)	
3-*O*-Caffeoylquinic acid	88.41	38.85	-*	Mikulic-Petkovsek et al., 2015.
4-*O*-Caffeoylquinic acid 1	28.68	-	-	
4-*O*-Caffeoylquinic acid 2	-	-	4.71	
5-*O*-Caffeoylquinic acid 1	153.80	85.25	150.99	
5-*O*-Caffeoylquinic acid 2	40.13	-	-	
3-Feruloylquinic acid	18.78	16.38	-	
p-coumaric acid hexoside	32.22	9.35	7.56	
Caffeic acid hexoside	-	23.78	5.22	
3-*p*-Coumaroylquinic acid	11.94	19.77	-	
4-*p*-Coumaroylquinic acid 1	10.20	5.78	-	
4-*p*-Coumaroylquinic acid 2	×*	×	-	
Dicaffeoylquinic acid 1	3.43	0.62	0.49	
Dicaffeoylquinic acid 2	2.29	1.94	-	
Flavanols				
Epicatechin	63.71	-	12.43	
Procyanidin dimer 1	-	-	19.87	
Procyanidin trimer 1	-	-	×	
Procyanidin tetramer 2	-	-	×	
Flavonols				
Quercetin 3-*O*-glucoside	43.52	48.18	8.62	
Quercetin 3-*O*-rutinoside	313.30	341.75	2.02	
Quercetin acetylhexoside 1	4.66	29.81	3.55	
Quercetin acetylhexoside 2	×	-	-	
Quercetin hexoside pentoside 1	3.36	-	-	
Quercetin hexoside pentoside 2	×	-	-	
Kaempferol 3-*O*-rutinoside	4.2	52.26	-	
Kaempferol 3-*O*-glucoside	-	×	×	
Kaempferol acetyl hexoside 1	-	×	×	
Isorhamnetin 3-rutinoside	2.03	4.47	-	
Isorhamnetin hexoside 1	-	-	13.23	
Isorhamnetin hexoside 2	-	-	×	
Isorhamnetin acetyl hexoside 1	-	-	×	

Isorhamnetin acetyl hexoside 2	-	-	×
Flavanone			
Naringenin hexoside 1	×	×	-
Naringenin hexoside 2	×	-	-

* "-" – not detected; "×" – present

Mikulic-Petkovsek et al. (2015) performed detailed analysis of *Sambucus* fruit. Quercetin-3-rutinoside and 5-caffeoylquinic acid were the major phenolic compounds in all elderberry species and hybrids which they analyzed. Such a diverse chemical composition of phenolic compounds classifies *S. nigra* fruit as a good source of bioactive and health-promoting food ingredients (Wang and Bohn, 2012).

b) HPLC-DAD-ESI-MS-MS

Firstly, polyphenols were isolated by solid-phase extraction using a C-18 Sep-Pak mini column (Kim and Lee, 2005). This method was used by Lee and Finn (2007) for the determination of individual polyphenolic compounds in *S. nigra* fruit from two cultivars (Korsor, Haschberg) in growing seasons 2004 and 2005. The column was Synergi Hydro-RP 80A˚ (150 × 2 mm, 4 μm) coupled with 4.0 × 3.0 mm guard column (Phenomenex), operated at 25 °C. The results for qualitative and quantitative analysis are presented in Table 9 (Lee and Finn, 2007).

Table 9. Polyphenolic compounds of *S. nigra* fruit from two cultivars (Korsor, Haschber) in two growing seasons.

Chemical compound	**Fruit of *S. nigra* cultivars**		**Reference**
Cinnamic acid derivatives	*S. nigra* (Korsor) 2004/2005	*S. nigra* (Haschberg) 2004/2005	
3-caffeoylquinic Acid	11/44	7/9	
5-caffeoylquinic Acid	264/359	281/347	
4-caffeoylquinic Acid	12/25	16/19	
Flavonols			Lee & Finn, 2007.
Quercetin 3-rutinoside	465/426	727/956	
Quercetin 3-glucoside	95/149	39/52	
Kaempferol 3-rutinoside	7/11	7/12	
Isorhamnetin 3-rutinoside	3/22	7/7	
Isorhamnetin	Traces/3	1/Traces	

Cinnamic acids expressed as chlorogenic acid mg/kg FW, and flavonol glycosides are expressed as rutin mg/kg FW

When results for total phenol contents obtained by spectrophotometric method are compared with those obtained by HPLC (which is taken as a reference method because it is free of interferences), it can be observed that spectrophotometric method overestimates the phenolic content (Escarpa and Gonzalez, 2001).

4.2.3. Individual anthocyanins

Elderberry fruit is mostly used as a fresh food in daily diet, or being processed into other food products and dietary supplements. As a part of the food we eat, it is useful to know anthocyanins composition and contents, which represents the dominant share in elderberry fruit.

Most widely used tool for the identification and quantification of anthocyanins is reversed-phase HPLC coupled with photodiode array detection. The difference in polarity of individual anthocyanins allows their separation. The anthocyanins can be quantitated with an external standard (cyanidin-3-glucoside or any purified anthocyanin standard) (Lee et al., 2008).

a) HPLC-DAD

For analysis of individual anthocyanins Veberic et al. (2009) prepared samples and performed HPLC analysis accordingly to the method previously published by Marks et al. (2007). The column used was Gemini C_{18} (150 × 4.6 mm, 3µm; Phenomenex) operated at 25 °C. As elution solvents 1% formic acid in twice distilled water (A) and 100% acetonitrile (B) were used. The results for anthocyanins of two cultivars and three selections of *S. nigra* fruit expressed as mg cyanidin glucoside equivalents (CGE) per 1 kg of fruit are presented in Table 10.

Table 10. Concentrations of anthocyanins of two cultivars and three selections of *S. nigra* fruit (mg CGE/kg FW)

Chemical compound	**Fruit of *S. nigra* cultivars and selections**					**Reference**
Cinnamic acid derivatives	Haschberg	Selection 13	Selection 14	Selection 25	Rubini	
Cyanidin 3-sambubioside-5-glucoside	332.9	195.2	219.1	534.9	256.3	Veberic et al., 2009
Cyanidin 3,5-diglucoside	94.7	74.1	113.5	232.9	201.8	
Cyanidin 3-sambubioside	3527	2708	3467	5928	6308	
Cyanidin 3-glucoside	3317	4562	2214	2851	5864	
Cyanidin 3-rutinoside	96.3	29.8	14.9	25.2	22.5	

By observing the results, it can be said that black elderberry fruits are rich in anthocyanin content with the domination of cyanidin 3-sambubioside and cyanidin 3-glucoside compared to other fruit varieties (Veberic et al., 2009).

b) HPLC-MS-MS

Sample preparation and HPLC analysis for the determination and quantification of anthocyanins in *S. nigra* fruit was performed as published by Wu et al. (2004). The column used for separation was Zorbax SB-C_{18} A 250 × 4.6 mm. Mobile phase A (5% formic acid aqueous solution) and mobile phase B (methanol) were used as mobile phases in elution process. Obtained results were expressed as anthocyanidin glucoside equivalents (AGE).

Table 11. Concentrations of anthocyanins in *S. nigra* fruit (mg AGE/kg FW)

Chemical compound	Concentration of anthocyanins mg kg^{-1} FW	Reference
Cyanidin 3-sambubioside-5-glucoside	826	Wu et al., 2004.
Cyanidin 3,5-diglucoside	Nit quantified	
Cyanidin 3-sambubioside	5459	
Cyanidin 3-glucoside	7398	
Cyanidin 3-rutinoside	44	
Pelargonidin 3-glucoside	18	
Pelargonidin 3-sambubioside	Trace	

Wu et al. (2004) identified 7 anthocyanins. It should be mentioned that cyanidin 3-rutinoside, pelargonidin 3-glucoside and pelargonidin 3-sambubioside are chemical compounds in black elderberry identified for the first time by Wu et al. (2004).

c) HPLC-UV-MS-MS

For the identification of anthocyanins, Duymus et al. (2014) used this equipment and extracts obtained with different solvents for the extraction of *S. nigra* fruit. The column was 250 × 4.6 mm, 5µm octadecyl silica gel analytical column (Supelco) operating at 40 °C. Solvent A was formic acid/water (8.5/91.5, v/v), and solvent B was tetrahydrofuran/formic acid/acetonitrile/methanol/water (5/8.5/22.5/22.5/41.5, v/v/v/v/v) (Duymus et al., 2014). Details about applied method are published by Bermudez-Soto and Thomas-Barberan (2004). Results for identified anthocyanins in extracts of *S. nigra*

fruit are presented in Table 12. However, Duymus et al. (2014) did not quantify the identified anthocyanins.

Table 12. Anthocyanin composition in different *S. nigra* fruit extracts

Anthocyanin	A	B	C	D	E	F	Reference
Cyanidin 3,5-diglucoside	+	+	+	+	+	+	Duymus et al., 2014
Cyanidin-3-sambubioside-5-glucoside	+	+	+	+	+	+	
Cyanidin 3-glucoside	+	+	+	+	+	+	
Cyanidin 3-sambubioside	+	+	+	+	-	+	
Quercetin-3-rutinoside Na^+ adduct	+	+	+	+	-	+	

A – water extract; B – 70% ethanol extract; C – methanol extract; D – 70% acetone extract; E – acidified methanol extract; F – infusion; "+" – present; "-" – not detected.

d) HPLC-DAD-ESI-MS

For the detailed anthocyanin profile of *S. nigra* and *S. racemosa* fruit analysis, Mikulic-Petkovsek et al. (2014) have used the HPLC-DAD-ESI-MS method. Sample preparation and detailed procedure for applied method are described (Mikulic-Petkovsek et al., 2014). The column was a Gemini C_{18} (150 × 4.6 mm, 3 μm; Phenomenex) operated at 25 °C. The elution solvents were aqueous 0.1% formic acid in double distilled water (A) and 0.1% formic acid in acetonitrile (B). They calculated concentrations of anthocyanins from peak areas of the sample and the corresponding standards and expressed in mg per 1 kg of fresh elderberry fruits.

Tabela 13. Anthocyanin composition of different *Sambucus* species. Some of the identified compounds are quantified (mg/kg FW)

Chemical compound	Fruit of *Sambucus* species			Reference
	***S. nigra* (nigra)**	***S. nigra* (viridis)**	***S. racemosa* (miquelli)**	
Cyanidin-3-*O*-sambubiosyl-5-*O*-glucoside	421.9	0.9	40.8	Mikulic-Petkovsek et al., 2014
Cyanidin-3,5-*O*-diglucoside	59.1	0.2	Not quantified	
Cyanidin-pentoside-hexoside 4	10.8	×	1.7	
Cyanidin-3-*O*-galactoside	3.2	×	×	

Cyanidin-3-*O*-sambubioside	3444.8	10.3	13.6
Cyanidin-3-*O*-glucoside	1906.4	5.5	1.3
Cyanidin-3-*O*-rutinoside	93.6	×	×
Cyanidin-sambubioside-malonylglucoside	×	×	2.3
Pelargonidin-3-*O*-glucoside	Not quantified	×	×
Pelargonidin-3-*O*-sambubioside	Not quantified	×	×
Cyanidin-3-(*Z*)-*p*-coumaroylsambubioside-5-glucoside	×	×	25.9
Cyanidin-3-(*E*)-*p*-coumaroylsambubioside-5-glucoside	×	×	113.1
Cyanidin-3-*p*-coumaroyl-sambubioside	×	×	6.1

” ×” – not detected.

Sambucus fruit from different species shows difference in chemical composition. The species have different anthocyanin and polyphenolic components. But, the cultivars within each species have similar anthocyanin and polyphenolic profiles (Lee and Finn, 2007).

e) HPLC-DAD-ESI-MS-MS

This method was used by Lee and Finn (2007) for the determination of individual anthocyanin compounds in *S. nigra* fruit from two cultivars (Korsor, Haschberg) in growing seasons 2004 and 2005. The column was Synergi Hydro-RP 80A˚ (150 × 2 mm, 4 µm) coupled with 4.0 × 3.0 mm guard column (Phenomenex), operated at 25 °C. The results for qualitative and quantitative analysis are presented in Table 14 (Lee and Finn, 2007).

Table 14. Anthocyanin composition of fruit of different *Sambucus nigra* cultivars. Some of the identified compounds are quantified (mg kg^{-1} FW)

Chemical compound	**Fruit of *S. nigra* cultivars**		**Reference**
Anthocyanin	*S. nigra* (Korsor) 2004/2005	*S. nigra* (Haschberg) 2004/2005	
Cyanidin 3-sambubioside-5-glucoside	160/373	322/592	Lee & Finn, 2007.
Cyanidin 3,5-diglucoside	82/183	112/195	
Cyanidin 3-Sambubioside	1222/2691	2537/2681	
Cyanidin 3-glucoside	2537/4814	2046/3097	
Cyanidin	Not detected	Trace	

3-rutinoside		
Pelargonidin 3-glucoside	Trace	Trace
Cyanidin based Anthocyanin	Not detected	Not detected
Delphinidin 3-rutinoside	Not detected	Trace
Cyanidin 3-(*Z*)-pcoumaroylsambubioside-5-glucoside	Not detected	Not detected
Cyanidin 3-*p*-coumaroyl glucoside	Not detected	Not detected
Petunidin 3-rutinoside	Not detected	Not detected
Cyanidin-3-(*E*)-pcoumaroylsambubioside-5-glucoside	Not detected	Not detected
Cyanidin 3-coumaroyl sambubioside	Not detected	Not detected

Overall, values from the HPLC are higher than anthocyanin content obtained by the pH differential method. This phenomenon is observed before. Measurements examined by HPLC were even 2 times higher than the values obtained by pH differential method (Lee and Finn, 2007). Despite that, results obtained for total anthocyanin content by using both methods are significantly correlated (Lee et al., 2008).

4.2.4. Proanthocyanidins – HPLC-ESI-MS-MS

Proanthocyanidins are defined as oligomeric and polymeric flavan-3-ols. Polymerization degree describes the size of proanthocyanidins molecules (Porter, 1994). Procyanidins or prodelphinidins are names for those proanthocyanidins which contains (epi)catechin or (epi)gallocatechin as subunits. Anthocyanins and proanthocyanidins are one of the dominant phytochemicals in berries (Gu et al., 2004), both of which have been shown to be effective antioxidants (Wang et al., 1997).

Identification and quantification of proanthocyanidins in *S. nigra* fruit was done by Wu et al. (2004) according to the methods described previously by Gu et al. (2004). The column used for separation was Zorbax SB-C_{18} A 250 × 4.6 mm. Elution was performed using mobile phase A (5% formic acid aqueous solution) and mobile phase B (methanol). Results of Wu et al. (2004) about quantities of proanthocyanidins are presented in Table 15 and expressed as mg kg^{-1} FW.

Table 15. Concentrations of proanthocyanidins in *S. nigra* fruit (mg kg^{-1} FW)

Proanthocyanidins	Concentration of proanthocyanins mg kg^{-1} FW	Reference
Monomers	14.4	Wu et al., 2004
Dimers	106.2	
Trimers	56.3	
4-6-mers	108	
7-10-mers	Not detected	
>10-mers	Not detected	
Total	233	
Type	Procyanidins, prodelphinidins	

Conclusion

Sambucus nigra and *Sambucus racemosa* are two elderberry species with several cultivars within. *S. nigra* was much more attractive for examination than *S. racemosa*. Scientists described black elderberry in more details because of its use as a processed food or dietary supplement. Although *S. nigra* fruit is much richer with phytochemicals within, *S. racemosa* fruit should be examined more. Extraction conditions, extraction solvent(s), make a significant contribution to the chemical composition of elderberry extracts. So far, all extractions that have been made have been done with conventional solvents. Deep eutectic solvents are is use for extraction of plant material, so, scientific community is waiting for its application for the extraction of *Sambucus* fruit. Spectrophotometric analytical methods provide valuable information for characterization of plant material. For determination of chemical composition of individual sugars, organic acids, phenol components, anthocyanins, proanthocyanidins, there are different methods available. Different HPLC apparatus in combination with appropriate detector provides detailed simultaneously multicomponent analysis. *Sambucus nigra* and *Sambucus racemosa* fruit should be commercialized more. The author's suggestion is that, basic on the chemical composition, *Sambucus* fruit extract is a source for an active component in cosmetic products.

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Conflict-of-Interest Statement

There are no conflicts of interest in this review article.

References

Akbulut, M., Ercisli, S., & Tosun, M. (2009). Physico-chemical characteristics of some wild grown European elderberry (*Sambucus nigra* L.) genotypes. Pharmacognosy Magazine, 5, 20, 320–323.

Anton, A. M., Pintea, A. M., Rugina, D. O., Sconta, Z. M., Hanganu, D., Vlase, L., & Benedec, D. (2013). Preliminary studies on the chemical characterization and antioxidant capacity of polyphenols from *Sambucus sp.* Digest Journal of Nanomaterials and Biostructures, 8, 3, 973–980.

ASE, 200 Accelerated Solvent Extractor Operator's Manual. (1995). Document No. 031149, Revision 01, Dionex, Sunnyvale, CA, Sect. 3–5.

Azmir, J., Zaidul, I. S. M., Rahman, M. M., Sharif, K. M., Mohamed, A., Sahena, F., Jahurul, M. H. A., Ghafoor, K., Norulaini, N. A. N., & Omar, A. K. M. (2013). Techniques for extraction of bioactive compounds from plant materials: A review. Journal of Food Engineering, 117, 426–436.

Badarinath, A. V., Mallikarjuna Rao, K., Madhu Sudhana Chetty, C., Ramkanth, S., Rajan, T. V. S., & Gnanaprakash, K. (2010). A review on in-vitro antioxidant methods: Comparisons, correlations and considerations. International Journal of PharmTech Research, 2, 2, 1276–1285.

Bermudez-Soto, M. J., & Thomas-Barberan, F. A. (2004). Evaluation of commercial red fruit juice concentrates as ingredients for antioxidant functional juices. European Food Research and Technology, 219, 133-141.

Brand-Williams, W., Cuvelier, M. E., & Berset, C. (1995). Use of a free radical method to evaluate antioxidant activity. Lebensmittel – Wissenschaft und Technologie, 28, 25–30.

Cannell, R. J. P. (1998). Natural Products Isolation (pp. 165–208). New Jersey: Human Press Inc.

Christensen, L., Kaack, K., & Frette, X. (2008). Selection of elderberry (*Sambucus nigra* L.) genotypes best suited for the preparation of elderflower extracts rich in flavonoids and phenolic acids. European Food Research and Technology, 227, 1, 293–305.

Cowan, M. M. (1999). Plant products as antimicrobial agents. Clinical Microbiology Reviews, 12, 4, 564–582.

Dawidowicz, A. L., Wianowska, D., & Baraniak, B. (2006). The antioxidant properties of alcoholic extracts from *Sambucus nigra* L. (antioxidant properties of extracts). Lwt – Food Science and Technology, 39, 3, 308–315.

Duymus, H. G., Göger, F., & Husnu Can Baser, K. (2014). *In vitro* antioxidant properties and anthocyanin compositions of elderberry extracts. Food Chemistry, 155, 112–119.

Enviromental Protection Agency, USA. <https://www.epa.gov/greenchemistry/basics-green-chemistry#definition >.

Escarpa, A., & Gonzalez, M. C. (2001). Approach to the content of total extractable phenolic compounds from different food samples by comparison of chromatographic and spectrophotometric methods. Analytica Chimica Acta, 427, 119–127.

Fan, X. H., Cheng, Y. Y., Ye, Z. L., Lin, R. C., & Qian, Z. Z. (2006). Multiple chromatographic fingerprinting and its application to the quality control of herbal medicines. Analytica Chimica Acta, 555, 217–224.

Giusti, M. M., & Wrolstad, R. E. (2000). Anthocyanins: Characterization and measurement with UV-visible spectroscopy. In R. E. Wrostad (Ed.), Current protocols in food analytical chemistry (pp. 1–13). New York, NY, Unit F1.2: John Wiley & Sons.

Gonzalez-Molina, E., Girones-Vilaplana, A., Mena, P., Moreno, D. A., & Garıa-Viguera, C. (2012). New beverages of lemon juice with elderberry and grape concentrates as a source of bioactive compounds. Journal of Food Science, 77, 6, C727–C733.

Gu, L., Kelm, M. A., Hammerstone, J. F., Beecher, G., Holden, J., Haytowitz, D., & Prior, R. L. (2004). Concentrations of oligomeric and polymeric of flavan-3-ols (proanthocyanidins) in common and infant foods and estimation of normal consumption. Journal of Nutrition, 134, 613–617.

Haminiuk, C. W. I., Maciel, G. M., Plata-Oviedo, M. S. V., & Peralta, R. M. (2012). Phenolic compounds in fruits—an overview. International Journal of Food Science & Technology, 47, 10, 2023–2044.

Hernandez, Y., Lobo, M. G., & Gonzalez, M. (2009). Factors affecting sample extraction in the liquid chromatographic determination of organic acids in papaya and pineapple. Food Chemistry, 114, 2, 734–741.

Jakobek, L., & Seruga, M. (2012). Influence of anthocyanins, flavonols and phenolic acids on the antiradical activity of berries and small fruits. International Journal of Food Properties, 15, 1, 122–133.

Kanner, J., Frankel, E., Granit, R., German, B., & Kinsella, J. E. (1994). Natural antioxidants in grapes and wines. Journal of Agricultural and Food Chemistry, 42, 1, 64–69.

Lee, J., Durst, R. W., & Wrolstad, R. E. (2005). Determination of total monomeric anthocyanin pigment content of fruit juices, beverages, natural colorants, and wines by the pH differential method: Collaborative study. Journal of the AOAC International, 88, 1269–1278.

Lee, J., & Finn, C. E. (2007). Anthocyanins and other polyphenolics in American elderberry (*Sambucus canadensis*) and European elderberry (*S. nigra*) cultivars. Journal of the Science of Food and Agriculture, 87, 2665–2675.

Lee, J., Rennaker, C., & Wrolstad, R. E. (2008). Correlation of two anthocyanin quantification methods: HPLC and spectrophotometric methods. Food Chemistry, 110, 782–786.

Majors, R. E. (1999). An overview of sample preparation methods for solids. LC-GC Europe, 17, 6, 8–13.

Manning, W. J. (2005). Pinus cembra, a long term bioindicator for ambient ozone in subalpine regions of the Carpathian Mountains. Polish Botanical Studies, 19, 59–64.

Marks, S. C., Mullen, W., & Crozier, A. (2007). Flavonoid and chlorogenic acid profiles of English cider apples. Journal of Science of Food and Agriculture, 87, 719–728.

Mikulic-Petkovsek, M., Schmitzer, V., Slatnar, A., Todorovic, B., Veberic, R., Stampar, F., & Ivancic, A. (2014). Investigation of anthocyanin profile of four elderberry species and interspecific hybrids. Journal of Agricultural and Food Chemistry, 62, 24, 5573–5580.

Mikulic-Petkovsek, M., Ivancic, A., Todorovic, B., Veberic, R., & Stampar, F. (2015). Fruit phenolic composition of different elderberry species and hybrids. Journal of Food Science, 80, 10, C2180–C2190.

Mikulic-Petkovsek, M., Ivancic, A., Schmitzer, V., Veberic, R., & Stampar, F. (2016). Comparison of major taste compounds and antioxidative properties of fruits and flowers of different Sambucus species and interspecific hybrids. Food Chemistry, 200, 134–140.

Milivojevic, J., Maksimovic, V., Nikolic, M., Bogdanovic, J., Maletic, R., & Milatovic, D. (2011). Chemical and antioxidant properties of cultivated and wild fragaria and rubus berries. Journal of Food Quality, 34, 1, 1–9.

Moure, A., Franco, D., Sineiro, J., Dominguez, H., Nunez, M. J., & Lema, J. M. (2001). Antioxidant activity of extracts from *Gevuina avellana* and *Rosa rubiginosa* defatted seeds. Food Research International, 34, 103–109.

Osorio, C., Hurtado, N., Dawid, C., Hofmann, T., HerediaMira, F. J., & Morales, A. L. (2012). Chemical characterisation of anthocyanins in tamarillo (*Solanum betaceum* Cav.) and Andes berry (*Rubus glaucus* Benth.) fruits. Food Chemistry, 132, 4, 1915–1921.

Paiva, A., Craveiro, R., Aroso, I., Martins, M., Reis, R. L. & Duarte, A. R. C. (2014). Natural deep eutectic solvents−solvents for the 21st century. ACS Sustainable Chemistry & Engineering, 2, 5, 1063–1071.

Paulsen, B. S. (2010). Highlights through the history of plant medicine. In: Proceedings from a Symposium Held at The Norwegian Academy of Science and Letters, Oslo, Norway.

Porter, L. J. (1994). Flavans and proanthocyanidins. In J. B. Harbone (Ed.), The flavonoids (pp. 23–53). Chapman and Hall: London, U.K.

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., & Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radical Biology and Medicine, 26 (9–10), 1231–1237.

Robards, K., & Antolovich, M. (1997). Analytical chemistry of fruit bioflavonoids a review. Analyst, 122, 2, 11R–34R.

Rutz, J. K., Voss, G. B., & Zambiazi, R. C. (2012). Influence of the degree of maturation on the bioactivecompounds in backberry (*Rubus spp.*) cv. Tupy. Food and Nutrition Sciences, 03, 10, 1453–1460.

Sasidharan, S., Chen, Y., Saravanan, D., Sundram, K. M., & Yoga Latha, L. (2011). Extraction, isolation and characterization of bioactive compounds from plant's extracts. African Journal of Traditional, Complementary and Alternative Medicines: AJTCAM, 8, 1, 1–10.

Sato, M., Ramarathnam, N., Suzuki, Y., Ohkubo, T., Takeuchi, M., & Ochi, H. (1996). Varietal differences in the phenolic content and superoxide radical scavenging potential of wines from different sources. Journal of Agricultural and Food Chemistry, 44, 1, 37–41.

Seeram, N. P. (2006). Berries. In D. Heber, G. Blackburn, G. V. L. W. Go, J. Milner (Eds.), Nutritional Oncology (pp. 615–625), 2nd edition, London: Academic Press.

Sellappan, S., Akoh, C. C., & Krewer, G. (2002). Phenolic compounds and antioxidant capacity of Georgia-grown blueberries and blackberries. Journal of Agricultural and Food Chemistry, 50, 8, 2432–2438.

Sidor, A., & Gramza-Michalowska, A. (2014). Advanced research on the antioxidant and health benefit of elderberry (*Sambucus nigra*) in food – A review. Journal of Functional Foods, 18, B, 941–958.

Singleton, V. L., Orthofer, R., & Lamuela-Raventos, R. M. (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin–Ciocalteu reagent. Methods in Enzymology, 299, 152-178.

Smith, R. M. (2003). Before the injection-modern methods of sample preparation for separation techniques. Journal of Chromatography A, 1000, 1–2, 3–27.

Tamer, C. E. (2012). A research on raspberry and blackberry marmalades produced from different cultivars. Journal of Food Processing and Preservation, 36, 1, 74–80.

Thole, J. M., Kraft, T. F. B., Sueiro, L. A., Kang, Y. –H., Gills, J. J., Cuendet, M., Pezzuto, J. M., Seigler, D. S., & Lila, M. A. A. (2006). A comparative evaluation of the anticancer properties of European and American elderberry fruits. Journal of Medicinal Food, 9, 498−504.

Veberic, R., Jakopic, J., Stampar, F., & Schmitzer, V. (2009). European elderberry (*Sambucus nigra* L.) rich in sugars, organic acids, anthocyanins and selected polyphenols. Food Chemistry, 114, 511–515.

Velioglu, Y. S., Mazza, G., Gao, L., & Oomah, B. D. (1998). Antioxidant activity and total phenolics in selected fruits, vegetables and grain products. Journal of Agriculture and Food Chemistry, 46, 10, 4113–4117.

Vinatoru, M. (2001). An overview of the ultrasonically assisted extraction of bioactive principles from herbs. Ultrasonics Sonochemistry, 8, 3, 303–313.

Vlachojannis, J. E., Cameron, M., & Chrubasik, S. (2009). A systematic review on the *Sambuci fructus* effect and efficacy profiles. Phytotherapy Research, 24, 1–8.

Wang, H., Cao, G., & Prior, R. L. (1997). The oxygen radical absorbing capacity of anthocyanins. Journal of Agricultural and Food Chemistry, 45, 304–309.

Wang, S. Y., Zheng, W., & Galletta, G. J. (2002). Cultural system affects fruit quality and antioxidant capacity in strawberries. Journal of Agricultural and Food Chemistry, 50, 6534–6542.

Wang, L., & Bohn, T. (2012). Health-promoting food ingredients and functional food processing. In J. Bouayed (Ed.), Nutrition, well-being and health (pp. 201–224), Croatia: InTech.

Wrolstad, R. E., Durst, R. W., & Lee, J. (2005). Tracking color and pigment changes in anthocyanin products. Trends in Food Science and Technology, 16, 423–428.

Wu, X., Gu, L., Prior, R. L., & McKay, S. (2004). Characterization of anthocyanins and proanthocyanidins in some cultivars of Ribes, Aronia, and Sambucus and their antioxidant capacity. Journal of Agricultural and Food Chemistry, 52, 7846–7856.

Zadernowski, R., Naczk, M., & Nesterowicz, J. (2005). Phenolic acid profile in some small berries. Journal of Agricultural and Food Chemistry, 53, 6, 2118–2124.

Zhang, T., Zhu, M., Chen, X., & Bi, K. (2010). Simultaneous analysis of seven bioactive compounds *in Sambucus chinensis* Lindl by HPLC. Analytical Letters, 43, 2525–2533.

Sambucus nigra i *Sambucus racemosa* plod: šematski prikaz hemijske karakterizacije

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SAŽETAK

Zova je biljka čiji se svi delovi koriste u svrhu lečenja. Po sadržaju hemijskih jedinjenja bogata je polifenolnim jedinjenjima (antocijanima, flavonolima, fenolnim kiselinama, proantocijanidinima). Crna zova je najbolje okarakterisana od svih vrsti zova. U ovom radu akcenat je na objavljenim rezultatima koji se tiču ploda crne zove (*Sambucus nigra*) i crvene zove (*Sambucus racemosa*), kao i genotipova unutar ovih vrsta. Prvi korak u hemijskoj analizi biljnog materijala je njegova ekstrakcija. Važno je izabrati odgovarajuću tehniku ekstrakcije i rastvarač(e) za ekstrakciju. Spektrofotometrijskim metodama određen je sadržaj ukupnih fenola, sadržaj ukupnih monomernih antocijana, antioksidativna aktivnost ($ABTS^{\cdot+}$, $DPPH^{\cdot}$, TEAC, β-karoten/linolna kiselina testovima). Tehnikom tečne hromatografije visokih performansi u kombinaciji sa odgovarajućim detektorima (za ugljene hidrate i organske kiseline: HPLC-PDA; za pojedinačna fenolna jedinjenja: HPLC-DAD-MS, HPLC-DAD-ESI-MS-MS; za pojedinačne antocijane: HPLC-DAD, HPLC-MS-MS, HPLC-UV-MS-MS, HPLC-DAD-ESI-MS, HPLC-DAD-ESI-MS-MS; za proantocijane: HPLC-ESI-MS-MS) određen je tačan hemijski sastav. Razlike u hemijskom sastavu su evidentne između crne i crvene zove, a manje među različitim sortama iste vrste. Primenom HPLC metode dobijaju se vrednosti za sadržaj ukupnih antocijana koje su dva ili više puta veće od onih dobijenih spektrofotometrijski. Isto se može reći i za rezultate određivanja fenolnih jedinjenja. Plod zove treba da bude više iskorišćen u komercijalne svrhe, a hemijski sastav ga kandiduje za kozmetički aktivnu supstancu.

Ključne reči*: zova, hemijski sastav, *Sambucus nigra*, *Sambucus racemosa

Sambucus nigra et *Sambucus racemosa* fruit : une revue schématique de la caractérisation chimique

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Résumé

Le sureau est une plante dont toutes les parties sont utilisées à des fins de guérison. Il est riche en composés polyphénoliques (anthocyanes, flavonols, acides phénoliques, proanthocyanidines). Le sureau noir est le plus caractérisé de tous les types de sureau. Dans cet article, l'accent est mis sur les résultats déjà publiés sur les fruits du sureau noir (*Sambucus nigra*) et du sureau rouge (*Sambucus racemosa*), ainsi que sur les différents cultivars au sein de ces espèces. La première étape de l'analyse chimique d'un matériel végétal est son extraction. Il est important de choisir la technique appropriée d'extraction et le(s) solvant(s) convenable(s) pour l'extraction. Les méthodes spectrophotométriques permettent de déterminer la teneur totale en phénol, la teneur totale en anthocyanes monomères et l'activité antioxydante (ABTS ˙ +, DPPH˙, TEAC, dosages β-carotène/acide linoléique). La technique de chromatographie liquide à haute performance combinée avec les détecteurs appropriés (pour les glucides et les acides organiques : HPLC-PDA ; pour les composés phénoliques individuels : HPLC-DAD-MS, HPLC-DAD-ESI-MS-MS; pour les anthocyanes individuelles: HPLC-DAD–, HPLC-MS-MS, HPLC-UV-MS-MS, HPLC-DAD-ESI-MS, HPLC-DAD-ESI-MS-MS; pour les proanthocyanines: HPLC-ESI-MS-MS) définit précisément la composition chimique. Les différences dans la composition chimique entre le sureau noir et le sureau rouge sont évidentes, tandis que celles entre les différents cultivars d'une même espèce sont moins apparentes. Les valeurs de la teneur totale en anthocyanes obtenues en utilisant la méthode HPLC sont deux fois ou beaucoup plus élevées que celles obtenues par spectrophotométrie. Il en va de même pour les résultats des composés phénoliques. Le fruit du sureau doit être plus utilisé à des fins commerciales et sa composition chimique en fait une source de substances cosmétiquement actives.

Mots-clés: sureau, composition chimique, *Sambucus nigra*, *Sambucus racemosa*.

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Плоды *Sambucus nigra* и *Sambucus racemosa*: схематический обзор химической характеристики

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Резюме

Бузина-это растение, части которого используются в лечебных целях. Он богат полифенольными соединениями (антоцианы, флавонолы, фенольные кислоты, проантоцианидины). Черная бузина наиболее характерна для всех видов бузины. В этой статье основное внимание уделяется опубликованным результатам о плодах черной бузины (*Sambucus nigra*) и красной бузины (*Sambucus racemosa*), а также о различных сортах этих видов. Первым этапом химического анализа растительного материала является экстракция. Важно выбрать подходящий метод экстракции и растворитель (и) для экстракции. Спектрофотометрические методы позволяют определять общее содержание фенола, общее содержание мономерного антоциана, антиоксидантную активность (ABTS $^{\cdot+}$, DPPH$^{\cdot}$, TEAC, β-каротин/линолевая кислота). Метод высокоэффективной жидкостной хроматографии в сочетании с соответствующими детекторами (для углеводов и органических кислот: ВЭЖХ-КПК; для отдельных фенольных соединений: ВЭЖХ-DAD-MS, ВЭЖХ-DAD-ESI-MS-MS; для отдельных антоцианов: ВЭЖХ-DAD-, ВЭЖХ-МС-МС, ВЭЖХ-УФ-МС-МС, ВЭЖХ-DAD-ESI-MS, ВЭЖХ-DAD-ESI-MS-MS; для проантоцианинов: ВЭЖХ-ESI-MS-MS) дает результаты о химическом составе, которые были определены. Различия в химическом составе очевидны между черной и красной бузиной и реже у разных сортов одного и того же вида. Значения общего содержания антоцианов, полученные с помощью метода ВЭЖХ, в два или более раз выше, чем те, которые получены спектрофотометрически. То же самое можно сказать и о результатах для фенольных соединений. Плоды бузины должны быть более коммерциализированными, так как химический состав делает их источником косметически активных веществб.

Ключевые слова: бузина, химический состав, Sambucus nigra, Sambucus racemosa

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Sambucus nigra- und *Sambucus racemosa*-Früchte: eine schematische Übersicht über die chemische Charakterisierung

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ABSTRAKT

Holunder ist eine Pflanze, deren Teile zu Heilzwecken verwendet werden. Es ist reich an Polyphenolverbindungen (Anthocyane, Flavonole, Phenolsäuren, Proanthocyanidine). Schwarzer Holunder ist der am meisten charakterisierte Holunder. In diesem Artikel liegt der Schwerpunkt auf veröffentlichten Ergebnissen über die Früchte der schwarzen Holunderbeere (*Sambucus nigra*) und der roten Holunderbeere (*Sambucus racemosa*) sowie über verschiedene Sorten innerhalb dieser Arten. Der erste Schritt bei der chemischen Analyse eines Pflanzenmaterials ist die Extraktion. Es ist wichtig, die geeignete Extraktionstechnik und die Lösungsmittel für die Extraktion auszuwählen. Spektrophotometrische Methoden ermöglichen die Bestimmung des Gesamtphenolgehalts, des gesamten Gehalts der monomeren Anthocyane und der Antioxidationsaktivität (ABTS+, DPPH˙, TEAC, β-Carotin/ Linolsäure-Assays). Unter Anwendung der Hochleistungsflüssigkeitschromatographie, kombiniert mit geeigneten Detektoren (für Kohlenhydrate und organische Säuren: HPLC-PDA; für einzelne phenolische Verbindungen: HPLC-DAD-MS, HPLC-DAD-ESI-MS-MS; für einzelne Anthocyane: HPLC-DAD–, HPLC-MS-MS, HPLC-UV-MS-MS, HPLC-DAD-ESI-MS, HPLC-DAD-ESI-MS-MS; für Proanthocyanine: HPLC-ESI-MS-MS) wurde die genaue chemische Zusammensetzung bestimmt. Unterschiede in der chemischen Zusammensetzung zwischen schwarzen und roten Holunderbeeren sind offensichtlich, und unter verschiedenen Sorten derselben Art weniger. Die Werte für den Gesamtgehalt an Anthocyanen, die unter Verwendung der HPLC-Methode erhalten wurden, sind zwei- oder mehrmals höher als diejenigen, die spektrophotometrisch erhalten wurden. Das Gleiche gilt für die Ergebnisse für Phenolverbindungen. Holunderfrüchte sollten stärker kommerzialisiert werden, da sie aufgrund ihrer chemischen Zusammensetzung eine Quelle für kosmetisch wirksame Substanzen darstellen.

Schlüsselwörter: Holunder, chemische Zusammensetzung, Sambucus nigra, Sambucus racemosa

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Chemical composition and biological activities of *Umbilicaria crustulosa* and *Umbilicaria cylindrica* extracts

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ABSTRACT

The main compounds of acetone, methanol, ether and ethyl acetate extracts of *Umbilicaria crustulosa* are tridepsides, gyrophoric acid and crustinic acid, and didepside, lecanoric acid. Chemical composition of *Umbilicaria cylindrica* extracts depends on the solvent used for the extraction and the major components are depsidones, salazinic acid and norstictic acid or depsides, gyrophoric acid and atranorin. Extracts of *U. crustulosa* and *U. cylindrica* have shown antibacterial, antioxidant, cytotoxic, antiproliferative and anticlastogenic activity. Acetone extracts of *U. crustulosa* and *U. cylindrica* are promising candidates for *in vivo* experiments considering antioxidant and anticlastogenic activity.

Keywords: Umbilicaria crustulosa, Umbilicaria cylindrica, antioxidant activity, antimicrobial activity, cytotoxic activity, micronucleus test, cholinesterase inhibition

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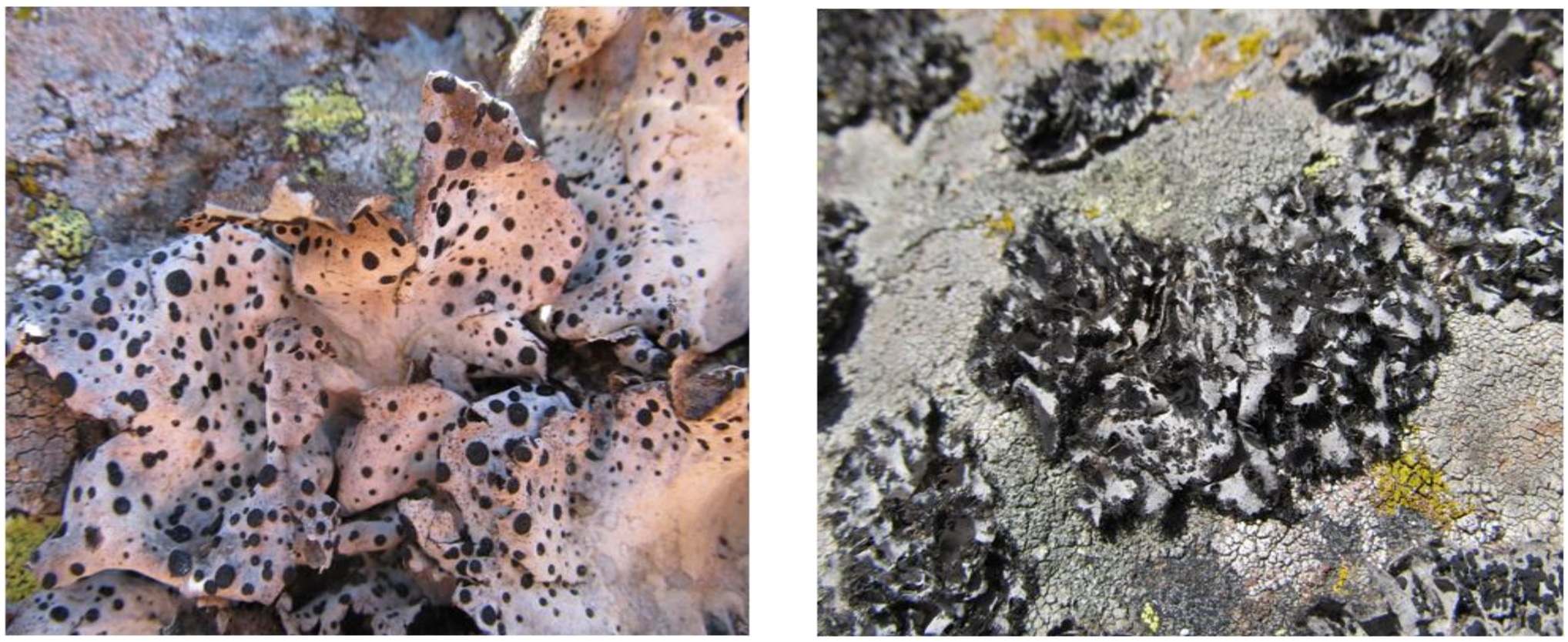
Introduction

Lichen represents symbiotic organisms consisted of algae (photobionts) which are responsible for photosynthesis and fungi (mycobionts) which produce specific secondary metabolites. Lichens are potential natural source of bioactive compounds which are used in folk medicine during centuries (Molnar and Farakas, 2010). *Umbilicaria* is a genus of lichenised fungi in the phylum Ascomycota within the family Umbilicariaceae. The lichen species belonging to genus *Umbilicaria* are commonly used in folk medicine as purgative and as a food during famine. The name of the genus is derived from Latin *umbilicaris*-umbilical and is also known as „rock tripe". *Umbilicaria crustulosa* (Figure 1) represents a foliose type of lichens with a common name crusty navel lichen and synonyms: *Gyrophora depressa* (Ach.) Schaer., *Omphalodiscus crustulosus* (Ach.) Schol., *Gyrophora crustulosa* (Ach.). Upper surface is flat and smooth, gray and lower surface is brown. *Umbilicaria cylindrica* L. Delise ex Duby (Figure 2) also belongs to a foliose type of lichens within light gray upper surface with black margins. These species are growing on siliceous rocks in Northern hemisphere mostly in Alpine vegetation zone (Frey, 1997).



(a) **(b)**

Figure 1. (a) *Umbilicaria crustulosa* (Ach.) Frey and (b) *Umbilicaria cylindrica* L. Delise ex Duby (photo by: Ivana Zlatanović, locality: Babin zub, Stara planina)

The majority of morphologically defined lichen species have constant chemical composition described in the literature. Lichens are characterized by the presence of cortical metabolite and one or more medullary metabolites (Culberson, 1969). Lichens metabolites are mostly crystalline compounds deposited on the surface of hyphae and can be isolated from the lichen matrix using different solvents for extraction (Huneck and Yoshimura, 1996).

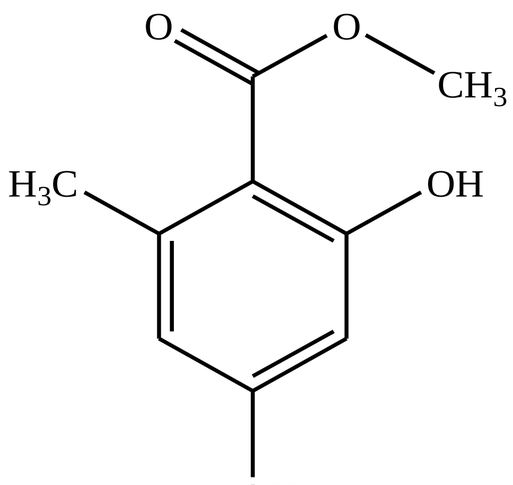
Many lichen secondary metabolites exhibited antioxidant, antimicrobial, cytotoxic and antiviral properties and could be used as active components of drugs (Molnar and Farakas, 2010; Stojanović et al., 2012). Having in mind the need to find new natural bioactive components the present study reviews bioactivities of *U. crustulosa* and *U. cylindrica* lichens extracts.

Chemical composition

Major secondary metabolites of lichens are depsides which are consisted of two, three or four hydroxybenzoic acid residues linked through ester bond and depsidones with additional ether bond between aromatic rings (Huneck and Yoshimura, 1996). Didepside, lecanoric acid and tridepside, gyrophoric acid, are formed by intermolecular coupling of two or three orsellinic acid units through ester bond in *para*-position (Posner et al., 1992). Meta-depsides appear less frequently similarly to crustinic acid, tridepside metabolite of *U. crustulosa*.

Serina and Arroyo (1996) distinguish two chemotypes of *U. crustulosa*: 1) gyrophoric acid type, with the gyrophoric acid as the main component followed by lecanoric acid and crustinic acid; 2) crustinic acid type, with crustinic acid as the main component followed by lecanoric acid and gyrophoric acid. The samples which have been examined by Zlatanović et al. (2017a) also belong to gyrophoric acid chemotype. The following constituents were also identified in these samples: methyl orsellinate, methyl lecanorate and atranorin (Zlatanović et al., 2016; Zlatanović et al., 2017a)

As well as for *U. crustulosa*, two chemical races of *U. cylindrica* were described in the literature, one without lichen substances and one characterized by different lichens substances (Huneck et al., 1991; Posner et al., 1992). Namely, Posner et al. (1992) were reported the presence of depsidones, norstictic acid and connorstictic acid while recent research of *U. cylindrica* samples from Serbia (Manojlović et al., 2012) were revealed salazinic acid, norstictic acid, methyl β-orcinol carboxylate, ethyl haematommate, atranorin and usnic acid as compounds of chloroform and methanol extracts. Zlatanović et al. (2017b) were detected norstictic acid as a main component followed by salazinic acid and atraric acid in ether and ethyl acetate extracts of *U. cylindrica* while atranorin was predominant component of dichloromethane extract. However, main compound of acetone extract of *U. cylindrica* was gyrophoric acid which was represented in smaller amounts in the other examined extracts (Zlatanović, 2019). The structural formulae of the constituents of *U. crustulosa* and *U. cylindrica* extracts are given in the Figure 2.



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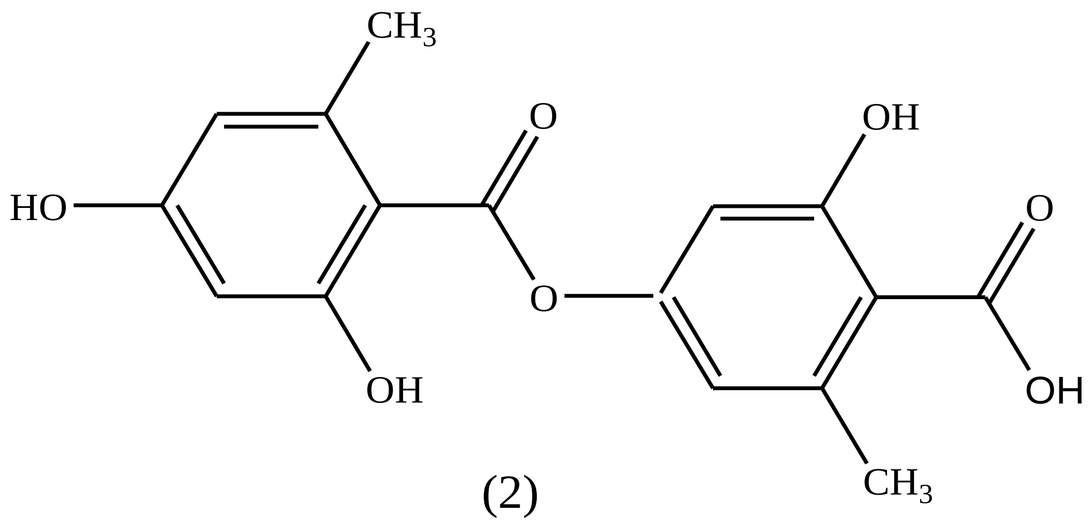

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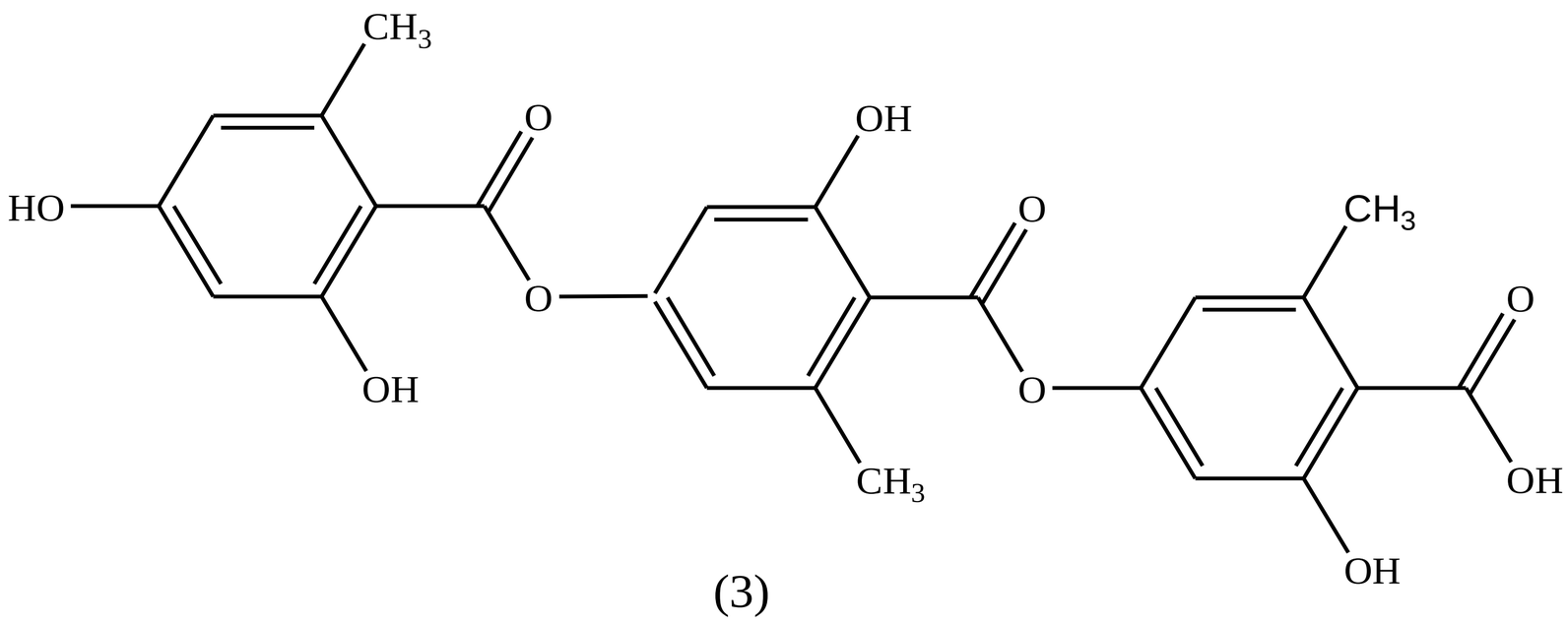

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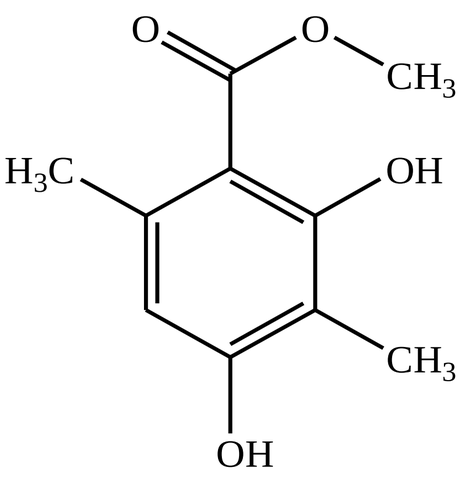

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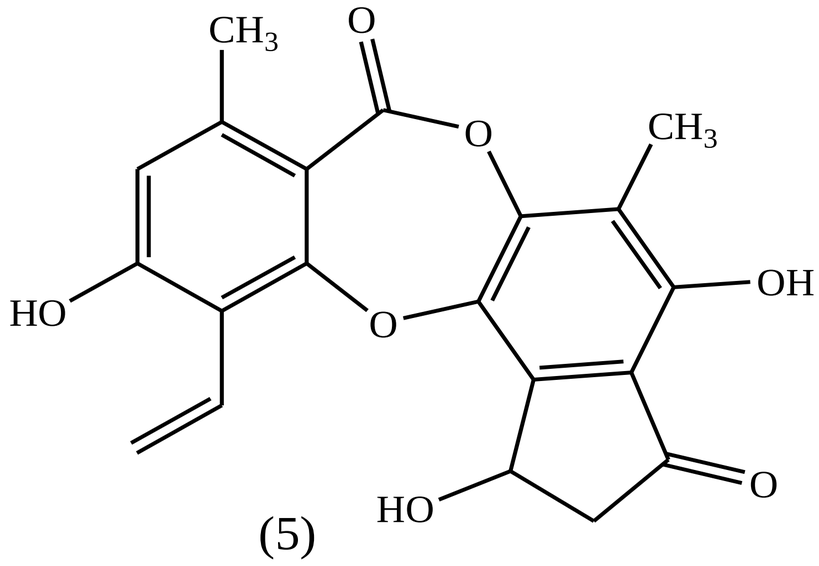

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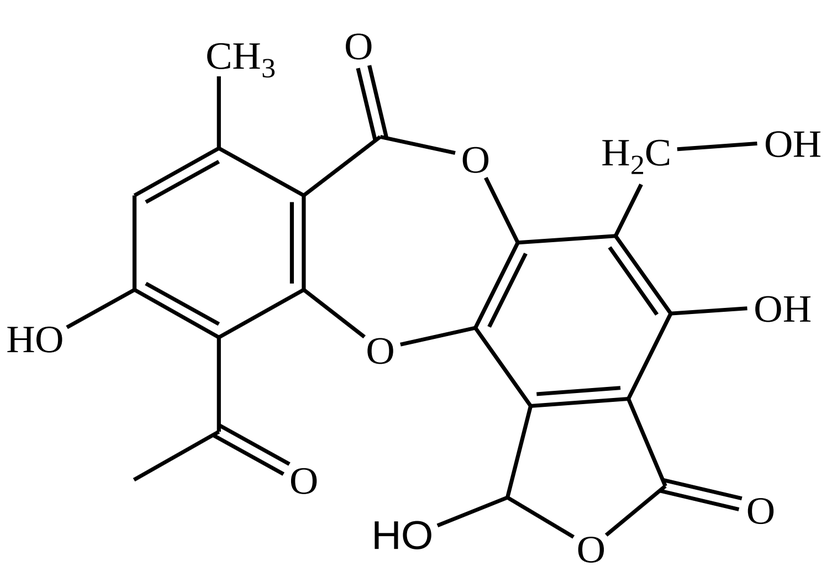

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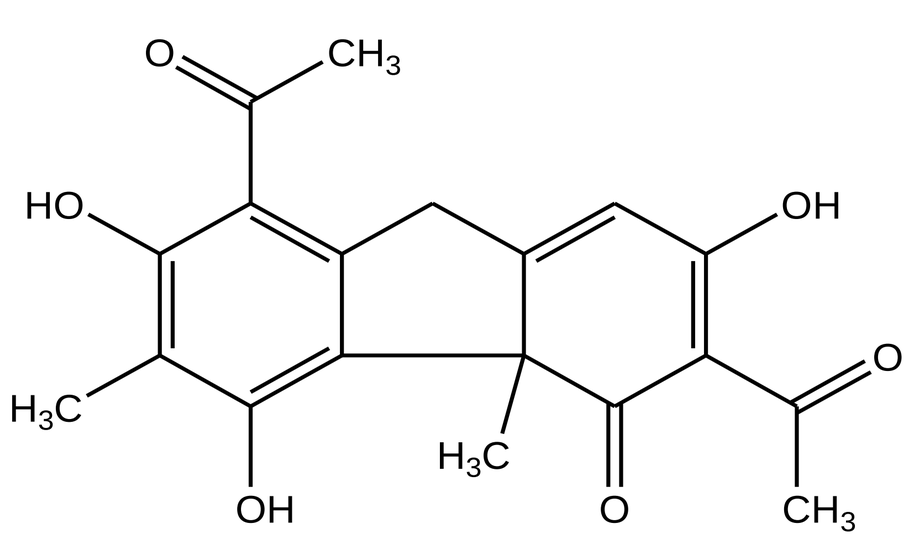

(7)

Figure 2. Structural formulae of (1) methyl orsellinate, (2) lecanoric acid, (3) gyrophoric acid, (4) atraric acid, (5) norstictic acid, (6) salazinic acid, (7) usnic acid

Biological activities

Review of published biological activity data is given in the Table 1.

Table 1. Biological activities of acetone and methanol extracts of *U. crustulosa* and *U. cylindrica*

***U. crustulosa* acetone extract**	***U. crustulosa* methanol extract**	***U. cylindrica* acetone extract**	***U. cylindrica* methanol extract**
Antioxidant (Kosanić et al., 2014; Zlatanović et al., 2017c) Antibacterial (Ranković et al., 2009) Anticlastogenic (Zlatanović, 2019)	Antibacterial (Ranković et al., 2009; Kosanić et al., 2014) Cytotoxic (Kosanić et al., 2014)	Antioxidant, Antibacterial, Anticlastogenic (Zlatanović, 2019)	Antioxidant (Manojlović et al., 2012) Cytotoxic (Kosanić et al., 2014) Antimicrobial (Ranković et al., 2009; Buçukoglu et al., 2013)

Total phenolic content and antioxidant activity

Reactive oxygen species could cause oxidative stress in lichen thallus. However, secondary metabolites of lichens provide protection due to high ability to scavenge free radicals (Molnar and Farakas, 2010). Antioxidant activity of *U. crustulosa* and *U. cylindrica* extracts has been the focus of numerous studies (Buçukoglu et al., 2013; Kosanić et al., 2014; Manojlović et al., 2012; Zlatanović et al., 2017a). Namely, Manojlović et al. (2012) were found that methanol and chloroform extracts of *U. cylindrica* were free radical scavengers (IC_{50} (μg mL^{-1}) 34.45 ± 1.15 and 31.34 ± 1.10, respectively). Also, Zlatanović et al. (2017a) found that DPPH scavenging activity of *U. crustulosa* and *U. cylindrica* acetone extracts was high and amounted 88.7% and 77%, respectively. In the same experiment, the assessment of ABTS scavenging activity showed that acetone extracts reduce the concentration of ABTS radicals for 96.2% and 78.4%, respectively (Zlatanović et al., 2017a; Zlatanović, 2019). Buçukoglu et al. (2013) found that umbilicaric acid exhibited stronger activity than gyrophoric and lecanoric acid at concentrations of 5 mg mL^{-1} (68.14%, 50.96% and 32.48%, respectively). Also, DPPH scavenging activity of mentioned lichen acids was higher than DPPH values of *U. cylindrica* methanol extract

(21.07%) (Buçukoglu et al., 2013). Kosanić et al. (2014) reported that methanol extracts of *U. crustulosa* have shown good scavenging activity on DPPH radical (79.85%).

Total phenolic content of acetone extracts of *U. crustulosa* and *U. cylindrica* were expressed as gallic acid equivalents (GAE) and amounted 350.4188±14.587 and 267.9710 ±8.011 μg GAE mg^{-1} of dry extract weight (Zlatanović et al., 2017a; Zlatanović, 2019). Kosanić et al. (2014) expressed the amount of total phenolic content of *U. crustulosa* methanol extract as the pyrocatechol equivalents (PE), and amounted 55.03 ± 1.096 μg PE mg^{-1} of dry extract weight.

The results of the total reducing power ability (TRP) for *U. crustulosa* and *U. cylindrica* acetone extracts were 0.6197±0.0166 and 0.6515±0.1846 μg ascorbic acid equivalents per mg dry extract weight (Zlatanović et al., 2017a; Zlatanović, 2019). Kosanić et al. (2014) have reported that methanol extract of *U. crustulosa* shows the weakest reducing power among the tested extracts. Measured values for absorbance of methanol extract were 0.066.

Obtained result for cupric reducing capacity (CUPRAC) of *U. crustulosa* acetone extract was 19.7641±0.01 μg TE mg^{-1} of dry extract. The higher CUPRAC value is observed for *U. cylindrica* acetone extract and amounted 21.9521 ± 0.23 μg TE mg^{-1} of dry extract (Zlatanović et al., 2017a; Zlatanović, 2019).

Antioxidant activity of *U. crustulosa* and *U. cylindrica* extracts assessed by the different systems could be attributed to their high total phenolic content. Acetone extract of *U. cylindrica* consisted of monoaromatic compound (atraric acid), depsidones (salazinic acid and norstictic acid) and depsides (gyrophoric acid and atranorin). On the other hand, acetone extract of *U. crustulosa* is characterised by the presence of depsides (lecanoric acid, crustinic acid, gyrophoric acid and atranorin) and monoaromatic compound methyl orsellinate (Zlatanović et al., 2017a, Zlatanović, 2019). Hidalgo et al. (1994) have reported stronger antioxidant activity of depsidones than depsides. This fact could be the reason why acetone extract of *U. cylindrica* was found to be more effective antioxidant in free radical scavenging as well as in reducing power assays. Salazinic acid possesses four hydroxyl groups (two phenolic groups) in the molecule while norstictic acid possesses two phenolic groups, and they, therefore, might contribute to the antioxidant activity of the tested extracts. Some authors believe that the higher efficiency of the depsidones was related to a larger incorporation into lipidic microdomains (Hidalgo et al., 1994).

Antibacterial activity

One of the possible roles of lichens secondary metabolites is to protect lichen from the pathogens in nature (Lawrey, 1989). It has been shown that lichens and their metabolites are quite effective against a

wide variety of microorganisms in many experiments conducted *in vitro* (Buçukoglu et al., 2013; Manojlović et al., 2012; Ranković et al., 2009; Turk et al., 2006). According to Manojlović et al. (2012) methanol and ethyl acetate extracts of *U. cylindrica* manifested strong antibacterial effect against *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Escherichia coli*, *Proteus vulgaris*, *Proteus mirabilis* and *Bacillus subtilis.* Also, the extracts were efficient against the yeast *Candida albicans* and *Aspergillus niger*. The MIC values were within the concentration range from 15.62 μg mL^{-1} to 62.50 μg mL^{-1}. Ranković et al. (2009) have reported that methanol extract of *U. cylindrica* did not show activity against the majority of tested microorganism except *S. aureus* and *C. albicans* whereas acetone extract showed activity only towards *C. albicans.* However, methanol extract of *U. cylindrica* tested by Bacukoglu et al. (2013) at concentration of 5% and 10% inhibited only *P. aeruginosa* among the five Gram-negative bacteria and *B. cereus,* among the three Gram-positive bacteria. In the same experiment, gyrophoric acid at concentration of 5% inhibited all tested Gram-negative bacteria, except *E. coli* and among Gram-positive bacteria only *B. subtilis* was resistant. Also, methanol extract of *U. cylindrica* and gyrophoric acid did not demonstrated activity towards fungi (Buçukoglu et al., 2013). On the other hand, Zlatanović (2019) was found that acetone extract of *U. cylindrica* possesses moderate activity against Gram-positive bacteria, such as *S. aureus* and *Bacillus subtilis subsp. spizizenii* and no activity on tested Gram-negative bacteria.

Manojlović et al. (2012) were reported that methanol and chloroform extracts of *U. cylindrica* consisted of salazinic acid, norstictic acid, methyl β-orcinol carboxylate, ethyl haematommate, atranorin and usnic acid while acetone extract of *U. cylindrica* analyzed by Zlatanović (2019) consisted mainly of gyrophoric acid (83.5%) followed by small amount of methyl β-orcinol carboxylate (2.8%), norstictic acid (1.7%), salazinic acid (2%), atranorin (5%) and usnic acid (0.2%). The significant antimicrobial activity of *U. cylindrica* extracts is probably due to the presence of depsidones.

Methanol extract of *U. crustulosa* was found to have moderate antimicrobial activity. Namely, Kosanić et al. (2014) reported no activity toward *E. coli*, *Botrytis cinerea* and *C. albicans*. The MIC values for *U. crustulosa* methanol extracts were 6.25 mg mL^{-1} against four species of bacteria and 12.5 mg mL^{-1} against three species of fungi. Ranković et al. (2009) examined the antimicrobial properties of acetone, methanol and aqueous extracts of *U. crustulosa*. Acetone and methanol extracts of *U. crustulosa* were inactive against *E. coli*, *B. cinerea* and *C. albicans* although both extracts were active against *Bacillus mycoides, Bacillus subtilis, Enterobacter cloacae, Klebsiella pneumoniae, Staphylococcus aureus, Aspergillus flavus, Aspergillus fumigatus, Fusarium oxysporum, Mucor mucedo, Paecilomyces variotii, Penicillium purpurescens, Penicillium verrucosum***,** *Saccharomyces cerevisiae, Trichoderma harzianum* (Ranković et al., 2009). However, acetone extract of *U. crustulosa* did not exhibit activity against tested Gram-positive and Gram-negative bacteria at concentration of 1 mg per disk (Zlatanović et

al., 2017a). Gyrophoric acid isolated from several species of the genus *Umbilicaria* did not show activity towards *E. coli*, *K. pneumoniae*, *P. aeruginosa* i *S. typhimurium* (Candan et al., 2006). Since gyrophoric acid showed no activity against tested bacteria and this compound represents 68.5% of the analyzed acetone extract of *U. crustulosa* these results can be explained due to its inactivity (Zlatanović et al., 2017a).

Citotoxic activity

Numerous studies have shown that lichen metabolites possess cytotoxic activity (Kosanić et al., 2014; Kumar and Müller, 1999b). Among the tested lichen extracts methanol extract of *U. crustulosa* manifested the weakest cytotoxic activity against colon cancer adenocarcinoma cell line HTC (Kosanić et al., 2014). Gyrophoric acid significantly inhibited the light-dependent synthesis of ATP and uncoupled electron transfer on the reducing side of photosystem II in freshly lysed illuminated spinach chloroplasts (Rojas et al., 2000), but it was inactive in the inhibition of leukotriene B4 biosynthesis (Kumar and Müller, 1999a). Also, it was found that gyrophoric acid possesses antiproliferative and cytotoxic activity and inhibited the growth of the human keratinocyte cell line HaCaT (Kumar and Müller, 1999b). Some depsidones and depsides (pannarin, 10-chloropannarin and sphaerophorin) were reported to have a higher cytotoxic effect than colchicine (Correché et al., 2002). Among fifteen lichen compounds, depsidones salazinic acid, stictic acid and psoromic acid were the most apoptotic active derivatives on primary cultures of rat hepatocytes. One of the most explored secondary metabolites of lichens is usnic acid which has manifested antitumor activity against Lewis Lung carcinoma (Kupchan and Kopperman, 1975) and antiproliferative effect against human HaCaT keratinocytes (Bezivin et al., 2004; Kumar and Müller, 1999b).

Anticlastogenic activity

The analysis of micronuclei (MN) in cultured lymphocytes is applied as a method to monitor human exposure to genotoxic agents (Fenech and Morley, 1993). The cytokinesis block micronuclei assay (CBMN) is used to test the impact of acetone extracts of *U. crustulosa* and *U. cylindrica* (at concentrations of 1.0, 2.0 and 3.0 μg mL^{-1}) for *in vitro* protective effect on chromosome aberrations in peripheral human lymphocytes. The cell cultures treated with amifostine WR-2721 (positive control) at concentration of 1 μg mL^{-1} gave a significant ($P < 0.01$) decrease in the MN frequency of 11.4% comparing to the control cell cultures. The *U. crustulosa* extract at concentration of 2 μg mL^{-1} gave a

decrease in the MN frequency of 16.3%, which was higher than amifostine, while at concentration of 1 μg mL^{-1} and 3 μg mL^{-1} the effect was lower than amifostine (Zlatanović et al., 2017a). Acetone extract of *U. cylindrica* at concentration of 2 μg mL^{-1} exhibited the most prominent effect of decreasing the MN frequency (11%) while at concentration of 1 and 3 μg mL^{-1} decreasing of the MN frequency was lower (5.3% and 1.6%, respectively) (Zlatanović, 2019). Secondary metabolites isolated from lichens such as atranorin, evernic acid and usnic acid showed significant anticlastogenic activity, reducing the frequency of MN to the same or greater extent than amifostine (11.1, 32.9 and 48.9%, respectively) (Stojanović et al., 2014). All tested extracts and metabolites showed the highest activity at concentration of 2 μg mL^{-1}.

Cholinesterase activity

Synthetic cholinesterase inhibitors represent the treatment of choice for Alzheimer's disease but finding of natural inhibitors of cholinesterase is the subject of many studies (Giacobini, 2004). Results obtained from the screening of the interaction of extracts with cholinesterase from pooled human serum, have shown that acetone extracts of *U. crustulosa* and *U. cylindrica* possess weak activation effect on cholinesterase activity (1.6% and 4.4%, respectively). In the same experiment gyrophoric acid isolated from *U. crustulosa* acetone extract has manifested weak inhibition effect on cholinesterase activity, -18.4% (Zlatanović, 2019). In conducted experiment neostigmine bromide (commercial cholinesterase inhibitor) inhibited cholinesterase to extent of -96.6%.

Conclusion

Two chemotypes of *U. crustulosa* acetone extracts are distinguished: 1) gyrophoric acid type with gyrophoric acid as the main constituent followed by lecanoric acid and crustinic acid; and 2) crustinic acid type with crustinic acid as the main constituent followed by lecanoric acid and gyrophoric acid. Chemical composition of *U. cylindrica* extracts depends on the solvent used for the extraction and the major constituents of methanol, chloroform, ether and ethyl acetate extracts are depsidones, salazinic acid and norstictic acid while main constituents of acetone and dichloromethane extracts are depsides, gyrophoric acid and atranorin, respectively. The present review has shown that *U. crustulosa* and *U. cylindrica* extracts possess antibacterial, antiproliferative, cytotoxic, antioxidant and anticlastogenic activity. Due to significant antioxidant activity and protective effect on human lymphocytes, acetone extracts of *U. crustulosa* and *U. cylindrica* are promising candidates for *in vivo* experiments.

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Conflict-of-Interest Statement

Authors declare no conflict of interest.

References

Bezivin, C., Tomasi, S., Rouaud, I., Delcros, J. G., & Boustie, J. (2004). Cytotoxic activity of compounds from the lichen: *Cladonia convoluta*. Planta Medica, 70, 874-877.

Buçukoglu, Z.T., Albayrak, S., Halici, G., & Tay, Y. (2013). Antimicrobial and antioxidant activities of extracts and lichen acids obtained from some *Umbilicaria* species from central Anatolia Turkey. Journal of Food Processing and Preservation, 37, 1103-1110.

Culberson, W.L. (1969). The use of chemistry in the systematics of the lichens. Taxon, 18, 152 – 166.

Correché, E., Carrasco, M., Giannini, F., Piovano, M., Garbarino, J., & Enriz, D. (2002). Cytotoxic screening activity of secondary lichen metabolites. Acta Farmaceutica Bonaerense, 21, 273-278.

Candan, M., Yilmaz, M., Tay, T., Kivanç, M., & Turk, H. (2006). Antimicrobial activity of extracts of the lichen *Xanthoparmelia pokornyi* and its Gyrophoric and Stenosporic acid constituents. Zeitschrift für Naturforschung. Section C: Journal of Biosciences, 61, 319-323.

Fenech, M., & Morley, A. A. (1993). The cytokinesis-block micronucleus technique: a detailed description of the method and its application to genotoxicity studies in human populations. Mutatation Research, 285, 35–44.

Frey, E. (1997). Die geographische Verbreitung der Umbilicariaceen und einiger alpiner Flechten. Berichte der Deutschen Botanischen Gesellschaft, 46, 412–444.

Giacobini, E. (2004). Drugs that target cholinesterase, In J.J. Buccafusco (Ed.), Cognitive Enhancing Drugs (pp. 11). Basel: Springer.

Hidalgo, M. E., Fernandez, E., Quilhot, W., & Lissi, E. (1994). Antioxidant activity of depsides and depsidones. Phytochemistry, 37, 6, 1585-1587.

Huneck, S., & Yoshimura, I. (1996). Identification of Lichen Substances. Springer-Verlag.

Lawrey, J. (1989). Lichen Secondary Compounds: Evidence for a Correspondence Between Antiherbivore and Antimicrobial Function. Bryologist, 92, 326–328.

Kupchan, S. M., & Kopperman, H. L. (1975). L-usnic acid: tumor inhibitor isolated from lichens. Experientia, 31, 6, 625.

Kumar, S., & Müller, K. (1999a). Lichen Metabolites. 1. Inhibitory Action Against Leukotriene B4 Biosynthesis by a Non-Redox Mechanism. Journal of Natural Products, 62, 817-820.

Kumar, S., & Müller, K. (1999b). Lichen Metabolites. 2. Antiproliferative and cytotoxic activity of gyrophoric, usnic, and diffractaic acid on human keratinocyte growth. Journal of Natural Products, 62, 821-823.

Kosanić, M., Šeklić, S., Marković, M., & Ranković, B. (2014). Evaluation of antioxidant, antimicrobial and anticancer properties of selected lichens from Serbia. Digest Journal of Nanomaterial and biostructures, 9 (1), 273-287.

Molnar, K., & Farkas, E. (2010). Current Results on Biological Activities of Lichen Secondary Metabolites: a Review. Zeitschrift für Naturforschung. Section C: Journal of Biosciences, 65, 157-173.

Manojlović, N., Vasiljević, P., Masković, P. Z., Jusković, M., & Bogdanović-Dusanović, G. (2012). Chemical composition, antioxidant, and antimicrobial ctivities of lichen *Umbilicaria cylindrica* (L.) Delise (Umbilicariaceae). Evidence-based Complementary and Alternative Medicine, 2012, 1-8.

Posner, B., Feige, G. B., & Huneck, S. (1992). Studies on the Chemistry of the Lichen Genus *Umbilicaria* Hoffm. Zeitschrift für Naturforschung. Section C: Journal of Biosciences, 47, 1 – 9.

Rojas, I., Lotina-Hennsen, B., & Mata, R. (2000). Effect of Lichen Metabolites on Thylakoid Electron Transport and Photophosphorylation in Isolated Spinach Chloroplasts 1. Journal of Natural Products, 63 (10), 1396-1399.

Ranković, B. Misic, M., & Sukdolak, S. (2009). The antimicrobial activity of substances derived from the lichens *Physcia aipolia*, *Umbilicaria polyphylla*, *Parmelia caperata* and *Hypogimnia physodes*. Byologia, 64 (1), 53-58.

Serina, E., & Arroyo, R. (1996). Lichen Substances and Their Intraspecifie Variability within Eleven *Umbilicaria* Species in Spain. Bryologist, 99, 335–342.

Stojanović, G., Stojanović, I., & Šmelcerović, A. (2012). Lichen Depsidones as Potential Novel Pharmacologically Active Compounds. Mini-reviews in Organic Chemistry, 9 (2), 1-7.

Stojanović, G., Stanković, M., Stojanović, I., Palić, I., Milovanović V., & Rančić, S. (2014). Clastogenic Effect of Atranorin, Evernic acid, and Usnic Acid on Human Lymphocytes. Natural product communication, 9 (4), 503-504.

Turk, H., Yilmaz, M., Tay, T., Turk, A.O., & Kivanc, M. (2006). Antimicrobial Activity of Extracts of Chemical Races of the Lichen *Pseudevernia furfuracea* and their Physodic Acid, Chloroatranorin, Atranorin and Olivetoric Acid Constituents. Zeitschrift für Naturforschung. Section C: Journal of Biosciences, 65, 499-507.

Zlatanović, I., Petrović, G., Jovanović, O., Zrnzević. I., & Stojanović, G. (2016). Isolation and identification of secondary metabolites of *Umbilicaria crustulosa* (Ach.) Frey. Facta Universitatis, Series Physics, Chemistry and Technology, 14 (2), 125-133.

Zlatanović, I., Stanković, M., Stankov-Jovanović, V., Mitić, V., Zrnzević. I., Djordjević, A., & Stojanović, G. (2017a). Biological activities of *Umbilicaria crustulosa* (Ach.) Frey acetone extract. Journal of Serbian Chemical Society, 82, 141-150.

Zlatanović, I., Zrnzević. I., Jovanović, O., Stojanović, I., Petrović, G., & Stojanović, G. (2017b). Chemical composition of *Umbilicaria crustulosa* (Ach.) Frey and *Umbilicaria cylindrica* (L.) Duby extracts. Natural Product Communication, 2017, 12 (7), 1105-1105.

Zlatanović, I., (2019). Chemical composition, antibacterial, antioxidant and genotoxic activity of lichens *Umbilicaria crustulosa* (Ach.) Frey, *Umbilicaria cylindrica* (L.) Delise, *Hypogymnia tubulosa* (Schaer.) Hav. and *Evernia prunastri* (L.) Ach. Doctoral dissertation, Faculty of sciences and mathematics, Niš: University of Niš.

Hemijski sastav i biološke aktivnosti ekstrakata *Umbilicaria crustulosa* i *Umbilicaria cylindrica*

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SAŽETAK

Glavne komponente acetonskog, metanolnog, etarskog i etil-acetatnog ekstrakta lišaja *Umbilica riacrustulosa* su tridepsidi, giroforna kiselina i krustinska kiselina i didepsid, lekanorna kiselina. Hemijski sastav ekstrakata lišaja *Umbilicaria cylindrica* zavisi od rastvarača koji se koristi za ekstrakciju, a najzastupljenije komponente su depsidoni, norstiktik kiselina i salazinska kiselina ili depsidi, giroforna kiselina i atranorin. Ekstrakti lišajeva *U. crustulosa* i *U. cylindrica* pokazali su antibakterijsku, antioksidativnu, citotoksičnu, antiproliferativnu i antiklastogenu aktivnost. Acetonski ekstrakti lišajeva *U. crustulosa* i *U. cylindrica* pokazuju izraženu antioksidativnu i antiklastogenu aktivnost što ih kvalifikuje za *in vivo* eksperimente.

Ključne reči: Umbilicaria crustulosa, Umbilicaria cylindrica, antioksidativna aktivnost, antimikrobna aktivnost, citotoksična aktivnost, mikronukleus test, inhibicija holinesteraze

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Composition chimique et activités biologiques des *Umbilicaria crustulosa* et *Umbilicaria cylindrica*

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Résumé

Les principaux composés d'acétone, de méthanol, d'éther et d'acétate d'éthyle d'*Umbilicaria crustulosa* sont les tridepsides – l'acide gyrophorique et l'acide crustinique – et le didepside – l'acide lécanorique. La composition chimique des extraits d'*Umbilicaria cylindrica* dépend du solvant utilisé pour l'extraction et ses principaux composants sont les depsidones, l'acide salazinique et l'acide norstictique ou les depsides, l'acide gyrophorique et l'atranorine. Des extraits des *U. crustulosa* et *U. cylindrica* ont montré une activité antibactérienne, antioxydante, cytotoxique, antiproliférative et anticlastogène. En raison de leur activité antioxydante et anticlastogène accentuée, les extraits à l'acétone des *U. crustulosa* et *U. cylindrica* sont des candidats prometteurs pour des expériences *in vivo*.

Mots-clés: Umbilicaria crustulosa, Umbilicaria cylindrica, activité antioxydante, activité antimicrobienne, activité cytotoxique, test du micronoyau, inhibition de la cholinestérase

Химический состав и биологическая активность экстрактов *Umbilicaria crustulosa* и *Umbilicaria cylindrica*

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Резюме

Основные соединения ацетоновых, метанольных, эфирных и этилацетатных экстрактов *Umbilicaria crustulosa* являются тридепсиды, гирофорная кислота и корастиновая кислота, а также дидепсиды, леканорная кислота. Химический состав экстрактов *Umbilicaria cylindrica* зависит от растворителя, используемого для экстракции, и основными компонентами являются депсидоны, салазиновая кислота и норстициновая кислота или депсиды, гирофорная кислота и атранорин. Экстракты *U. crustulosa* и *U. cylindrica* проявили антибактериальную, антиоксидантную, цитотоксическую, антипролиферативную и антикластогенную активность. Ацетоновые экстракты *U. crustulosa* и *U. cylindrica* являются многообещающими кандидатами для экспериментов *in vivo* с учетом антиоксидантной и антикластогенной активности.

Ключевые слова: Umbilicaria crustulosa, Umbilicaria cylindrica, антиоксидантная активность, антимикробная активность, цитотоксическая активность, микроядерный тест, ингибирование холинэстеразы.

Chemische Zusammensetzung und biologische Aktivitäten von Extrakten aus *Umbilicaria crustulosa* und *Umbilicaria cylindrica*

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ABSTRAKT

Die Hauptkomponenten der Aceton-, Methanol-, Ether- und Ethylacetatextrakte der Flechte *Umbilicaria crustulosa* sind Tridepside, Gyrophor- und Crustinsäure sowie Didepside, Lecanorsäure. Die chemische Zusammensetzung von Extrakten aus *Umbilicaria cylindrica* hängt von dem für die Extraktion verwendeten Lösungsmittel ab, und die am häufigsten verwendeten Hauptkomponenten sind Depsidone, Salazinsäure und Norstictinsäure oder Depside, Gyrophorsäure und Atranorin. Extrakte der Flechten *U. crustulosa* und *U. cylindrica* zeigten antibakterielle, antioxidative, zytotoxische, antiproliferative und anticlastogene Wirkung. Acetonextrakte der Flechten *U. crustulosa* und *U. Cylindrica* zeigen ausgeprägte antioxidative und anticlastogene Aktivität, was sie für In-Vivo-Experimente qualifiziert.

Schlüsselwörter: Umbilicaria crustulosa, Umbilicaria cylindrica,antioxidative Aktivität, antimikrobielle Aktivität, zytotoxische Aktivität, Mikronukleustest, Cholinesterasehemmung

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Textbook presentation: "Rational Design of Bioactive Compounds: From Theoretical to the Practical Approach" by authors: Milan Mladenovic, Rino Ragno, Nevena Stankovic, Nezrina Mihovic,

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The textbook entitled: "RATIONAL DESIGN OF BIOACTIVE COMPOUNDS: From Theoretical to Practical Approach" was created as a result of the scientific and teaching-pedagogical collaboration between the Faculty of Science and Mathematics of the University of Kragujevac and the Faculty of Pharmacy and Medicine of the Sapienza University of Rome. The authors of the textbook have incorporated their knowledge of biochemistry, pharmacy and medical chemistry into a textbook that will bring medical chemistry closer to the broader academic community in Serbia and present the basic concepts of rational design of bioactive compounds with the ultimate goal of designing compounds that will be used as pharmaceuticals.

The textbook is an upgrade of biochemistry knowledge and answers the basic question in biochemistry: How does a bioactive molecule (substrate, inhibitor, agonist, partial agonist, antagonist ...) interact with its molecular target (enzyme, receptor or nucleic acid)? The authors, through the concept of crystal structures of co-crystallized complexes of biomolecules and proteins, present to the reader the activity of biomolecules as a direct consequence of the interactions that the biomolecule exerts in the

crystal structure with the active center of the enzyme or receptor. The specificity of this textbook is to consider biomolecule-protein interactions by using accredited and recognized computer methods, with a simple premise: biomolecule-protein interactions will be better understood if viewed on a monitor screen. In this way, future readers will easily adopt a new way of understanding biochemistry and fully understand the importance of biomolecule interactions with proteins. The manuscript focuses not on the metabolic pathways of biomolecules, but on the interactions of biomolecules with proteins and the consequences that these interactions have on human physiology.

The book is thematically divided into nine chapters:

Chapter I: Crystallography as a method for the definition of bioactive conformations of inhibitors and antagonists of enzymatic reactions

Chapter II: Preparation of crystal structures of biomolecules in the complex with native protein

Chapter III: Three-dimensional quantitative correlation between the bioactive conformation and the activity of biomolecules

Chapter IV: Generating 3-D QSAR studies

Chapter V: Generation of bioactive conformations by molecular target structure: molecular docking

Chapter VI: Practical applications of molecular docking

Chapter VII: Defining bioactive conformations according to crystal structure of inhibitors or agonists: alignment biomolecules

Chapter VIII: Practical application of biomolecule alignment

Chapter IX: Rational design of bioactive compounds

The major emphasis in the textbook is on the understanding the biophysical phenomena that lead to the generation of the bioactive conformation (Chapters III and IV). In other words, it explains in detail how the interaction between biomolecules and proteins occurs. The textbook is based on the recently published study of the rational design of novel monoamine oxidase B (MAO B) inhibitors (Chapter III), an enzyme whose disruption of activity leads to the oxidative stress at the level of mitochondrial DNA and the subsequent development of Parkinson's disease. The authors presented to readers simply and understandably how diversity in the structure of MAO B inhibitors affects activity. MAO B inhibitor activity was quantified through the concept of Three-Dimensional Structure Activity Relationships (3-D QSAR) and presented in detail how statistically significant 3-D QSAR models are generated and how they are interpreted, with minimal use of complex mathematical formulations of chemometrics, at the level at which it is necessary to understand how chemoinformatic methods are used in medicinal chemistry.

The authors' view is that medical chemistry cannot be learnt only by reading university and scientific literature, but mainly through practical work; all the methods of generating 3-D QSAR studies and other manuscript methods that are described in the scientific literature are descriptively presented in a case study of a human dihydrofolate reductase inhibitor (Chapters II, IV, VI, and VIII), an enzyme whose disruption in catalytic activity leads to the development of leukemia. Within the practical chapters, the authors, using Linux as an operating system, bash and Python programming, present to readers in detail the most popular practical methods for the rational design of bioactive compounds.

The practical chapters represent the greatest value of this textbook. All of the molecular modeling tools that are presented are completely free to the academic community. Generation of 3-D QSAR studies will be learnt by readers through the use of Open3DALIGN (Chapter IV).

From the theoretical and practical point of view, both molecular docking methods (Chapter V) and the alignment of three-dimensional structures of biomolecules (Chapter VII) are addressed in the textbook. Methods that are complementary to 3-D QSAR studies and used to predict the bioactive conformations of compounds whose activities are known but not their interactions with proteins of interest are also presented. Although the methods presented are supported by appropriate mathematical formulations, greater emphasis is placed on understanding of the methods and the purpose of their applications. Readers are told how to choose the best method to predict the bioactive conformation of a new molecule based on the structure of the molecular target or known inhibitors or antagonists. Molecular docking requires the practical application of AutoDock4.2, AutoDock Wines and DOCK6 (Chapter VI), while the Balloon/ShaEP and Obconformer/Open3DALIGN programs (Chapter VIII) are used to compare the three-dimensional structures of biomolecules.

The textbook concludes with the presentation of the rational design concepts of novel monoamine oxidase B inhibitors, using all the methods learnt (Chapter IX). By understanding the content, readers will understand that the rational design of new biomolecules is based solely on the interactions that the molecule will accomplish with the molecular target of interest, and after adopting all the methods presented in the manuscript, readers will be able to critically review the biomolecular-molecular interaction target and to experimentally work (synthesis and biochemical evaluation of compounds) based on the rational knowledge.

The concept that each theoretical title is followed by a chapter in which the methods of computational medicinal chemistry are practically

taught is certainly different from the usual formats of university textbooks, forcing the reader to apply what has been learnt previously. Mastering the methods presented in the book, readers will understand that the activity of biomolecules and future drugs is a result of the interactions that the biomolecule has with the active center of the enzyme or a receptor and will acquire the ability to rationally design new bioactive molecules.

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In silico study on the apicoplast L4 ribosomal protein and three domains from 23S rRNA from *Plasmodium falciparum* and comparison with the existing co-crystal structures

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ABSTRACT

We performed preliminary computational studies on the construction of a segment of ribosomal protein L4 from the apicoplast ribosome of *Plasmodium falciparum*. With a Z-score of -3.404, it is arguably the best constructed model of this drug target so far. Three domains from 23S rRNA were made from scratch using the software RNA2D3D: domain II, IV and V. They were not validated but show reasonable similarity with bacterial 23S rRNA. This model has technical limitations but is a starting point; refined models are expected to find use in antimalarial drug design.

Keywords: *in sillico, Plasmodium falciparum,* ribosome

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Introduction

Malaria is a disease caused by several strains of protozoa from the *Plasmodium* genus. Many attempts have been made to control the disease including vaccination, vector control and parasiticidal drugs (Ralph et al., 2001).Currently, parasiticides constitute the most routinely applied approach in combating malaria, complementing the widely popular use of insecticide-treated nets (World Health Organization, 2019). However, there is widespread resistance to existing anti-malarial drugs (World Health Organization, 2019). Also, many studies have been conducted into the development of prophylactic treatments (especially vaccines) (World Health Organization, 2019). The most promising vaccine to date is a recombinant protein-basedRTS, S/AS01 vaccine.One of the drawbacks of this vaccine is that children treated with this medicine show an elevated risk of meningitis infection (Moorthy and Okwo-Bele, 2015).

Plastid can be defined as any organelle that is the site of manufacture and storage of important chemical compounds used by the cell. The apicoplast originates from the same endosymbiosis as other plastids (Gleeson, 2000), so it can be regarded as a mini-bacterium living inside the malaria parasite. All familiar processes happening inside apicoplast are bacterial in nature (DNA replication, transcription, translation, post-translational modification, catabolism, and anabolism), and can be potential drug targets.

In addition to the standard treatments, there are several families of drugs in experimental use or under investigation as potential medicines against the malaria parasite. Some of them act on metabolic targets such as DNA replication (Gozalbes et al., 2000), RNA transcription (Pukrittayakamee et al., 1994), protein translation (Clough et al., 1999; McConkey et al., 1997; Pfefferkorn and Borotz, 1994; Rogers et al., 1998; Woods et al., 1996), amino acid biosynthesis (Roberts et al., 1998), isopentenyl diphosphate biosynthesis (Clastre et al., 2007; Jomaa et al., 1999) and fatty acid biosynthesis (Surolia and Surolia, 2001). However, only a few of them show significant inhibitory activity against *P. falciparum* (rifampicin, azithromycin, thiostrepton, tetracycline, amythiamicin and fosmidomycin). Rifampicin inhibits transcription from the 35kb apicoplast genome by targeting the plastid-encoded RNA polymerase (McConkey et al., 1997; Uddin et al., 2018). Azithromycin has activity on plastid 23S rRNA, but nevertheless there is no substantial evidence of its mode of action (Dahl and Rosenthal, 2007; Sidhu et al., 2007; Yeo and Rieckmann, 1995).

Azithromycin is a potent anti-bacterial agent that exhibits mild anti-malarial activity (Arsic et al., 2014). Schlunzen et al. (2003) reported the crystal structure of azithromycin bound to the ribosome of *D. radiodurans* and postulated two binding sites. It was found that the first azithromycin molecule has interactions mostly with domains IV and V of 23S rRNA,while the second azithromycin molecule interacts with the ribosomal proteins L4,L22and domain II of 23S rRNA. We wanted to investigate whether other macrolide antibiotics possess anti-malarial activity but we were hindered by the lack of a

crystal structure of the exit tunnel of the apicoplast ribosomal exit tunnel from *P. falciparum* (caused in part by the difficulty of separating apicoplasts from mitochondria). We therefore explored the use of the bacterial crystal structure of *D. radiodurans* as a template for superpositions of the modelled *P. falciparum* exit tunnel and *D. radiodurans*(Figure 1).

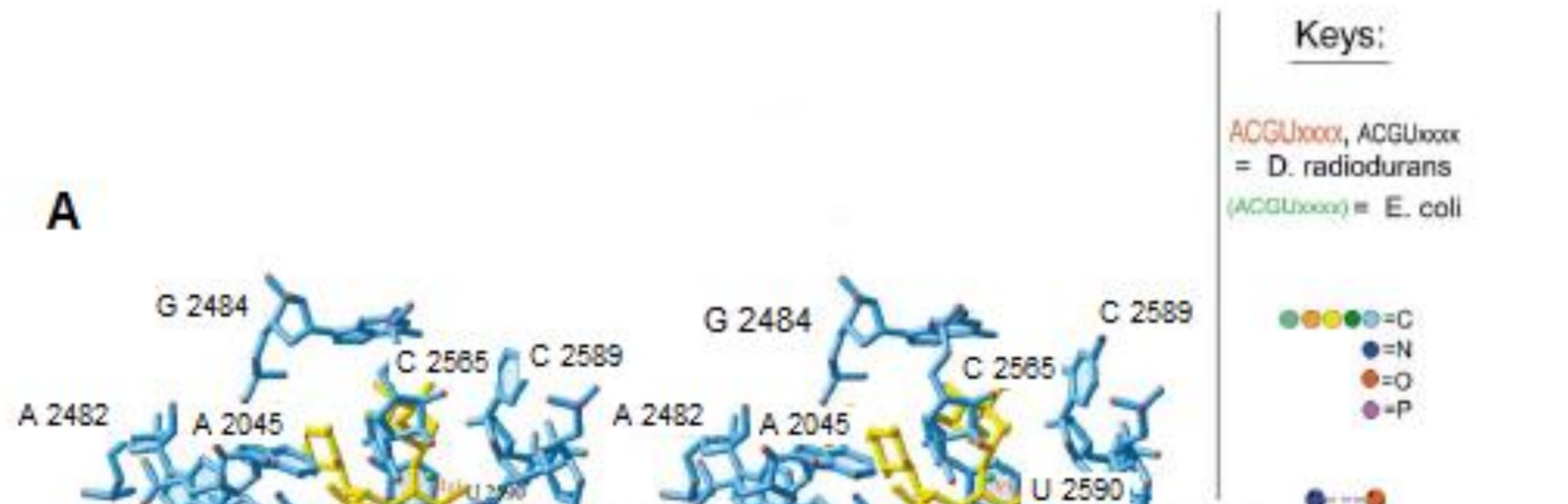


Figure 1. (**A**) Stereoview of the local environment around the two azithromycin molecules. (**B**) Two-dimensional sketch of interactions between the two azithromycin molecules, 23S rRNA, and the ribosomal proteins L4 and L22. Nucleotides or amino acids contributing to hydrophobic interactions are indicated; those contributing to hydrogen bonds or electrostatic interactions are represented by their structures (Schlunzen et al., 2003)

Sidhu et al.(2007)modelled the L4 segment from *P. falciparum* (Lys^{57} to Pro^{97}) using MODELLER and *E. coli* and *D. radiodurans* as templates. However, no quantitative scores of the quality of the model were provided, so its precision cannot be estimated (Sidhu et al., 2007).

Experimental

Homology modelling using the SWISS Model server (Arnold et al., 2006)

Homology modelling using the SWISS Model server was performed by submitting the sequence of the target sequence of ribosomal protein L4 from *P. falciparum*, and template pdb files of L4 from *D. radiodurans* and *E. coli* extracted using Molecular Viewer.

Ab initio molecular modelling using I-TASSER server (Roy et al., 2010; Roy et al., 2011;Zhang, 2008)

Ab initio molecular modelling using I-TASSER server of L4 ribosomal protein from *P. falciparum* was performed by submitting the sequence of this protein and using the option of modelling without the template.

Modelling of 23S rRNA using RNA2D3D (http://www-lmmb.ncifcrf.gov/~bshapiro/software.html)

The molecular modelling of 23S rRNA from *P. falciparum* was performed making two separate input files containing the RNA sequences of 5' and 3'-half of 23S rRNA and information of the base pairs obtained from the secondary structure of 23S rRNA. The obtained crude models were refined according to the manual given by the creators of the software RNA2D3D (http://www-lmmb.ncifcrf.gov/~bshapiro/software.html). Energy refinement was performed using the TINKER software available in RNA_2D3D, and two separate models were generated.

PyMOL (https://pymol.org/2/)superposition of 23S rRNA of *D. radiodurans* domains and constructed domains of 23S rRNA from *P. falciparum*

L4 and 23S rRNA were modelled separately as described in previous sections above. These were now aligned with the corresponding *D. radiodurans* moieties using Pymol. Three domains of 23S rRNA (II, IV, and V) are important for macrolide binding, and each was aligned separately with the

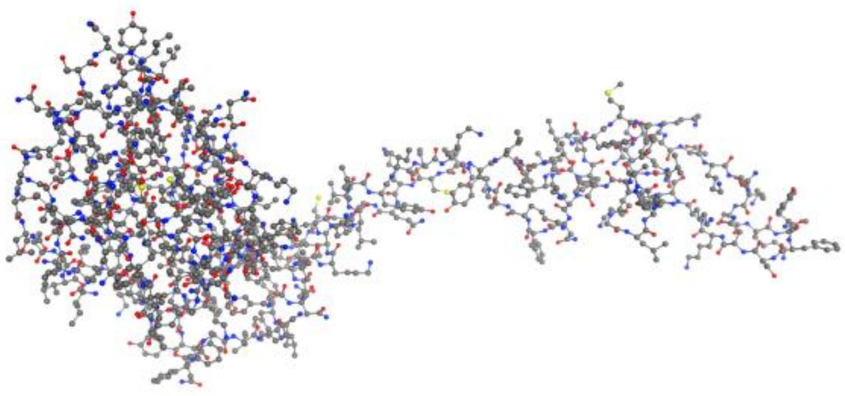
corresponding domains from *D. radiodurans*. The smallest RMS was shown with the alignment of domain IV (RMS=14.528). Domain IV was now superimposed on the *D. radiodurans*RNA structure, and the *D. radiodurans* domain IV deleted. This procedure was repeated sequentially with domains II and V to give a hybrid 23S rRNA.

Results and Discussion

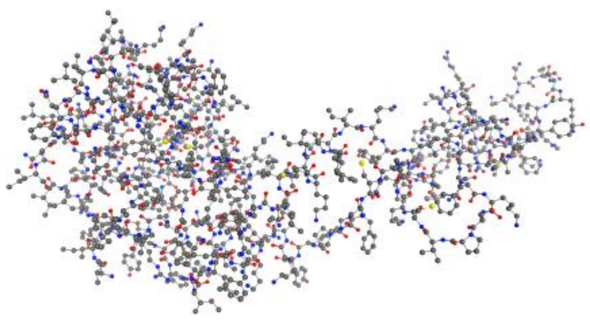
Modelling of apicoplast-encoded L4

Ribosomal protein L4 from the *P. falciparum* apicoplast (PfRpl4) shows only modest similarity in primary sequence with reported crystal structures of bacterial L4, even in the protein region responsible for macrolide activity against bacteria (Sidhu et al., 2007). It was reported that *E.coli* and *D.radiodurans*L4 proteins, share 39% and 32% sequence identity with PfRpl4 in this loop region (Sidhu et al., 2007).

In our study, we used SWISS Model (Arnold et al., 2006), and *E. coli*(Mitra et al., 2006) and *D. radiodurans*(Harms et al., 2008)as templates, and the whole L4 protein from *P. falciparum* was constructed from each template separately. The two models show similar structural characteristics. The model obtained using *D. radiodurans* as a template analyzed by YASARA (www.yasara.org) yielded 29.9% Helix, 11.4% Sheet, 9.6% Turn, 41.2% Coil, 0.0% 3-10 Helix, and 7.9% of the model was not organized into the common motifs. Whilst, the model obtained using *E. coli* as a template analyzed by YASARA gave 24.2% Helix, 5.2% Sheet, 13.4% Turn, 50.5% Coil, and 6.7% 3-10 Helix. Unfortunately, the Ramachandran Z-scores (Hooft et al., 1997) obtained from these two models were low (model obtained using *D. radiodurans* as a template has the value of -7.192 and model using *E. coli* as a template has the value of -5.982) showing that they are not satisfactory models (Figure 2).



A



B

Figure 2. A An apicoplast ribosomal L4 protein of *Plasmodium falciparum* obtained using SWISS model and *D. radiodurans* as a template; **B** An apicoplast ribosomal L4 protein of *Plasmodium falciparum* obtained using SWISS model and *E. coli* as a template

Ab initio calculations were performed using an I-TASSER server (Roy et al., 2010; Roy et al., 2011; Zhang, 2008) by submitting the sequence of the protein to be modelled in FASTA format and using the option for modelling without the template (*ab initio*). The whole sequence of L4 ribosomal protein from *P. falciparum* apicoplast was used. The obtained model (Figure 3) shows c-score=-2.26 and Ramachandran Z-score=-4.081. Again, the model was not satisfactory because the Z-score was on the border of acceptability.

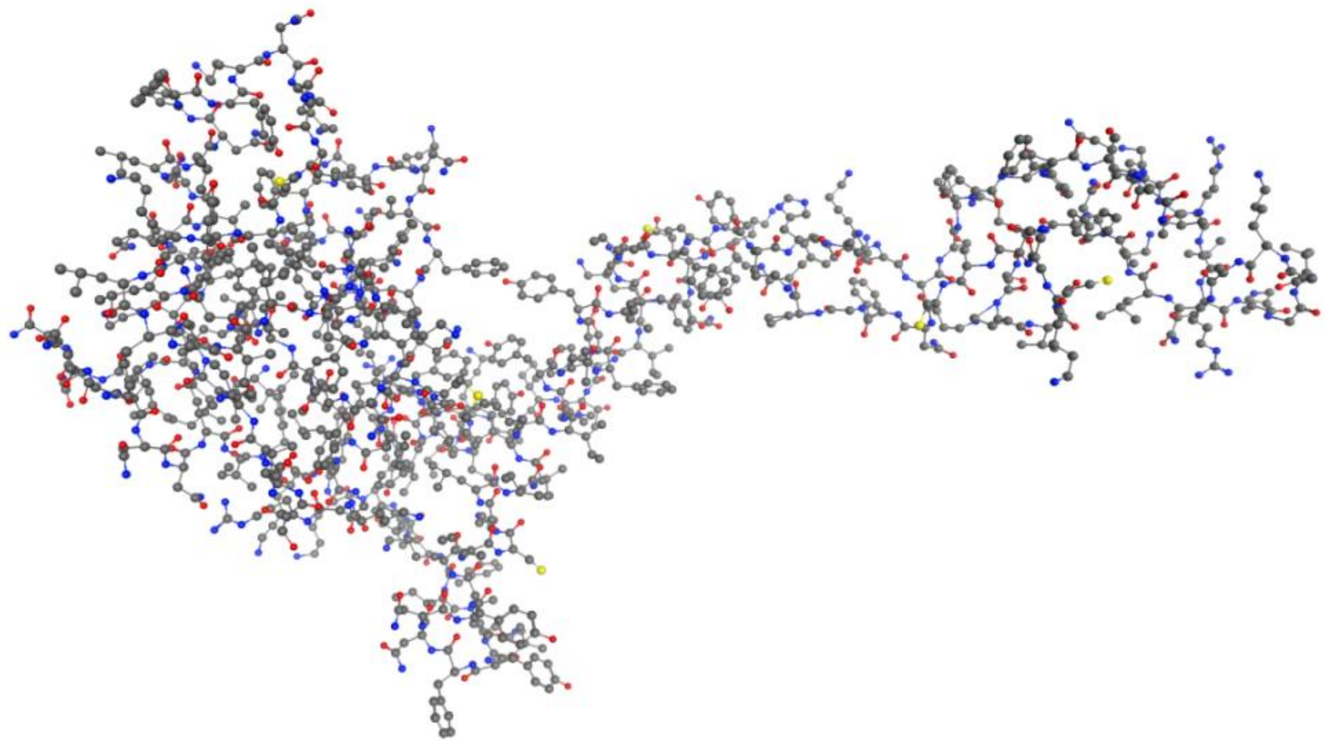


Figure 3. L4 protein from *P. falciparum* obtained using *ab initio* method on I-TASSER

In order to improve the modelling, *ab initio* modelling was used on the I-TASSER server to design the L4 ribosomal protein segment (Lys^{57} to Pro^{97}) also previously modelled by Sidhu et al. (2007). A model was obtained with a corresponding Ramachandran Z-score of -3.404, which is satisfactory (Figure 4).

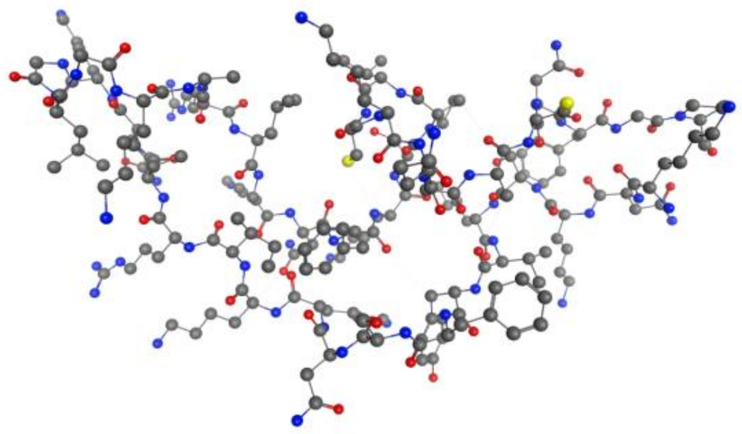


Figure 4. A segment of L4 ribosomal protein from *P. falciparum* obtained using *ab initio* method on I-TASSER

The results demonstrated that the homology modelling on the apicoplast proteins from *P. falciparum* had little similarity to the templates (*D. radiodurans* and *E. coli*) and all known and available crystal structures of L4 protein was less successful than *ab initio* modelling of the same structure. The modelled segment of L4 apicoplast ribosomal protein (Lys^{57} to Pro^{97} in *D. radiodurans*) from *P. falciparum* using *ab initio* method represents a reliable model on the basis of its Ramachandran Z-score and could be useful for the construction of the exit tunnel of the apicoplast ribosome from *P. falciparum*.

Modelling on 23S rRNA from P. falciparum

Modelling of the 23S rRNA presented an additional challenge. In order to model the sequence, due to its length the sequence had to be divided into two halves. Modelling was subsequently performed, and refinement and energy minimization of the two halves was performed separately. The division of the RNA into two halves was somewhat arbitrary and it is difficult to judge whether this necessary simplification could be justified. Unfortunately, there is no method similar to protein methods for checking the quality of generated structures. However, there was high similarity between the *P. falciparum* apicoplast LSU rRNA and the *E. coli* 23 S rRNA (they have 70% sequence identity (Sidhu et al., 2007)), particularly in hairpins; therefore, we are quite confident that the separation into two halves did not affect significantly the conformations of the hairpin regions involved in the binding to macrolides.

Comparison of the sequence and secondary structure of *P. falciparum* and organisms with known crystal structures yielded a significant similarity in domain V, but very small similarity in sequence and base pairs of domains II and IV of 23S rRNA. The final models

(Figure 5) appeared to be satisfactory, but there were concerns about the folding because of the mutual influence of one modelled 23S rRNA residue on other.

A **B**

Figure 5. A Model of large subunit ribosomal RNA-3' half of *P. falciparum*; **B** Model of large subunit ribosomal RNA-5' half of *P. falciparum*

Conclusion

We have made a first attempt to model the apicoplast ribosome exit tunnel, which may be an important drug target in the fight against malaria. *Ab initio* modelling of apicoplast ribosomal L4 gave models with acceptable Ramachandran Z-scores, whereas homology modelling did not. The challenging task of modelling the long sequence of RNA was performed by splitting the RNA into two fragments *in silico.* Comparison of the sequence and secondary structure of *P. falciparum* and organisms with known crystal structures yielded a significant similarity in domain V, but very small similarity in sequence and base pairs of domains II and IV of 23S rRNA. The obtained modelscouldbe good starting point for docking drugs.

Acknowledgment

The authors want to thank Dr Richard Bryce from The University of Manchester for provided computational facilities, and all advices given during the performed work.

Conflict-of-Interest Statement

There is no conflict of interest.

References

Arnold, K., Bordoli, L., Kopp, J., &Schwede, T. (2006). The SWISS-MODEL workspace: a web-based environment for protein structure homology modelling. Bioinformatics, 22,195-201.

Arsic, B., Awan, A., Brennan, R., Aguilar, J., Ledder, R., McBain, A. J., Regan, A. C., & Barber, J. (2014). Theoretical and experimental investigation on clarithromycin, erythromycin A and azithromycin and descladinosyl derivatives of clarithromycin and azithromycin with 3-*O*-substitution as anti-bacterial agents. MedChemComm, 5(9), 1347-1354.

Clastre, M., Goubard, A., Prel, A., Mincheva, Z., Viaud-Massuart, M.-C., Bout, D., Rideau, M., Velge-Roussel, F., &Laurent, F. (2007). The methylerythritol phosphate pathway for isoprenoid biosynthesis in coccidia: Presence and sensitivity to fosmidomycin. Experimental Parasitology, 116, 375-384.

Clough, B., Rangachari, K., Strath, M., Preiser, P. R., & Wilson, R. (1999). Antibiotic inhibitors of organellar protein synthesis in *Plasmodium falciparum*. Protist, 150, 189-195.

Dahl, E. L., & Rosenthal, P. J. (2007). Multiple antibiotics exert delayed effects against the *Plasmodium falciparum* apicoplast. Antimicrobial Agents and Chemotherapy, 51, 3485-3490.

Gleeson, M. T. (2000). The plastid in Apicomplexa: what use is it?International Journal of Parasitology, 30, 1053-1070.

Gozalbes, R., Brun-Pascaud, M., Garcia-Domenech, R., Galvez, J., Girard, P.-M., Doucet, J.-P., & Derouin, F. (2000). Anti-*Toxoplasma* activities of 24 quinolones and fluoroquinolones in vitro: prediction of activity by molecular topology and virtual computational techniques. Antimicrobial Agents and Chemotherapy, 44, 2771-2776.

Harms, J. M., Wilson, D. N., Schluenzen, F., Connell, S. R., Stachelhaus, T., Zaborowska, Z., Spahn, C. M. T, &Fucini P. (2008). Translational regulation *via* L11: Molecular switches on the ribosome turned on and off by Thiostrepton and Micrococcin. Molecular Cell, 30, 26-38.

Hooft, R. W., Sander, C., &Vriend, G. (1997). Objectively judging the quality of a protein structure from a Ramachandran plot. Computer Applications in the Biosciences, 13, 425-430.

http://www-lmmb.ncifcrf.gov/~bshapiro/software.html, accessed 19/11/2018

https://pymol.org/2/, accessed 19/11/2018

https://www.who.int/malaria/en/, accessed 24/09/2019

Jomaa, H., Wiesner, J., Sanderbrand, S., Altincicek, B., Weidemeyer, C., Hintz, M., Turbachova, I., Eberl, M., Zeidler, J., Lichtenthaler, H. K., Soldati, D., & Beck, E. (1999). Inhibitors of the nonmevalonatepathway of isoprenoid biosynthesis as antimalarial drugs. Science, 285, 1573-1576.

McConkey, G.A., Rogers, M. J., &McCutchan, T. F. (1997). Inhibition of *Plasmodium falciparum* protein synthesis. Targeting the plastid-like organelle with thiostrepton. The Journal of Biological Chemistry, 272, 2046-2049.

McConkey,G. A., Rogers, M. J., &McCutchan, T. F. (1997). Inhibition of *Plasmodium falciparum* protein synthesis. The Journal of Biological Chemistry, 272, 2046-2049.

Mitra, K.,Schaffitzel, C., Fabiola, F., Chapman, M. S., Ban, N., & Frank, J. (2006). Elongation arrest by SecM*via* a cascade of ribosomal RNA rearrangements. Molecular Cell, 22, 533-543.

Moorthy, V. S., &Okwo-Bele, J-M. (2015). Final results from a pivotal phase 3 malaria vaccine trial. Lancet, 386, 5-7.

Pfefferkorn, E. R., &Borotz, S. E. (1994). Comparison of mutants of *Toxoplasma gondii* selected for resistance to azithromycin, spiramycin, or clindamycin. Antimicrobial Agents and Chemotherapy, 338, 31-37.

Pukrittayakamee, S., Viravan, C., Charoenlarp, P., Yeamput, C., Wilson, R. J. M., & White, N. J. (1994). Antimalarial effects of Rifampin in *Plasmodium vivax*malaria. Antimicrobial Agents and Chemotherapy, 38, 511-514.

Ralph, S. A., D'Ombrain, M. C., & McFadden, G. I. (2001). The apicoplast as an antimalarial drug target.Drug Resistance Update, 4, 145-151.

Roberts, F., Roberts, C. W., Johnson, J. J., Kyle, D. E., Krell, T., Coggins, J. R., Coombs, G. H., Milhous, W. K., Tzipori, S., Ferguson, D. J. P., Chakrabarti, D., &McLeod, R. (1998). Evidence for the shikimate pathway in apicomplexan parasites. Nature, 393, 801-805.

Rogers, M. J., Cundliffe, E., &McCutchan, T. F. (1998). The antibiotic Micrococcinis a potent inhibitor of growth and protein synthesis in the malaria parasite. Antimicrobial Agents and Chemotherapy, 42, 715-716.

Roy, A., Kucukural, A., & Zhang, Y. (2010). I-TASSER: a unified platform for automated protein structure and function prediction. Nature Protocols, 5, 725-738.

Roy, A., Xu, D., Poisson, J., & Zhang, Y. (2011). A protocol for computer-based protein structure and function prediction. Journal of Visualized Experiments, 57, e3259.

RTS,S Clinical Trials Partnership. (2015). Efficacy and safety of RTS,S/AS01 malaria vaccine with or without a booster dose in infants and children in Africa: Final results of a phase 3, individually randomized, controlled trial. The Lancet, 386 (9988), 31-45.

Schlunzen, F., Harms J. M., Franceschi, F., Hansen, H. A. S., Bartels, H., Zarivach, R., &Yonath, A. (2003). Structural basis for the antibiotic activity of ketolides and azalides. Structure, 11, 329-338.

Sidhu, A. B. S., Sun, Q., Nkrumah, L. J., Dunne, M. W., Sacchettini, J. C., & Fidock, D. A. (2007). *In vitro* efficacy, resistance selection, and structural modelling studies implicate the malarial parasite apicoplast as the target of azithromycin. The Journal of Biological Chemistry, 282, 2494-2504.

Surolia, N., & Surolia, A. (2001). Triclosan offers protection against blood stages of malaria byinhibiting enoyl-ACP reductase of *Plasmodium falciparum*. Nature Medicine, 7, 167-173.

Uddin, T., McFadden, G.I., & Goodman, C.D. (2018). Validation of putative apicoplast-targeting drugs using a chemical supplementation assay in cultured human malaria parasites. Antimicrobial Agents and Chemotherapy, 62 (1), e01161-17.

Woods, K. M., Nesterenko, M. V., &Upton, S. J. (1996). Efficacy of 101 antimicrobials and other agents on the development of *Cryptosporidium parvumin vitro*. Annals of Tropical Medicine and Parasitology, 90, 603-615.

World Health Organization. (2019). World malaria Report 2019.

www.yasara.org, accessed 19/11/2018

Yeo, A. E. T., & Rieckmann, K. H. (1995). Increased antimalarial activity of azithromycin during prolonged exposure of *Plasmodium falciparum in vitro.* International Journal of Parasitology, 25, 531-532.

Zhang, Y. (2008). I-TASSER server for protein 3D structure prediction. BMC Bioinformatics, 9, 40.

In silico istraživanje na L4 ribozomalnom proteinu i tri domena iz 23S rRNA apikoplasta iz *Plasmodium falciparum-a* i upoređivanje sa postojećim ko-kristalnim strukturama

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SAŽETAK

Sproveli smo preliminarna kompjuterska istraživanja u vezi s konstrukcijom segmenta ribozomalnog proteina L4 iz ribozoma apikoplasta iz *Plasmodium falciparum*-a. To je, verovatno, dosad najbolje konstruisani model ove mete sa Z-skorom-3.404. Tri domena 23S rRNA: II, IV i V, napravljena su bez šeme, korišćenjem softvera RNA2D3D. Oni nisu validirani, ali pokazuju značajnu sličnost sa bakterijskom 23S rRNA. Ovaj model poseduje tehnička ograničenja, ali je početna tačka za poboljšane modele od kojih se očekuje da nađu primenu u dizajnu antimalarijskog leka.

Ključne reči: *in sillico, Plasmodium falciparum,* ribozom

Étude *in silico* sur la protéine ribosomale apicoplaste L4 et les trois domaines de l'ARNr 23S du *Plasmodium falciparum* et la comparaison avec les structures du co-cristal existant

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Résumé

Nous avons effectué les études informatiques préliminaires concernant la construction d'un segment de la protéine ribosomique L4 à partir du ribosome apicoplaste de *Plasmodium falciparum*. Avec un score Z de -3,404, c'est sans doute le modèle le mieux construit de cette cible médicamenteuse jusqu'à présent. Les trois domaines de l'ARNr 23S ont été créés à partir de zéro en utilisant le logiciel RNA2D3D: les domaines II, IV et V. Ils n'ont pas été validés, mais ils présentent une similitude raisonnable avec l'ARNr 23S bactérien. Ce modèle a des limites techniques, mais il sert de point de départ pour les modèles raffinés qui, pour leur part, devraient trouver une utilisation dans la conception de médicaments antipaludiques.

Mots-clés: *in sillico*, *Plasmodium falciparum*, ribosome

In silico исследование рибосомального белка апикопласта L4 и трех доменов из 23S рРНК из *Plasmodium falciparum* и сравнение с существующими сокристаллическими структурами

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Резюме

Мы провели предварительные вычислительные исследования по построению сегмента рибосомного белка L4 из рибосомы апикопласта *Plasmodium falciparum*. С Z-баллом -3.404 это, пожалуй, лучшая модель этой мишени для лекарств на данный момент. Три домена из 23С пПНК были созданы с нуля с использованием программного обеспечения RNA2D3D: домены II, IV и V. Они не были подтверждены, но демонстрируют разумное сходство с бактериальной 23S рРНК. Эта модель имеет технические ограничения, но является отправной точкой; Ожидается, что изысканные модели найдут применение в разработке противмалярийных препаратов.

Ключевые слова: in silico, Plasmodium falciparum, рибосома

In-Silico-Studie zum ribosomalen Apikoplast-L4-Protein und zu drei Domänen aus 23s rRNA aus *Plasmodium Falciparum* und Vergleich mit den vorhandenen Kokristallstrukturen

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ABSTRAKT

Wir führten vorläufige Computerstudien zur Konstruktion eines Segmentes des ribosomalen Proteins L4 aus dem Ribosom des Apikoplasten aus *Plasmodium falciparum* durch. Mit einem Z-Score von -3,404 ist es wohl das am besten konstruierte Modell für dieses Medikamentenziel. Drei 23S-rRNA-Domänen, II, IV und V, wurden ohne Schema unter Verwendung von RNA2D3D-Software hergestellt. Sie wurden nicht validiert, weisen jedoch eine deutliche Ähnlichkeit mit der bakteriellen 23S-rRNA auf. Dieses Modell weist technische Einschränkungen auf, ist jedoch ein Ausgangspunkt für verfeinerte Modelle, von denen erwartet wird, dass sie beim Design von Antimalariamedikamenten Anwendung finden.

Schlüsselwörter: in sillico, Plasmodium falciparum, Ribosom

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Analytical Problem-Solving Procedures for Undergraduates by ^{1}H NMR

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ABSTRACT

^{1}H NMR Spectroscopy is widely used technique, but until recently was of limited practical importance in pharmaceutical and chemical education. Teaching^{1}H NMR spectroscopy remains a challenge in all the chemistry labs, as the number of facts obtained from each experiment is easily overwhelming for the students. We developed four different experimental settings for the undergraduates which connect interdisciplinary problem-solving approaches with the hands-on experience in NMR. The set of the experiments consists of amino acids identification, log*P* value determination, quantitative determination of the marketed over the counter drugs, and p*K*a value determination. We could show that our approach to teach NMR has significantly improved the understanding of the technique among our students.

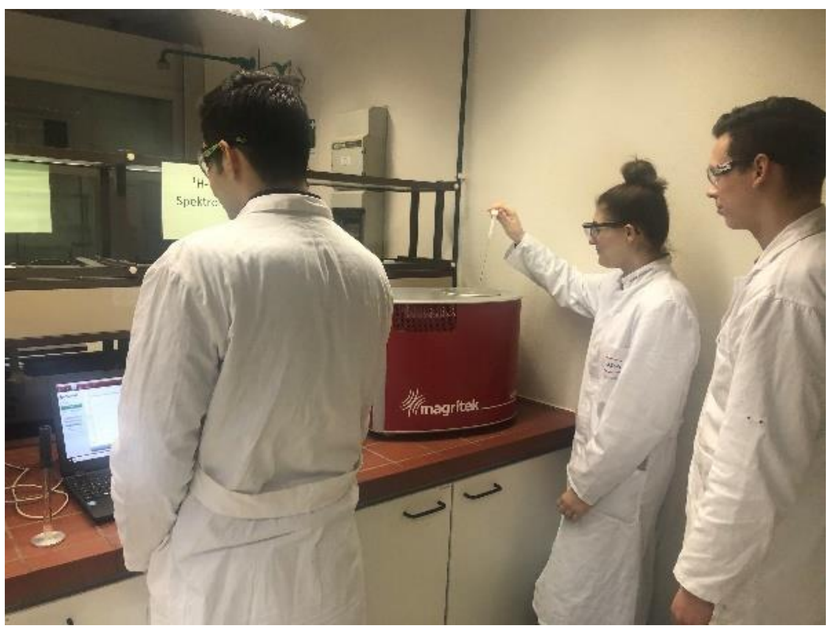

Photo: A. Zivkovic

Keywords: ^{1}H NMR, hands on experience, analytical chemistry, problem solving approach, qualitative analysis, quantitative analysis

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Introduction

Nuclear Magnetic Resonance spectroscopy (NMR) is a fundamental identification and structural determination technique in any chemical lab. This technique became more important with its introduction in the European Pharmacopoeia (*Ph. Eur)*. In 2008 about 900 cases of adverse events associated with the use of heparin were reported in Germany, resulting in the urgent need for increased analytical/quality control of complex compounds (Beyer et al., 2010). As a necessity, the first NMR-based identification control *via* ^{1}H NMR measurement has been introduced into *Ph. Eur*. (Beyer et al., 2010). This highlights the need for teaching NMR techniques in pharmacy and related sciences. Due to the high costs of NMR instrumentations, teaching NMR in the majority of academic practical courses in analytics has for a long time been restricted on the theoretical evaluation of NMR spectra, which have been recorded by technicians or other scientists, but not by the students themselves. Although high-field NMR spectrometers up to 1000 MHz or higher are extremely expensive to purchase as well as to run, the practical student courses can nowadays take advantage of the availability of low-field benchtop spectrometers (Anon n.d.).This technique development made experiments in NMR for students possible having a great variety of analytical aspects (Edgar et al., 2019; Kennedy et al., 2019; Kent and Bell 2019; Swartz et al., 2018; Yearty et al., 2017; Yennie et al., 2017). Recently, we developed and performed four different experiments using ^{1}H NMR benchtop instruments in the practical instrumental analytics course for undergraduate pharmacy students. The experiments are part of an interactive scientific puzzle where each element supports theunderstanding of a set of information obtained from ^{1}H NMR measurements. Identification of natural amino acids consists of using increment calculations, multiplicity predictions and chemical shifts in order to identify an unknown amino acid (Zivkovicet al., 2017). Another experiment consists of log*P* determination, where signal integration is used for the calculation of lipophilicity (Soulsby and Chica, 2017; Zivkovic et al., 2018). Furthermore, for the purpose of understanding the relative signal integration, the experiment where the quantitative analysis of multicomponent mixtures of over-the-counter (OTC) pain killer drugs as an example for nonsteroidal anti-inflammatory drugs (NSAIDs) is determined (Zivkovicet al., 2017). In order to deepen the understanding of the connection betweenacidity as altered protonation level and chemical shift, we developed an experiment where the p*Ka* value of the known drugs is determined (Zivkovicet al., 2017). Pedagogical goals of the battery experiments are to learn problem-based interpretation of ^{1}H NMR data (in one or two dimensions (COSY)), to process their data, understand the connection between chemical character and chemical shift, understand integration, use of D_2O exchange as well as in which way one can use the ^{1}H NMR for quantitative evaluation. Interdisciplinary, problem-orientedgroup learning should also increase motivation for learning.

Experimental

All of the measurements have been performed on the Magritek Spinsolve Benchtop (42.5 MHz) (Aachen, Germany) with the following resolution parameters: 50% linewidth <0.7 Hz (16 ppb), 0.55% line width<20 Hz. The measurements have been done in standard 5 mm NMR tubes in either D_2O for amino acids identification and p*Ka* determination, H_2O for log*P* value determination or DMSO-d_6 for qualitative and quantitative analysis of the OTC mixtures (and subsequent addition of D_2O).

The procedures are simple and can be performed and reproduced by inexperienced lab students. Undergraduate students have performed all parts of the experiment: dissolution, measurement, processing of the data (using MNova software) and identification.

As first milestone in a battery of experiments, the students have identified an unknown amino acid. We developed(Zivkovicet al., 2017) an easy algorithm thatstudents can follow (Figure 1).

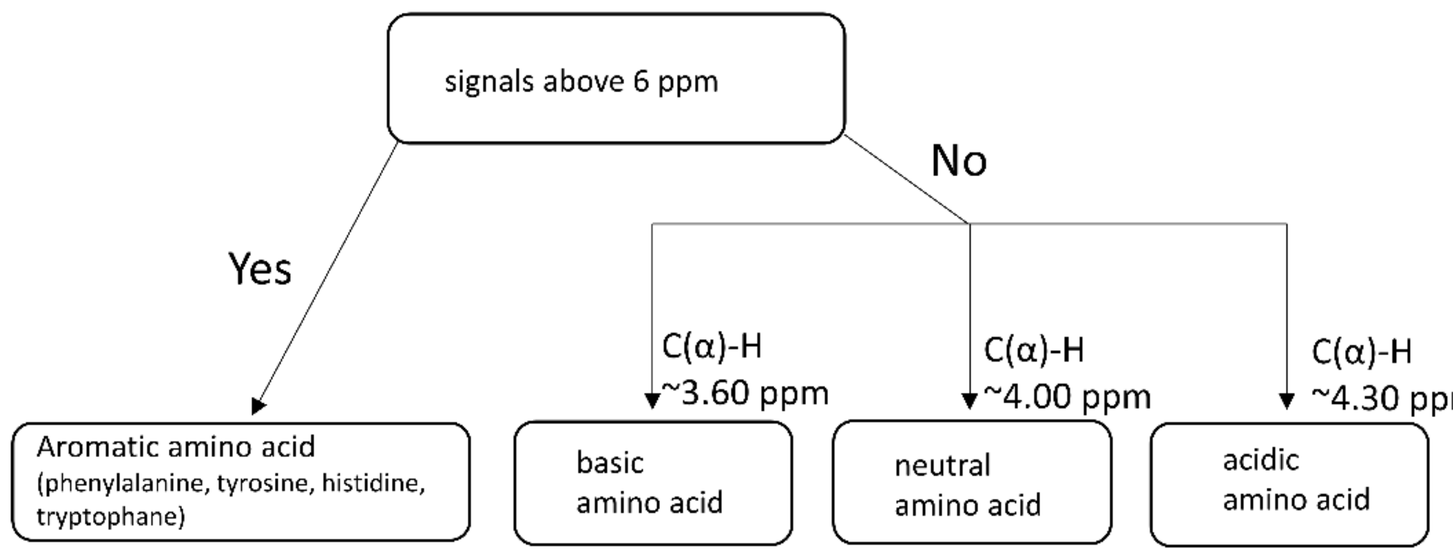
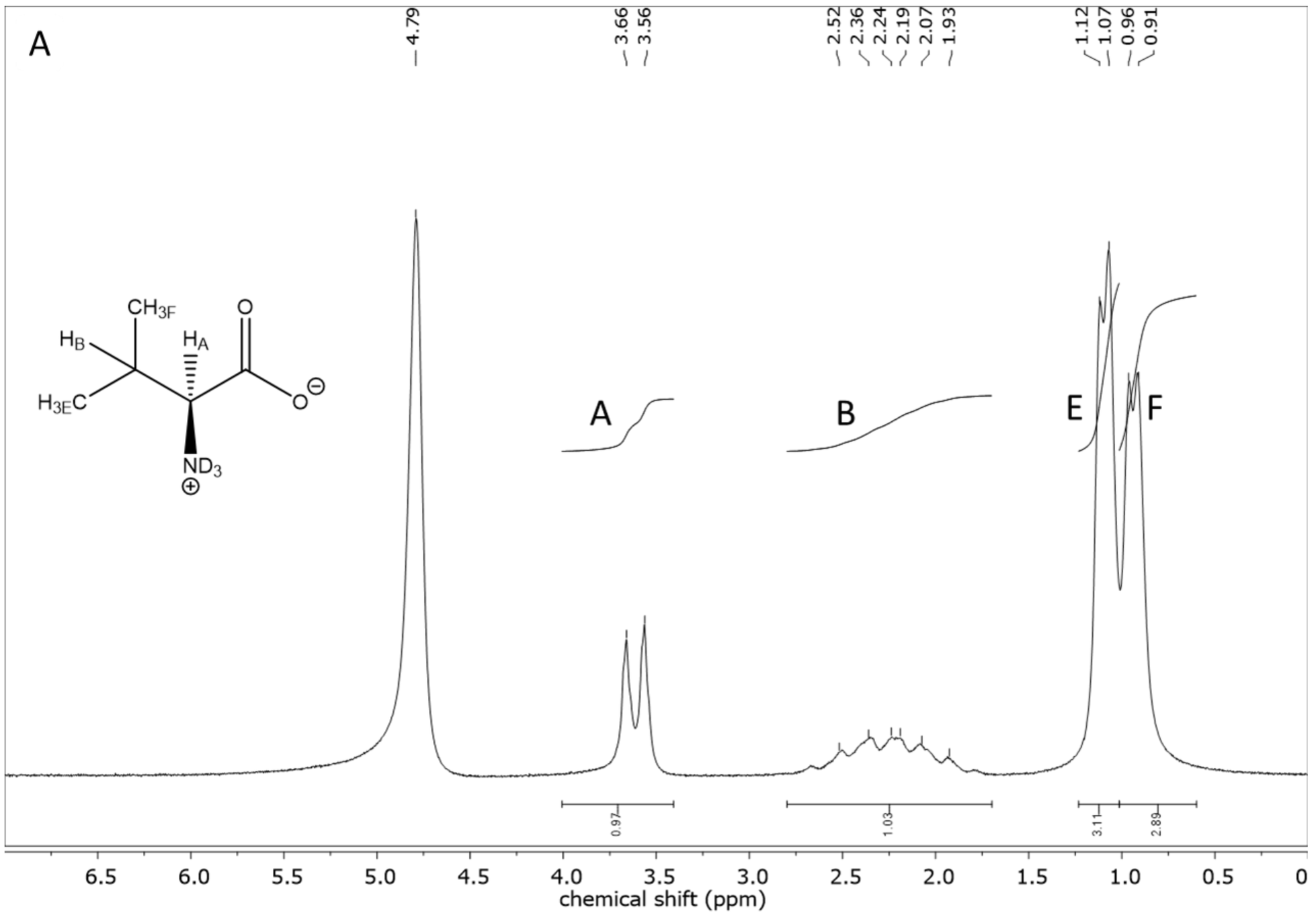


Figure 1. Algorithm for amino acidsidentification

To illustrate our measurements, we demonstratein Figure 2 the ^{1}HNMR spectrum of L-valine (20 mgmL^{-1} in D_2O), with ^{1}H-^{1}H COSY measurement.


A
4.79
3.66
3.56
2.52
2.36
2.24
2.19
2.07
1.93
1.12
1.07
0.96
0.91
CH3F
HB
HA
H3EC
O
O
ND3
A
B
E
F
0.97
1.03
3.11
2.89
6.5
6.0
5.5
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2.0
1.5
1.0
0.5
0
chemical shift (ppm)

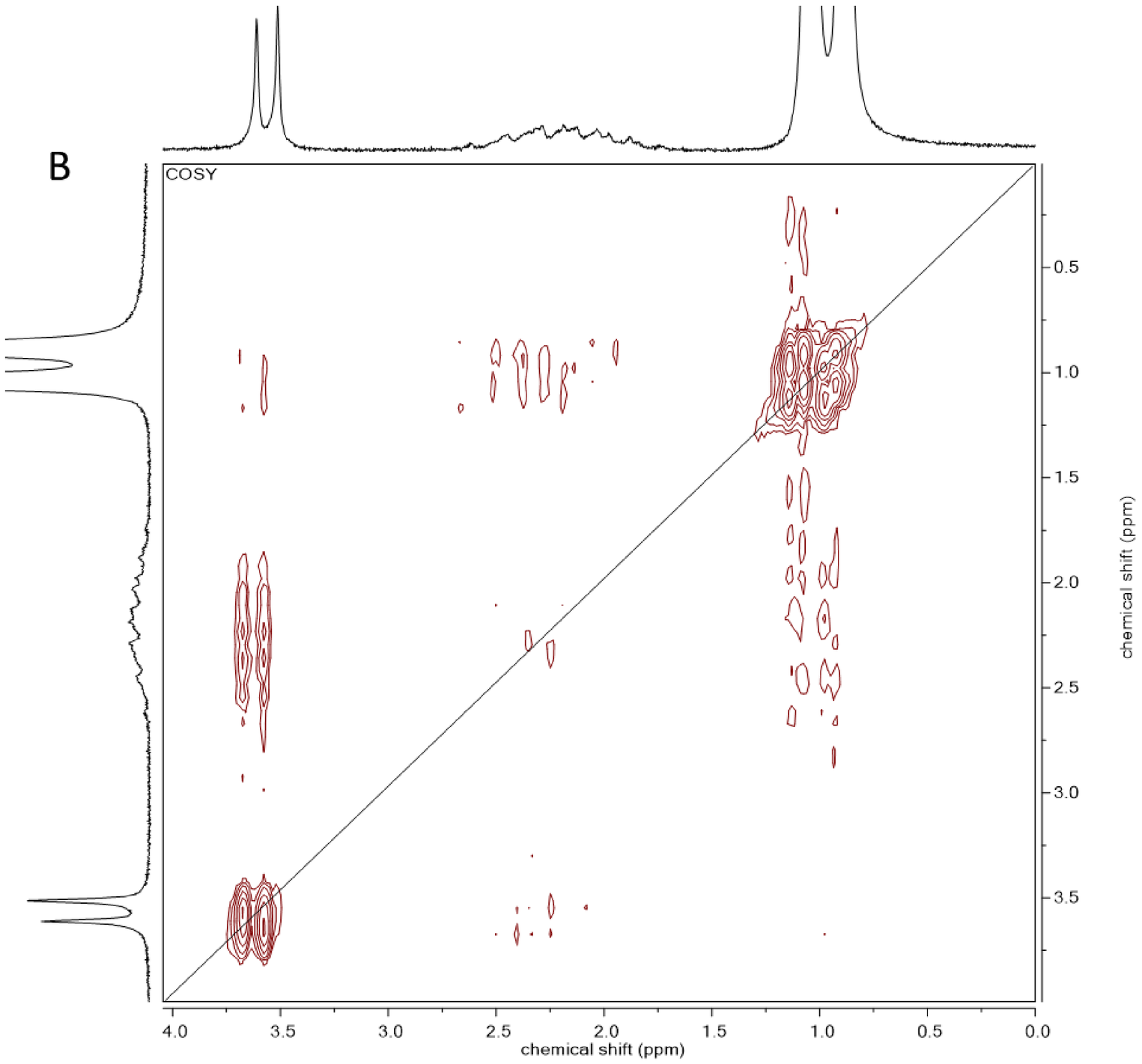

Figure 2. ^{1}HNMR (A) and ^{1}H-^{1}H COSY (B) measurement of L-valine in D_2O (~20 mgmL^{-1}).

In the second experiment, the students determined the log*P* value of one of the common solvents (acetone, methanol, ethanol, dimethylformamide,*etc.*). The log*P* parameter together with the p*K*a value is of high importance in the design and synthesis ofpharmaceuticallyactive compounds, especially for lipophilicity, solubility, protein binding, and permeability (Manallack, 2007; Manallack et al., 2014). To keep an experiment simple for the beginners, ithas been done with frequently used solventsin water and water/octanol mixture. In the first measurement,^{1}H NMR of water and the solvent mixture has been measured. Then the integration has been done to the water peak integral as a reference, which is always set at 1.000.00. After thismeasurement, 1-octanol has been added to the mixture, shaken and after separation of the phases directly transferred into the NMR tube, the second measurement has been done. When water is integrated on the same value (1.000.00), the integral reduction of the solvent (from which log*P* is determined) fits to the concentration that is now present in 1-octanol.The experiment is replicated thrice. The mean value and the standard deviation are calculated. The measurement of ethanol in water and water/octanol mixture is shownin Figure 3. The log*P* value was calculated as follows (A_w (solvent integral in water) and A_{ow} (solvent integral in water/octanol) are taken from the NMR in Figure 3):

$$logP = log\left(\frac{A_w - A_{ow}}{A_{ow}}\right)$$

$$\log P = log\left(\frac{203.18 - 136.50}{136.50}\right) = -0.31$$

The log*P* value obtained corresponds with literature data for ethanol log*P* values (Erhart et al., 2015; Wasserkort and Koller, 1997).

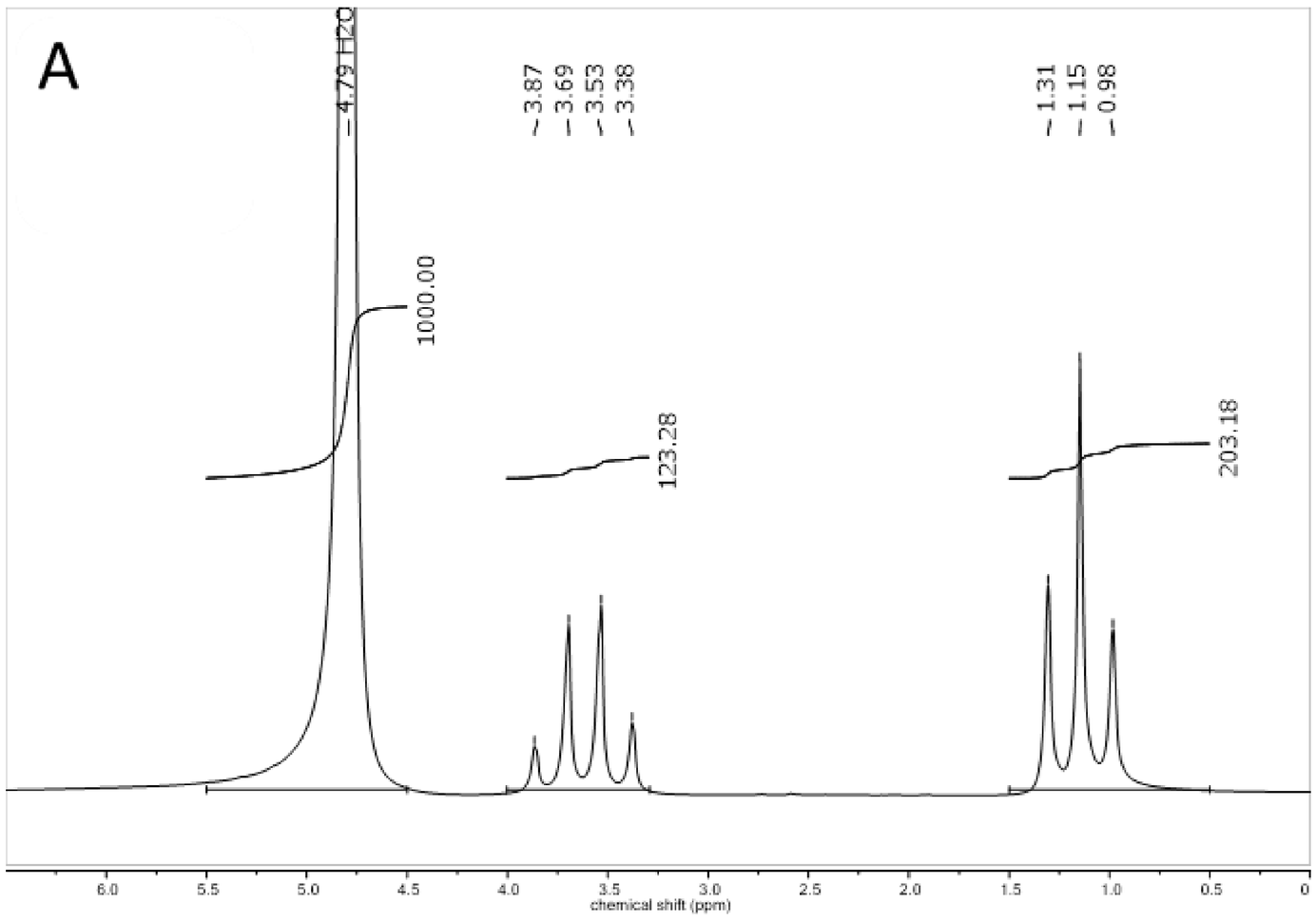

Figure 3.[1]HNMR measurement of ethanol in water (A) and water/octanol (B)

A slightly moreadvanced experiment (performed as the third in this series) is the qualitative and quantitative determination of one compound and two-component mixtures using common OTC drugs as shown in Figure4.

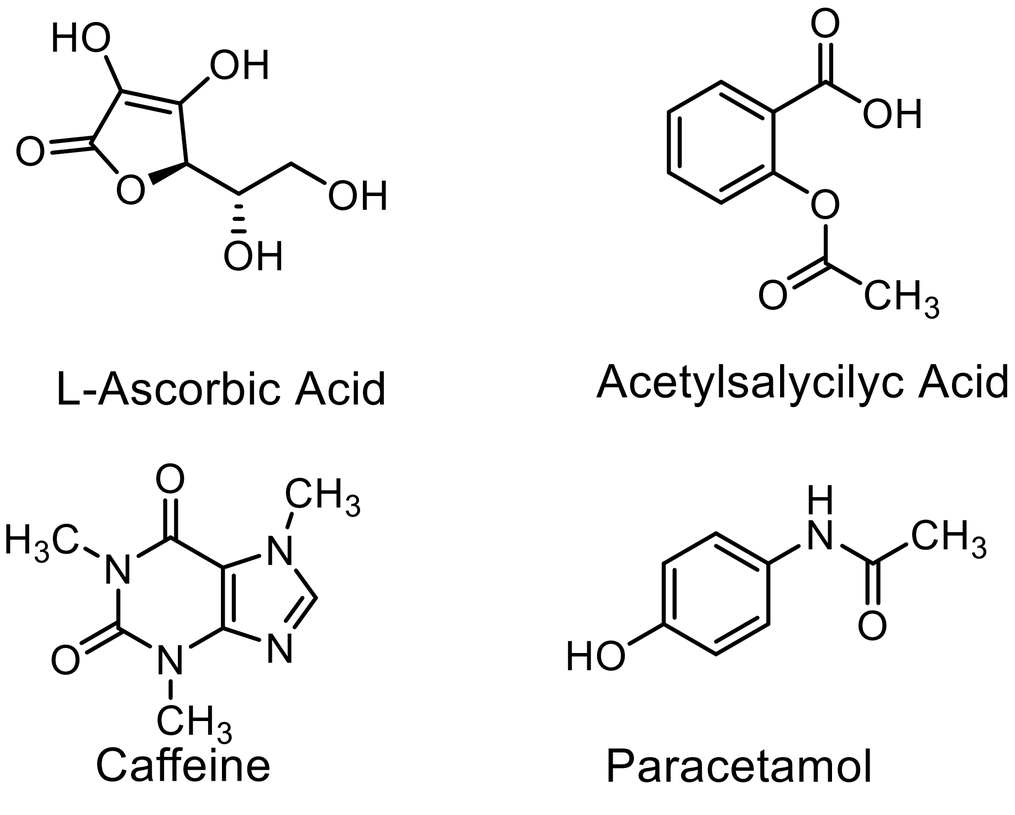

Figure 4. The structures of the common OTCs in quantitative determination

In this analysis step, it is important tofirst identify the substances in the mixture and assign all the signals (in both NMRs, with and without D_2O) to the correspondingprotons in the structures. After this, the students need to choose one signal from each OTC drug that is going to be used for relative quantitative determination. The chosen NMR signal cannot be exchangeable with D_2O;it has to be isolated and clearly has to belong to only one of the substances. If there are more than one possibility, the signal belonging to the largest number of protons should be chosen. The signal/noise ratio would have the smallest influence on the result, so these selection criteria are the most efficient. In this challenging experiment for NMR beginners, each group has to find the problem solving way on their own, the best for their measurements (Zivkovic et al., 2017). One measurement of a compound mixture with the corresponding calculations from the quantification is shown in Figure 5 and Table 1.

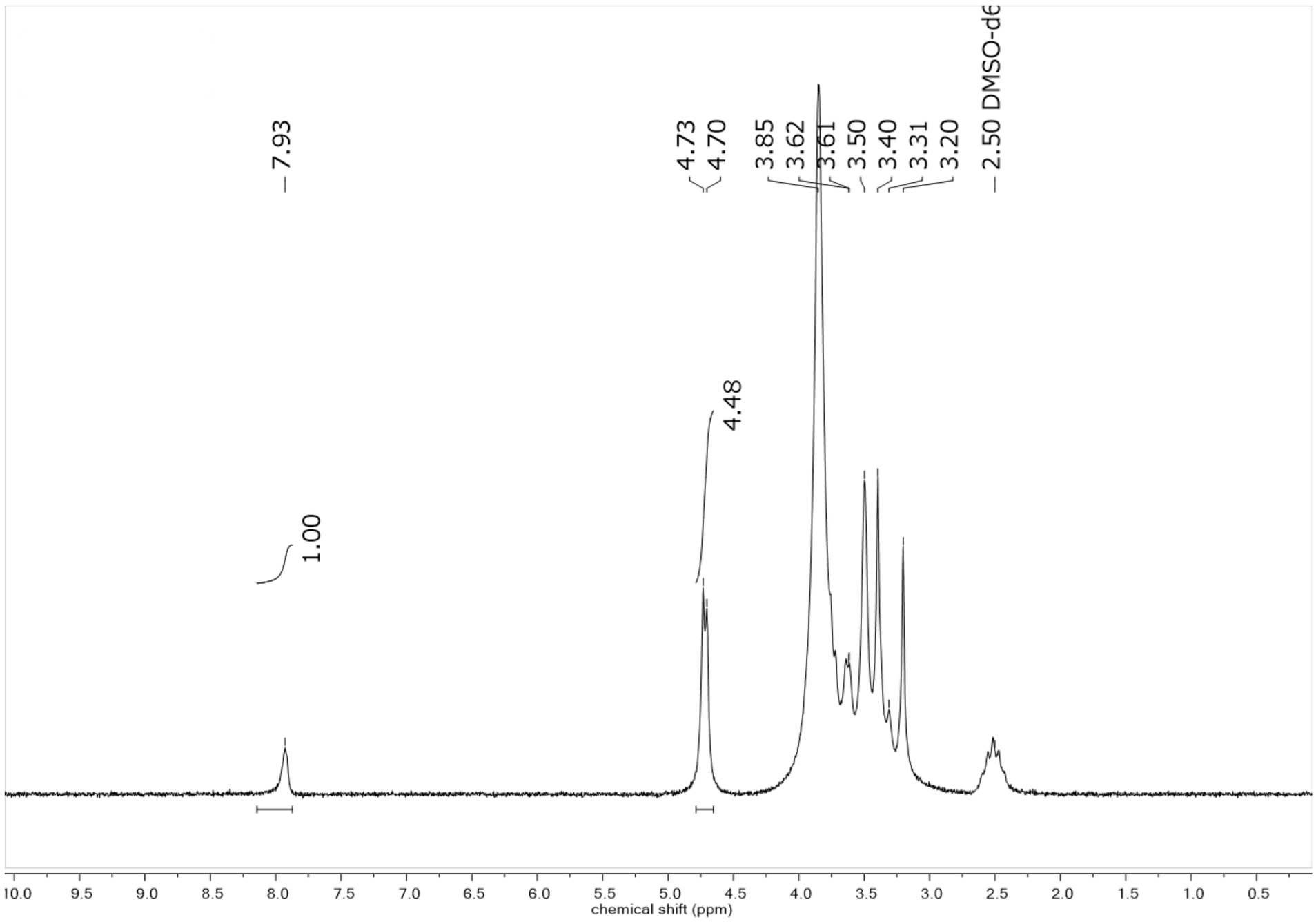

Figure 5. ^{1}H NMR measurement of the mixture consisting of caffeine (20%) and L-ascorbic acid (80%) in DMSO-d_6 (~20 mgmL^{-1}) after addition of few drops of D_2O (exchangeable protons)

The aromatic proton in caffeine (at 7.93 ppm) and the doublet signal of one proton at 4.72 ppm is used for the quantification in this mixture.

Table 1. Quantification from the ^{1}H NMR measurement of the mixture in Figure 5

Compound	*L-ascorbic acid*	*Caffeine*
Molecular Weight (Mr)	176.1	194.2
Integral Height (I)	4.48	1.00
Number of Protons (P) that belongs to Integral (I)	1	1
Ratio I/P	4.48	1.00
Mass Ratio (Mr•I/P)	789.0	194.2
Mass Ratio in %	80.2	19.8
Actual Composition (mg)	80	20

In the last experiment in ^{1}H NMR spectroscopy in this series, the students have determined the p*K*a value of one of the following drugs: nicotinamide, isoniazid and pyridoxine hydrochloride. Therefore, the change in the chemical shift in one of the aromatic protons was documented upon p*H* change (achieved with the addition of either acid or base). Results were represented as the function of the chemical shift (ppm) of p*D* value (Figure 6) (De Almeida Drumond Dos Santos et al., 2010; Gift et al., 2012; Mumcu and Küçükbay, 2015; Zivkovicet al., 2017). The titration curve was evaluated in the form of a classical titration curve with MS-Excel or related programs. The obtained results for all the probes agree with literature values for the corresponding drugs (Becker et al., 2007; De Almeida Drumond Dos Santos et al., 2010; Perrin, 1969)

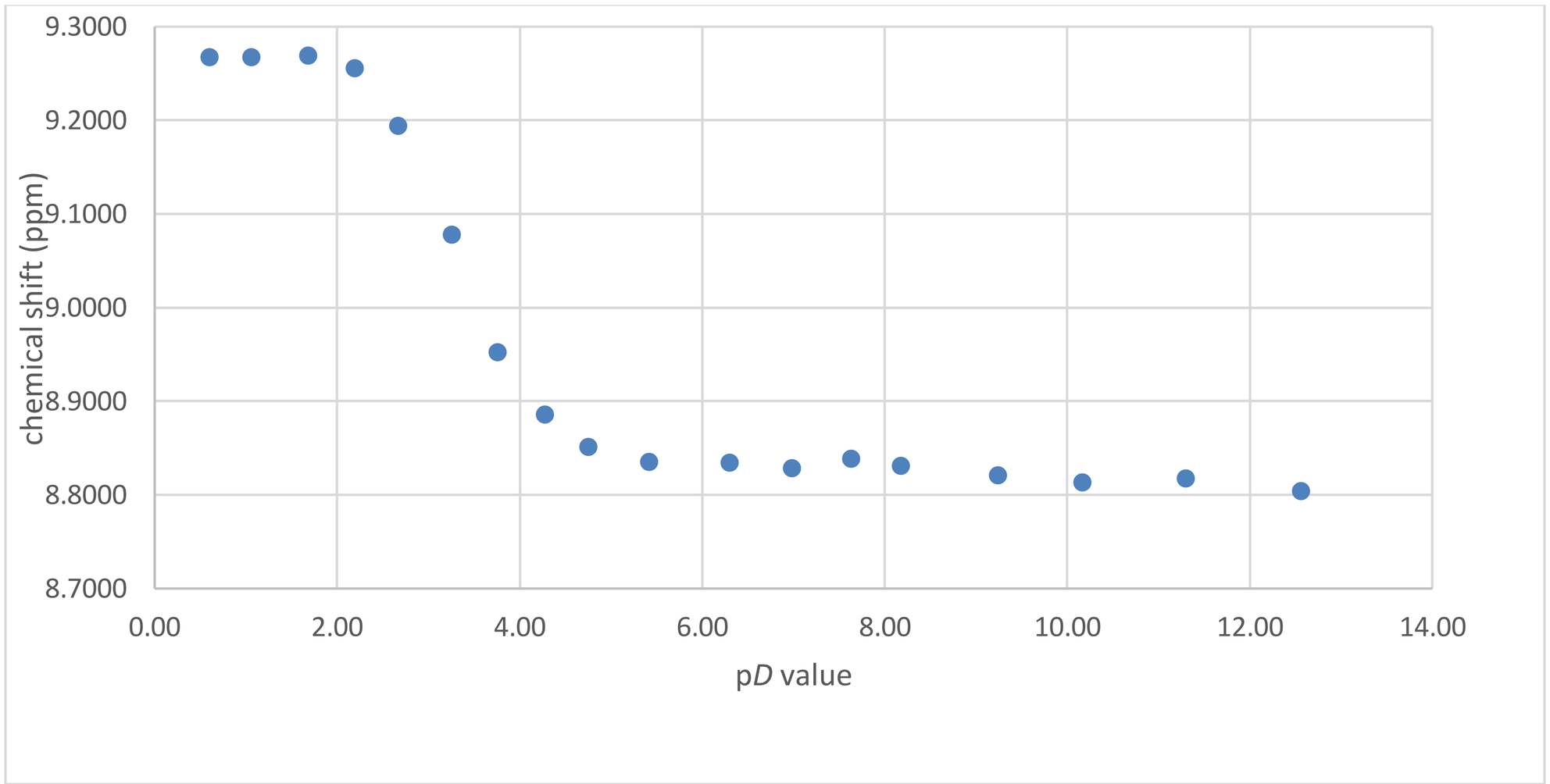

Figure 6. An example plot of the ^{1}H NMR chemical shift of aromatic proton of nicotinamide as a function of p*D*

From the titration curve in Figure 6, the calculated pKa value of 3.54 was in good accordance with the literature value of 3.35 (Perrin, 1969).

Results and Discussion

We have developed and implemented four experiments with different difficulty level in the undergraduate pharmacy student's lab that are easily transferable to related compound studies in other scientific fields. In the last five years more than 500 students performed the experiments. Each of the experiments was conducted up to today more than 400 times. The first three experiments, amino acid identification, log*P* determination and quantification of the mixtures,were performed in one 5 h laboratory course, and the pKa determination in the second 5 h course. Amino acid determination was incorrect on the first try in 13 determinations (3.1%). In the second try all of the students have correctly determined the natural amino acid. In the log*P* determination experiments, of all the determinations performed, 12.5%

had to be repeated, mostly due to the incorrect experimental performance. In the slightly more sophisticated experiment with OTC drugs, the correct mixture composition was given only once improperly. The students' quantification evaluation failed in 20.1% of cases. Here one should mention that not all of the mixtures show the same tendency for a failure, mostly depending on the signal overlap and student detection of exchangeable protons. In the second try, only 2.1% of the students failed, and on the third try, all of the determinations were done properly. The p*Ka* determination experiment requires precise and focused practical work.

Conclusion

We were able to develop and perform four experiments in order to deepen our students'understanding of ^{1}H NMR principles as well as potential applications. After the introduction of spectroscopy experience in practice, the average of 56% points in the NMR spectroscopy questioning (test/exam) increased to almost 81%. Pharmacy students in Germany take the state exam in instrumental analytics after the fourth semester, where approximately ten of the 45 questions are NMR related. Since the introduction of the experiment into our lab course, we observe that at the NMR state exam, our students achievestatistically significantly better results than the average (all pharmacy faculties in Germany).

Acknowledgment

Funding of this project was kindly provided by the fund for teaching support (Lehrförderfonds) of Heinrich Heine University Düsseldorf, number L020401/15. We also thank all our students for theirexperimental data, especially Jan Josef Bandolik, Alexander Skerhut and Christina Coesfeld for developing the experiments.

Conflict-of-Interest Statement

The authors declare no competing financial interests.

References

De Almeida Drumond Dos Santos, T., Oliveira Da Costa, D., Silva Da Rocha Pita, S., &Semaan, F. S. (2010). Potentiometric and conductimetric studies of chemical equilibria for pyridoxine hydrochloride in aqueous solutions: simple experimental determination of pKa values and analytical applications to pharmaceutical analysis. Ecletica Quimica, 35(4), 81–86.

Anon. n.d. Benchtop Nuclear Magnetic Resonance Spectrometer - Wikipedia.Retrieved December 10, 2019 (https://en.wikipedia.org/wiki/Benchtop_nuclear_magnetic_resonance_spectrometer).

Becker, C., Dressman, J. B., Amidon, G. L., Junginger, H. E., Kopp, S., Midha, K. K., Shah,

V.,Stavchansky, S.,&Barends, D. M. (2007). Biowaiver monographs for immediate release solid oral dosage forms: isoniazid. Journal of Pharmaceutical Sciences, 96(3), 522–531.

Beyer, T., Matz, M., Brinz, D., Rädler, O., Wolf, B., Norwig, J., Baumann, K., Alban, S., &Holzgrabe, U. (2010). Composition of OSCS-contaminated heparin occurring in 2008 in batches on the German market. European Journal of Pharmaceutical Sciences, 40(4), 297–304.

Edgar, M., Percival, B. C., Gibson, M., Masania, J., Beresford, K., Wilson, P. B., &Grootveld, M. (2019). Benchtop NMR spectroscopy and spectral analysis of the *cis*- and *trans*-stilbene products of the Wittig reaction. Journal of Chemical Education, 96(9), 1938–1947.

Erhart, S. E., McCarrick, R. M., Lorigan, G. A., & Yezierski, E. J. (2016). Citrus quality control: an NMR/MRI problem-based experiment. Journal of Chemical Education, 93 (2), 335-339.

Gift, A. D., Stewart, S. M., & Bokashanga, P. K. (2012). Experimental determination of pKa values by use of NMR chemical shifts, revisited. Journal of Chemical Education, 89(2), 1458–1460.

Kennedy, J. S., Larson, G. E., Blumenfeld, A., &Waynant, K. V. (2020). Carbohydrate characterization through multidimensional NMR: an undergraduate organic laboratory experiment. Journal of Chemical Education, 97 (1), 195-199.

Kent, J. E., & Bell, N. G. A. (2019). Molecular properties of caffeine explored by NMR: a benchtop NMR experiment for undergraduate physical-chemistry laboratories. Journal of Chemical Education, 96(4), 786–791.

Manallack, D. T. (2007). The pK(a) distribution of drugs: application to drug discovery. Perspectives in Medicinal Chemistry, 1, 25–38.

Manallack, D. T., Prankerd, R. J., Yuriev, E., Oprea, T. I., & Chalmers, D. K. (2014). The significance of acid/base properties in drug discovery. Chemical Society Reviews, 42(2), 485–496.

Mumcu, A., & Küçükbay, H. (2015). Determination of pKa values of some novel benzimidazole salts by using a new approach with 1H NMR spectroscopy. Magnetic Resonance in Chemistry, 53(12), 1024–1030.

Perrin, D. D. (1969). Dissociation contants of inorganic acids and bases in aqueous solution. Pure and Applied Chemistry 20, 133–236.

Soulsby, D.,& Chica, J. A. M. (2017). Determination of partition coefficients using 1H NMR spectroscopy and time domain complete reduction to amplitude-frequency table (CRAFT) analysis. Magnetic Resonance in Chemistry, 55(8), 724–729.

Swartz, M. A., Tubergen, P. J., Tatko, C. D., & Baker, R. A. (2018). Experimental determination of pKa values and metal binding for biomolecular compounds using 31 P NMR spectroscopy. Journal of Chemical Education, 95 (1), 182-185.

Wasserkort, R., & Koller, T. (1997). Screening toxic effects of volatile organic compounds using *Drosophila melanogaster*. Journal of Applied Toxicology, 17(2), 119–125.

Yearty, K. L., Sharp, J. T., Meehan, E. K., Wallace, D. R., Jackson, D. M., & Morrison, R. W. (2017). Implementation of PicoSpin benchtop NMR instruments into organic chemistry teaching laboratories through spectral analysis of Fischer esterification products. Journal of Chemical Education, 94(7), 932–935.

Yennie, C. J., Hopson, R., & Hess, K. M.. (2017). Quantifying the product distribution of a chemical reaction by 1H NMR spectroscopy: acooperative learning approach for the undergraduate organic chemistry laboratory. Journal of Chemical Education, 94(9), 1383–1387.

Zivkovic, A., Bandolik, J. J., Skerhut, A. J., Coesfeld, C., & Stark, H. (2018). Govi Onlineshop - PZ PRISMA: NMR-Basierte Lipophiliebestimmungen. Prizma, 25, 25–32.

Zivkovic, A., Bandolik, J. J., Skerhut, A. J., Coesfeld, C., Prascevic, M., Zivkovic, Lj., & Stark, H. (2017). Quantitative analysis of multicomponent mixtures of over-the-counter pain killer drugs by low-field NMR spectroscopy. Journal of Chemical Education, 94(1), 121–125.

Zivkovic, A., Bandolik, J. J., Skerhut, A. J., Coesfeld, C., Zivkovic, N., Raos, M., & Stark, H. (2017). Introducing students to NMR methods using low-field 1H NMR spectroscopy to determine the structure and the identity of natural amino acids. Journal of Chemical Education, 94(1), 115–120.

Zivkovic, A., Bandolik, J., Skerhut, A., Coesfeld, C., Raos, M., Zivkovic, N., Nikolic, V., & Stark, H. (2017). Low field NMR determination of pKa values for hydrophilic drugs for students in medicinal chemistry. Magnetochemistry, 3(3), 29.

Chemia Naissensis, Vol 2, Issue 2, RESEARCH PAPER, 78

Procedura za rešavanje analitičkog problema za studente osnovnih studija pomoću ^{1}H NMR

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Sažetak

^{1}H NMR spektroskopija je široko korišćena tehnika, ali je sve do nedavno bila ograničenog praktičnog značaja u farmaceutskom i hemijskom obrazovanju. Predavanje ^{1}H NMR spektroskopije i dalje je izazov u svim laboratorijama hemije, jer je broj činjenica dobijenih iz svakog eksperimenta preobiman za studente. Za studente smo razvili četiri različita eksperimentalna okruženja koja povezuju interdisciplinarne pristupe rešavanju problema sa praktičnim iskustvom u NMR-u. Skup eksperimenata sastoji se od identifikacije aminokiselina, određivanja logP vrednosti, kvantitativnog određivanja lekova na tržištu i određivanja vrednosti pKa. U radu pokazujemo da je naš pristup podučavanju NMR-a značajno poboljšao razumevanje tehnike među našim studentima.

Ključne reči: ^{1}H NMR, praktično iskustvo, analitička hemija, prilaz rešavanju problema, kvalitativna analiza, kvantitativna analiza

Chemia Naissensis, Vol 2, Issue 2, RESEARCH PAPER, 79

Procédures analytiques de résolution de problèmes par RMN du proton pour les étudiants du premier cycle d'études

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Résumé

La spectroscopie RMN du proton est une technique largement utilisée, mais jusqu'à récemment, elle avait une importance pratique limitée dans l'enseignement pharmaceutique et chimique. L'enseignement de la spectroscopie RMN du proton reste un défi dans tous les laboratoires de chimie car le nombre de faits obtenus à partir de chaque expérience se montre encombrant pour les étudiants. Pour les étudiants du premier cycle d'études, nous avons développé quatre différents paramètres expérimentaux qui relient les approches interdisciplinaires de résolution des problèmes à l'expérience pratique en RMN. L'ensemble des expériences comprend l'identification des acides aminés, la détermination de la valeur log*P*, la détermination quantitative des médicaments en vente libre et la détermination de la valeur p*Ka*. Nous pourrions montrer que notre approche pour enseigner la RMN a considérablement amélioré la compréhension de la technique chez nos étudiants.

Mots-clés: RMN 1H, expérience pratique, chimie analytique, approche de résolution de problémes, analyse qualitative, analyse quantitative

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Процедуры решения аналитических задач для магистрантов методом ЯМР 1Н

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Резюме

1H ЯМР-спектроскопия является широко используемым методом, но до недавнего времени имела ограниченное практическое значение в фармацевтическом и хемическом образовании. Обучение 1H ЯМR спектросакопии остается проблемой во всех химических лабораториях, так как количество фактов, полученных в каждом эксперименте, легко подавляет студентов. Мы разработали четыре различных экспериментальных условия для студентов, которые связывают междисциплинарные подходы к решению проблем с практическим опытом в ЯМР. Набор экспериментов состоит из идентификации аминокислот, определения значения logP, количественного определения продаваемых через счетчик лекарств и определения значения pKa. Мы могли бы показать, что наш подход к обучению ЯМР значительно улучшил понимание техники среди наших студентов.

Ключевые слова: 1H ЯМР, практический опыт, аналитическая химия, подход к решению проблем, качественний анализ, количественный анализ

Analytische Problemlösungsverfahren für Studierende mittels 1H-NMR

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ABSTRACT

Die ^{1}H NMR -Spektroskopie ist eine weit verbreitete Technik, diebis vor kurzem nur eine begrenzte praktische Bedeutung in der pharmazeutischen und chemischen Ausbildung hatte. Das Unterrichten von ^{1}H NMR-Spektroskopie bleibt in allen Chemielabors eine Herausforderung, da die Anzahl der Fakten, die aus jedem Experiment erhalten werden, für die Studenten leicht überwältigend ist. Für die Studierenden haben wir vier verschiedene experimentelle Umgebungen entwickelt, die interdisziplinäre Problemlösungsansätze mit der praktischen Erfahrung in der NMR verbinden. Die Versuchsreihe besteht aus der Identifizierung von Aminosäuren, der Bestimmung von logP-Werten, der Quantifizierung von vermarkteten Arzneimitteln und der Bestimmung von pKa. Wir könnten zeigen, dass unser Ansatz beim Unterrichten von NMR das Verständnis der Technik bei unseren Schülern erheblich verbessert hat.

Schlüsselwörter: 1H-NMR, praktische Erfahrung, analytische Chemie, Problemlösungsansatz, qualitative Analyse, quantitative Analyse

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Photostability of bacteriochlorophyll *a* and bacteriopheophytin *a* against UV-A, UV-B and visible light treatments in methanol solutions

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ABSTRACT

Bacteriochlorins as the porphyrins derivatives are well known photosensitizers with great potential for use in various fields of pharmacy and medicine. Photostability of selected bacteriochlorins, bacteriochlorophyll *a* and bacteriopheophytin *a*, in different methanol solutions (with and without lipids) during continual UV-A, UV-B and visible light treatments were studied using absorption UV-VIS spectroscopy providing kinetic analysis. Applied irradiation treatments resulted in irreversible degradation of both selected bacteriochlorins obeying the first order of kinetics. Bacteriopheophytin *a* showed significantly higher photostability in comparison to bacteriochlorophyll *a* for all applied irradiation treatments, for about one to three orders of magnitude. Photochemical degradation of bacteriochlorins is energy dependant process, governed by photons energy input.Lipid environment play stability role for both bacteriochlorins against all, UV-A, UV-B and visible light treatments. Bacteriopheophytin *a* induced lipid peroxidation processduring UV-A irradiation treatment.

Keywords: photostability, bacteriopheophytin a, bacteriochlorophyll a, irradiation, lipids, lipid peroxidation

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Introduction

Bacteriochlorophyll *a* (BChl*a*) is a bacteriochlorin type chlorophyll which is the most widely distributed. It is present in the reaction center (RC) and the core-antennas of most anoxygenic bacteria, as well as in the peripheral antennas of the purple bacteria (Grimm et al., 2007, Permentier et al., 2001). A major bacteria pigment bacteriochlorophyll*a* (BChl*a*), with an isocyclic cyclopentanone ring fused to a C-pyrrole ring of the porphyrin core between the C-13 and C-15 positions (Figure 1A) contains one magnesium in the center which coordinates four pyrrole rings by two covalent and two coordination bonds (Kay and Gräcel, 1993). Bacteriopheophytin *a* (BPheo*a*) is a derivative of BChl*a* with only difference in the central position of macrocycle, the absence of Mg (Figure 1B).

In recent decades, there has been a growing interest in the use of porphyrins and their derivates as well as BChls and their derivates in medical field by giving consideration for photophysical and photochemical properties as efficient and promising sensitizers in photodynamic therapy known as Photodynamic Therapy (PDT) (Brandis et al., 2006; Henderson et al., 1990; Pandey and Zheng, 2000). Photodynamic Therapy is a successful treatment method for cancer and premalignant conditions that leads to the selective destruction of tumor through photodynamic process and it is based on the induction of tissue and cellular damage by the combined effects of three components including a photosensitizer (PS), light and oxygen (Kübler, 2005). Porphyrins and their derivates have interesting and good photochemical characteristics and exhibit desirable properties for drug candidates in PDT (Pandey and Zheng, 2000; Rinco et al., 2009). They also have some drawbacks (Yano et al., 2011), but in general, porphyrins and their derivates, as well as chlorins and bacteriochlorins, have en excellent PDT efficacy (Sternberg et al., 1998) and some of them have been approved for clinical use (Henderson and Dougherty, 1992).

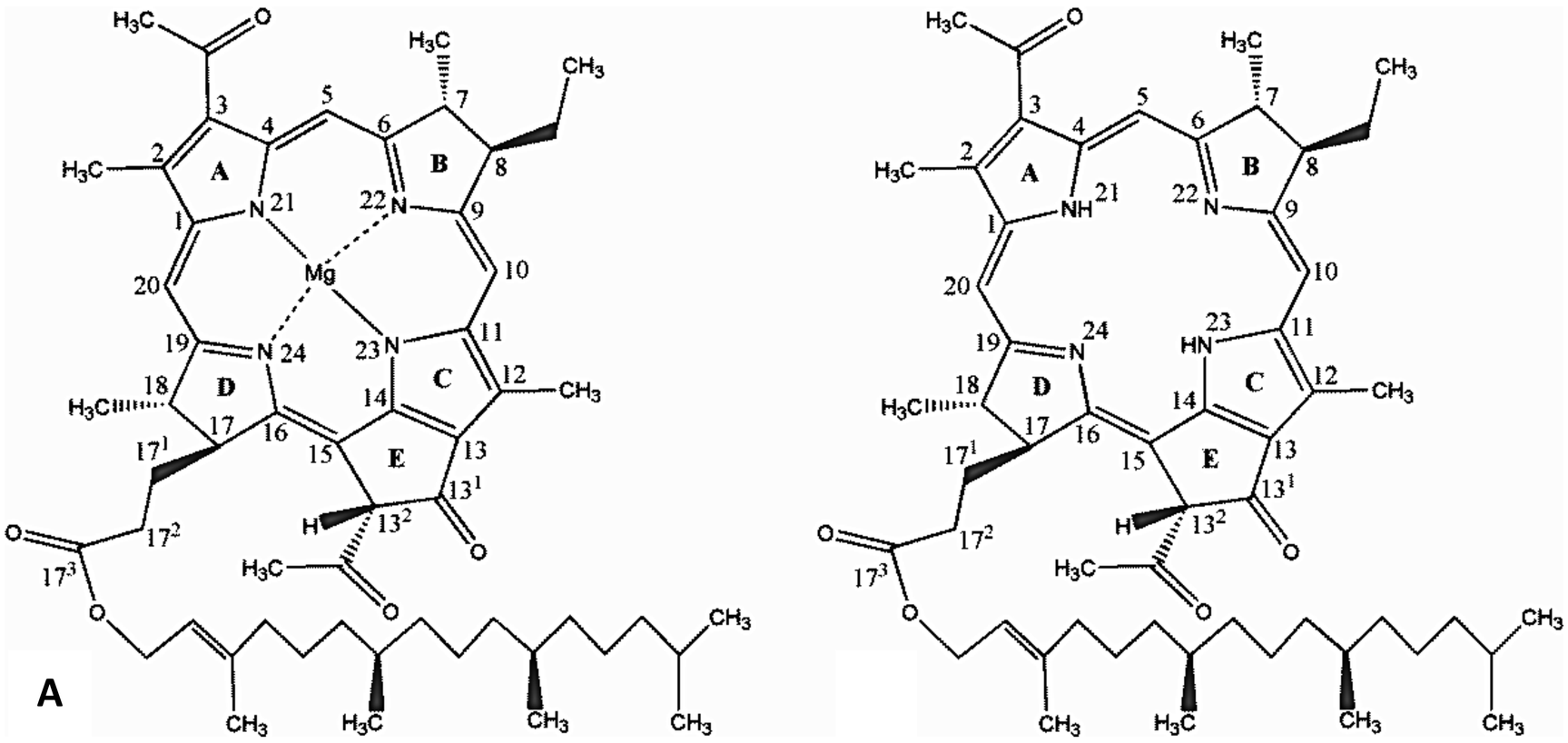


Figure 1. Structure of BChl*a* (A) and BPheo*a* (B).

It is important to examine the connection between photosensitizers and lipid peroxidation process because lipid peroxidation is considered as the main molecular mechanism involved in the oxidative damage to cell structures and in the destruction and

cell death in PDT and generally in biological processes (Repetto et al., 2012; Yin et al., 2011). In earlier experiments with bacteriochlorophyll analogues chlorophylls, it was found that UV-irradiation induces degradation of these compounds. Chlorophyll degradation obeys a first-order law and it is highly dependant on the used energy input and also dependant on the used solvents (Petrovic et al., 2017; Zvezdanović and Marković, 2008; Zvezdanović et al., 2009). The interaction of bacteriochlorin derivatives with oxygen under UV-VIS light (to produce harmful reactive oxygen species) and their photostability against different irradiation treatments could play important role in various fields of research and their applications. The objective of this study was to determine photostability of two chosen bacteriochlorins, BChl*a* and BPheo*a*, during continual UV-A, UV-B and VIS irradiation treatments in methanol solutions - without and with phospholipid mixture, PL90, in order to determine the corresponding influence of lipid medium, and possibly, their photosensitizing potential for the induction of lipid peroxidation process under the given conditions.

Experimental

All experiments were performed under dim light and equipment covered with aluminium foil, preventing possible BChl*a* and BPheo*a* photooxidation during sample preparation experiments.

The samples of BChl*a* and BPheo*a* used in this study are a gift from professor Fiedor Leszek, Faculty of Biochemistry, Biophysics and Biotechnology, Jagellonian University of Krakow, Poland. Phospholipid mixture, Phospholipon®90,PL90† is a gift from Phospholipid, GMBH, Cologne, Germany. All solvents used in the experiments were HPLC or LC/MS grade purity.

Samples preparation

For the purpose of this study, four different solutions of BChl*a* and BPheo*a* in methanol were made: BChl*a*,BPheo*a*,BChl*a*+PL90 and BPheo*a*+PL90 solutions.

Bacteriochlorophyll *a* and BPheo*a* were dissolved in methanol to concentration $2.5x10^{-5}$ M in all solutions; BChl*a* and BPheo*a* concentration was adjusted to the chosen by Beer's law in UV-VIS spectrophotometric method, using extinction coefficients values in methanol at 772 and 530 nm, respectively (Kobayashi et al., 2006; Pandey and Zheng, 2000). In addition, lipids concentration for BChl*a*+PL90 and BPheo*a*+PL90 solutions were 1.0×10^{-4} M. Additionally, the solution of lipids, PL90 in methanol without bacteriochlorins (the same concentration 1.0×10^{-4} M) was made for the control experiments. Control experiments with PL90 methanol solutions were obtained to give better insight to possible changes of lipids itself under continual treatment with UV-B, UV-A and visible light, especially in the area of lipid peroxides formation (at 234 nm in the absorbance spectra).

†Declaration: phosphatidylcholine 94.7%, lysophosphatidylcholine 0.9%, tocopherol 0.21% andfatty acids: palmitoleic 12.0±2%; stearic 3±1%; oleic 3±3%; linoleic 66±5% and linolenic 5±2%. Peroxide value of mixture is 1.4 (max. 5.0), acid value is 0.2 (max. 0.5) the content of ethanol and water 0.0% (max. 0.2%) and 0.3% (max. 1.5%), respectively.

UV irradiation treatments

Continuous irradiation of the samples(BChl*a*, BPheo*a*, BChl*a*+PL90 and BPheo*a*+PL90 and the controls) in methanol were performed in a cylindrical photochemical reactor "Rayonet", with 8 symmetrically placed lamps having emission maxima at 350 nm (UV-A) and 300 nm (UV-B). The samples were irradiated in quartz cells (1×1×4.5 cm^3) placed on the rotating circular holder. The total measured energy flux (hitting the samples) was about 10.3 W m^{-2} for 350 nm and 12.0 W m^{-2} for 300 nm.

Visible light treatment

Continuous illumination of bacteriochlorins solutions with visible light in the visible range (200-800 nm), was performed in hand-made cylindrical photochemical reactor equipped with symmetrically placed LED lamps at 10 cm distance from the samples (number of LED lamps was 60 com./m, distance between lamps 16 mm, light color" Pure White", emitting angle 120° spherical). The total measured energy flux received by the samples was 14 W m^{-2}.

UV-VIS spectrophotometry

Absorption UV-VIS spectra of all samples in the methanol were recorded on VARIAN Cary-100 Spectrophotometer equipped with 1.0 cm quartz cells. All spectra, before and after illumination/irradiation treatments (visible light / UV-A and UV-B) were recorded in the range from 200 to 800 nm, with 1.0 bandwidth. Degradation of BChl*a* and BPheo*a* in different methanol solutions induced by visible light, UV-A and UV-B irradiation treatments has been studied by using Q_y absorption band of these compounds as a sensible indicator of detected changes and providing kinetic analysis.

Data analysis was performed by the OriginPro 8 software. For the presentation of selected bacteriochlorins degradation, the dynamic plots based on the data from the corresponding UV-VIS spectra were used, *i.e*. absorbance values at Q_y-bands maximums (A_{772nm} for BChl*a* and A_{754nm} for BPheo*a*). Percent of BChl*a*and BPheo*a* retained in the treated samples were calculated by using equation: *Content of bacteriochlorins* (%) = (A_t×100)/A_0, where A_0 and A_t were the absorbance maximum values of BChl*a* and BPheo*a* for different t_{irr} periods and for t_{irr}= 0 min, respectively (for*y*-axis); $t_{irr.}$ given in min representing *x*-axis. For kinetic analysis,calculated rate constants were obtained from linear dependence of the corresponding lnA_{772nm} and lnA_{754nm} values during time of treatment, t_{irr} (min).

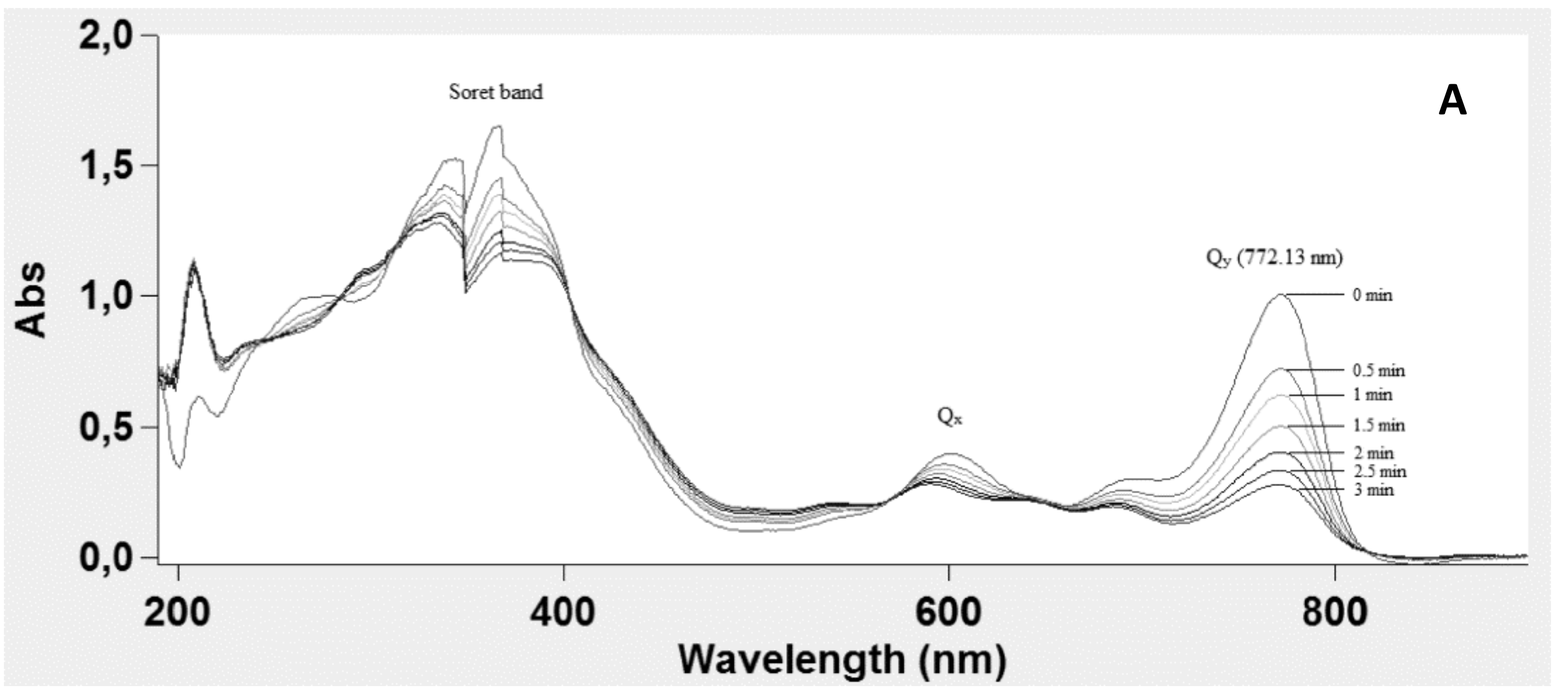
Results and Discussion

Absorption spectra of bacteriochlorins show the electronic transitions along the x-axis of the molecule running through two nitrogen (N) atoms of rings B and D, and along the *y*-axis through the N atoms of rings A and C (Grimm et al., 2007). The two pairs of absorption bands in the blue and red spectral regions of bacteriochlorins are called *B* (or Soret) and Q bands, respectively, and arise from $\pi \rightarrow \pi^*$ transitions of the four frontier orbitals (Weiss, 1978). One band of each pair is polarized along the *x*-axis (B_x, Q_x), the

other along the *y*-axis (B_y, Q_y) (Grimm et al., 2007). Rings B and D in bacteriochlorins are reduced by two hydrogens (Figure1); the conjugated system is longer along the *y*-axis than the *x*-axis (Ke, 2001; Weiss, 1978)and the spectroscopic consequence of this hydrogenation of rings B and D (Figure 1) is a considerably increased gap among the absorption bands (Grimm et al., 2007). The Q_y-absorption band is red-shifted to 750-800 nm in mono-disperse solutions, and even more (800-1020 nm) *in situ*, while the Soret-band is a blue-shifted (<400 nm) and split (Drews and Giesbrecht, 1966). Bacteriopheophytin *a* can be easily distinguished from BChl*a* by the shape of its *B* bands, by its higher *B*/Q_y band ratio (ca. 1.6 in BPheo*a* and ca. 0.75 in BChl*a*) and by its blue shifted Q_x maximum (520 nm in BPheo*a* and 570 nm in BChl*a*), as shown in Figure 2 (Grimm et al., 2007).

Photostability of selected bacteriochlorins were monitored on the basis of the change of the maxima of absorption Q_y band ($Q_{y,max}$) during the UV-A, UV-B and visible light treatment.

Absorption spectra of BChl*a* and BPheo*a* solutions treated with UV-B and UV-A irradiations, and BChl*a* and BPheo*a* in mixture with lipids treated with UV-A and visible light,were shown in Figures 2A,B, C and D, respectively. The rest of UV-A, UV-B and visible light treated solutions of BChl*a* and BPheo*a* as well as BChl*a*+PL90 and BPheo*a*+PL90 showed similar spectral behaviour (spectra not shown). The corresponding time dynamic of bacteriochlorins (photo)degradation during the treatments were shown in Figures 3A, B and C, for all solutionsunder UV-B, UV-A and VIS irradiation regime, respectively, by using absorbance values recorded at Q_y-band maximums - 772 nm for BChl*a* and 754 nm for BPheo*a*, A_{752nm} and A_{754nm}. The corresponding ln-plots of A_{752nm} and A_{754nm} valuesfor increasing UV-A, UV-B and VIS treatment periods ($t_{irr.}$), with linear fitting (mainly, R^2 values > 0.94) were used for kinetic analysis, and the corresponding calculated (photo)degradation rate constants (in min^{-1}), determined as the slopes of linear plots, were listed in Table 1.



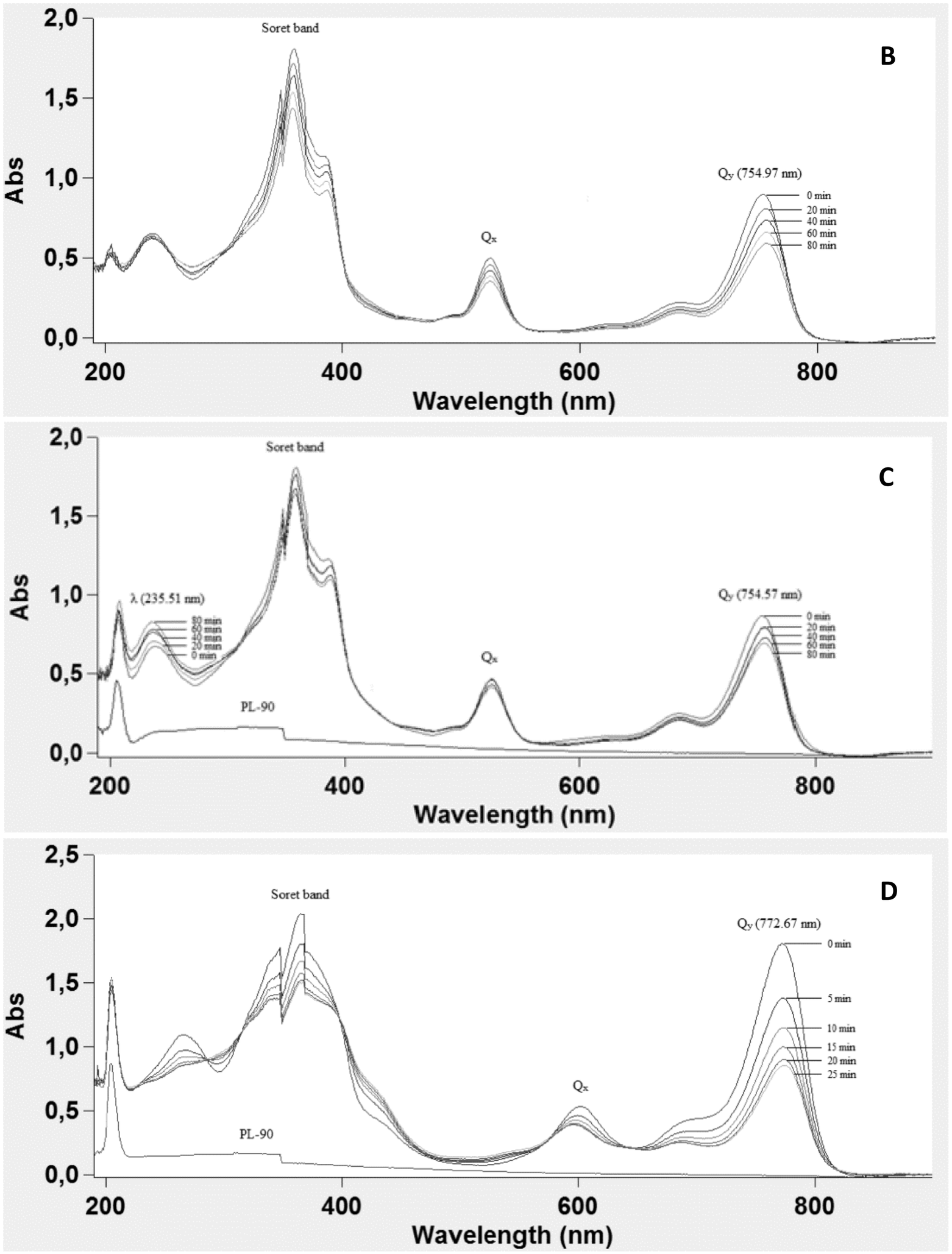


Figure 2.Absorption spectra of BChl*a* and BPheo*a* solutions treated with continual UV-B and UV-A irradiations (A and B, respectively) and BPheo*a*+PL90 and BChl*a*+PL90 solutions treated with UV-A and visible light (C and D, respectively). The time periods of treatments were shown on the figure. From the control experiments, UV-A and visible light treated PL-90 in methanol, the corresponding UV-VIS spectra were shown and marked on the figures (C and D, respectively).

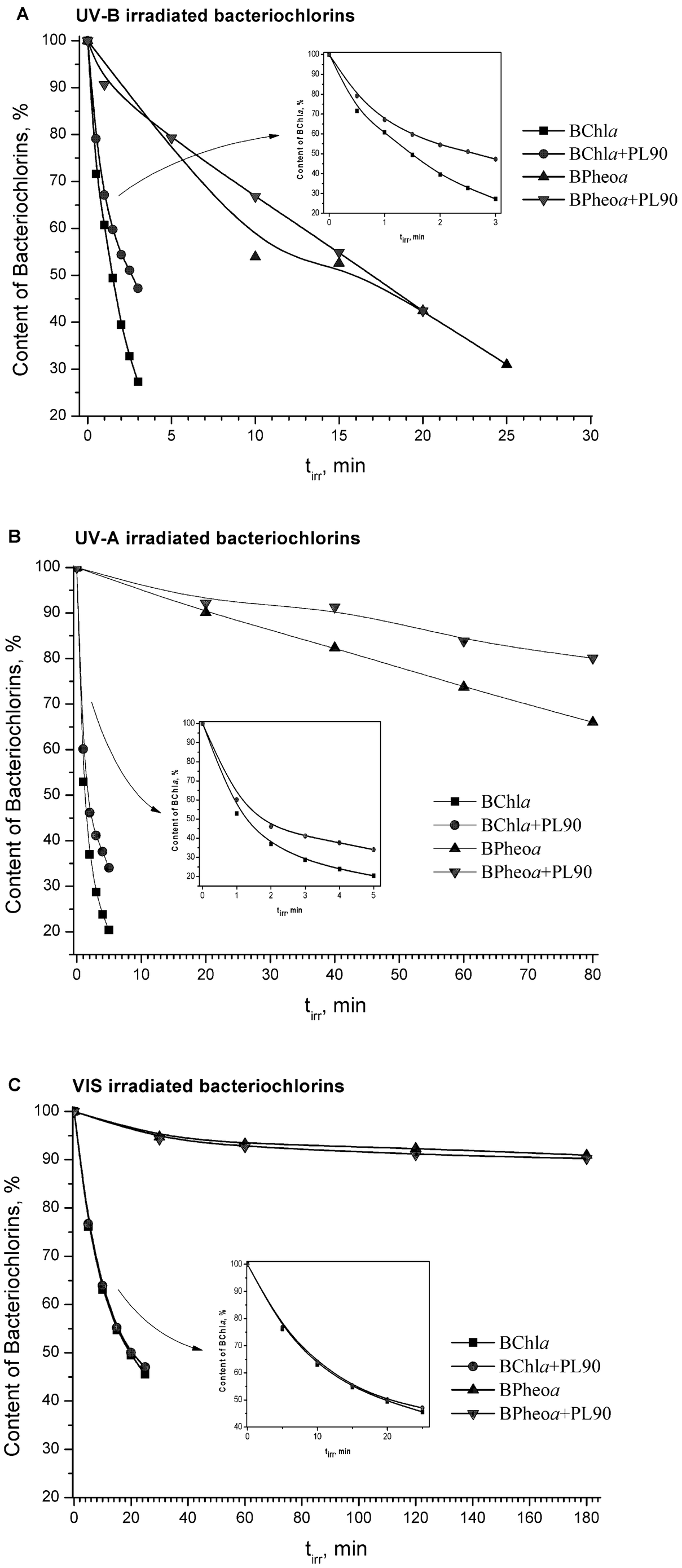

A
UV-B irradiated bacteriochlorins
Content of Bacteriochlorins, %
t_irr, min
Content of BChl a, %
t_irr, min
BChla
BChla+PL90
BPheoa
BPheoa+PL90
B
UV-A irradiated bacteriochlorins
Content of Bacteriochlorins, %
t_irr, min
Content of BChl a, %
t_irr, min
BChla
BChla+PL90
BPheoa
BPheoa+PL90
C
VIS irradiated bacteriochlorins
Content of Bacteriochlorins, %
t_irr, min
Content of BChl a, %
t_irr, min
BChla
BChla+PL90
BPheoa
BPheoa+PL90

Figure 3. The dynamic plots of bacteriochlorophyll *a* and bacteriopheophytin *a* degradation in both mixtures (with and without lipidsin methanol solutions) under continuous UV-B, UV-A and visible light irradiation regime (A, B and C, respectively), expressed as the changes in percent of BChl*a* and BPheo*a* (compared to not-irradiated) for different irradiation time periods (t_{irr}).

Table 1. List of calculated photodegradation rate constants for selected bacteriochlorins

	k_{BChla} (min^{-1})	R^2	$k_{BChla+PL90}$(min^{-1})	R^2	k_{BPheoa} (min^{-1})	R^2	$k_{BPheoa+PL90}$ (min^{-1})	R^2
UV-B	0.421	0.99	0.263	0.94	0.044	0.95	0.040	0.99
UV-A	0.348	0.92	0.203	0.88	0.005	0.99	0.003	0.95
White light	0.035	0.95	0.034	0.96	$^{*}\approx 10^{-4}$	0.70	$^{*}\approx 10^{-4}$	0.70

*Due to small R^2 values, the corresponding rate constants could only considerate as roughly approximate values.

According to obtained results, the continual UV-A, UV-B and visible light treatment of BChl*a* and BPheo*a* in methanol solutions result in their irreversible degradation. The corresponding absorption spectra were changed, showing continuous decrease in whole measured spectral range (190-900 nm) during the treatments (Figure 2). Mixtures of lipids as the controls in methanol are not considerably changed during irradiation (Figures 2C and D).

Degradation of BChl*a* and BChl*a*+PL90 with UV-A irradiation treatment is significant even in the first minute of irradiation (only 50% and 60 % of BChl*a* is retained in the samples, respectively, Figure 3B). On the other hand, BPheo*a* and BPheo*a*+PL90 solutions showed much slower degradation during continuous UV-A irradiation (even 70% and 85% of BPheo*a* are retained after 80 min of treatment, respectively, Figure 3B). Similarly, BChl*a* in all solutions (with and without PL90), treated with UV-B and visible light,has shown degradation trend during time of irradiation, more intensively in solutions without PL90 (Figures 3A,C, respectively). Calculated degradation rate constants for UV-B, UV-A and visible light treated BChl*a* are for about one, two and three orders of magnitude higher than the same for treated BPheo*a* in methanol (Table 1). Similar relation can be established for the treated BChl*a* and BPheo*a* in the mixture with PL90 in methanol. Even more, chosen bacteriochlorins are more stable in the mixtures with lipids, PL90. This is in accordance with calculated degradation rate constants (Table 1) confirming the stability order: BChl*a*<BChl*a*+PL90<BPheo*a*<BPheo*a*+PL90, for all three used treatments. And finally, photostability of chosen bacteriochlorins is energy dependent. Starting from the visible light (the lowest photons energy input), through UV-A to UV-B (the highest photons energy input) irradiation treatments, degradation of both bacteriochlorins rising in all mixtures - their stability decreasing (Table 1). Detected differences are more noticeable for BPheo*a* in comparison to the corresponding ones for BChl*a* in both solutions (with PL90 and without PL90). For example, calculated rate constant values for UV-B, UV-A and visible light treated BPheo*a* in methanol are 0.044 min^{-1}, 0.005 min^{-1} and $\approx 10^{-4}$ min^{-1}, respectively. So,the corresponding UV-B induced degradation rate is almost one order of magnitude faster than UV-A, and two orders of magnitude faster than visible light induced degradation of BPheo*a* (Table 1). On the other hand, the corresponding values calculated for BChl*a* are 0.421 min^{-1}, 0.348 min^{-1} and 0.035 min^{-1} for UV-B, UV-A and visible light induced degradation, respectively, meaning

only one order magnitude faster UV-B and also, UV-A induced degradation in comparison to visible light, and almost equal UV-A and UV-B degradation rates (Table 1).

The answer to arising question “why?” is a more complex and out of the experimental data used in this study. But we will try to give some directions in better understanding of the obtained results for chosen two (bacterio)chlorins, BChl*a* and BPheo*a* treated by light. It is known that chlorophylls (bacteriochlorophylls as well) in solutions - *in vitro* in general are very unstable, much more in comparison to the corresponding their pheophytin derivatives (bacteriopheophytins), not only when exposed to the light, UV-irradiation, room and higher temperature values, oxygen in the dark and light, different solvents, acids *etc.* (Grimm et al., 2007). The stability of chlorophyll *a*wealready studied and the results were published (Petrović et al., 2017; Zvezdanović and Marković, 2008;Zvezdanović et al., 2009). The main explanation for the detected changes can be found in the structural differences between (B)Chl*a* and (B)Pheo*a*. (Bacterio)chlorophyll *a* has a central, labile bonded metal Mg; (B)Pheo*a* is without Mg (Figure 1), and this can be very important explanation for the photostability differences between selected compounds. Based on the previous research (Katz et al., 1978; Scheer, 1991), it can be supposed that central metal Mg in BChl*a* extends the half-life of a molecule excited triplet state after interaction with the irradiation (Melkozernov and Blankenship, 2006) resulted in a larger production of reactive oxygen species. In turn, formed reactive oxygen species could directly participate in the degradation of selected bacteriochlorins. Another important factor is the role of solvent. Methanol is a polar protic solvent and can be an electron donor. In that environment (solvents molecules) central metal Mg in BChl*a* can be an electron-acceptor, surrounded with molecules of solvent above and below bacteriochlorin tetrapyrrole macrocycle (Cotton and Van Duyne, 1979) somehow acting as an instability factor.

On the other hand, lipid environment playsa stability role to BChl*a* and BPheo*a*. Lipids used in this study in general could play a stability role to irradiated molecules (Stanojevic et al., 2013; Zvezdanovic et al., 2012). In the treated mixtures of bacteriochlorins, they can be “target” molecules for both, action ofdirect irradiation and also indirect irradiation treatments. Indirectly, the lipids can interact with molecules of singlet oxygen formed in photosensitizer (such as bacteriochlorin) - ground-state oxygen interactions under photon energy inputs (from UV-B, UV-A and visible light) and form lipid peroxides which can be detected in the UV-VIS spectra as rising absorbance at 234-235 nm. Namely, lipid peroxides, peroxidative dienes structures are lipid peroxidation products of lipids under many oxidative treatments (Cvetković and Marković, 2011; Repetto, 2010), including photooxidative were used in this study. In case of UV-A irradiated mixture BPheo*a*+PL90 in methanol, its clearly observed lipid peroxidation process (Figure 2C), implicated BPheo*a* photosensitizing properties under the given conditions. It is in accordance with known fact that porphyrins in general are well known photosensitizing molecules when they are exposed to light, especially visible, in the Q_y-band area of absorption (DeRosa, 2002). Also, from the absorption spectra of BChl*a* and BPheo*a* (Figure 2), it can be observed significant absorption in the area of UV-A radiation (320-380 nm) implying a possibility of photosensitization reaction upon UV-A absorption and as a consequence, lipid peroxides formation (Figure 2C).

Conclusion

The chosen bacteriochlorophylls, BChl*a* and BPheo*a* in methanol and methanol-lipid solutions undergo light/irradiation induced photochemical degradation. Photochemical degradation of bacteriochlorins is energy dependant process, governed by photons energy input. On the other hand, bacteriochlorophyll *a* and bacteriopheophytin *a* showed different photostability: BPheo*a* is more stable against all applied treatments in comparison to BChl*a* due to the structural differences. The impact of the environment on photostability is also an important factor. Experiments showed that BPheo*a* in mixture with lipid induce lipid peroxidation process, but only with UV-A irradiation is clear observed. This is probably because BPheo*a* absorption is the strongest in UV-A irradiation area (350 nm).Studied bacteriochlorins BChl*a* and BPheo*a* are photosensitive compounds and they show potential for future investigations in different areas.

Acknowledgement

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Conflict-of-Interest Statement

Declarations of interest: none.

References

Brandis, A. S., Salomon, Y., & Scherz, A. (2006). Bacteriochlorophyll sensitizers in photodynamic therapy. In B. Grimm, J. Porra, W. Rudiger and H. Scheer (Eds.) Chlorophylls and Bacteriochlorophylls: Biochemistry, Biophysics, Functions and Applications (pp. 485–494). Dordrecht: Springer.

Cotton, T. M., & Van Duyne, R. P. (1979). An electrochemical investigation of the redox properties of bacteriochlorophyll and bacteriopheophytin in aprotic solvents. Journal of the American Chemical Society, 101 (25), 7605-7612.

Cvetković, D., & Marković, D. (2011). Beta-carotene suppression of benzophenone-sensitized lipid peroxidation in hexane through additional chain-breaking activities. Radiation Physics and Chemistry, 80 (1), 76-84.

DeRosa, M. C., & Crutchley, R. J. (2002). Photosensitized singlet oxygen and its applications. Coordination Chemistry Reviews, 233, 351-371.

Drews, G., & Giesbrecht, P. (1966). Rhodopseudomonas viridis, nov. spec., ein neu isoliertes, obligat phototrophes Bakterium. Archiv für Mikrobiologie, 53 (3), 255-262.

Grimm, B., Porra, R. J., Rüdiger, W., & Scheer, H. (Eds.). (2007). Chlorophylls and bacteriochlorophylls: biochemistry, biophysics, functions and applications (Vol. 25). Springer Science & Business Media.

Henderson, B. W., & Dougherty, T. J. (1992). How does photodynamic therapy work? Photochemistry and Photobiology, 55 (1), 145-157.

Henderson, B. W., Potter, W. R., Sumlin, A. B., Owczarczak, B., Nowakowski, F. S., & Dougherty, T. J.(1990). Bacteriochlorophyll-a as photosensitizer for photodynamic treatment of transplantable murine tumors. Photodynamic Therapy: Mechanisms II, 1203, 211–222.

Katz, J. J., Shipman, L. L., Cotton, T. M., & Janson, T. R. (1978). Chlorophyll aggregation: coordination interactions in chlorophyll monomers, dimers, and oligomers. The Porphyrins, 5, 401-458.

Kay, A., & Gräcel, M. (1993). Artificial photosynthesis. 1. Photosensitization of 2 solar cells with chlorophyll derivatives and related natural porphyrins. Journal of Physical Chemistry, 97, 6272-6277.

Ke, B. (2001). Photosynthesis photobiochemistry and photobiophysics (Vol. 10). Springer Science & Business Media.

Kobayashi, M., Akiyama, M., Kano, H., & Kise, H. (2006). Spectroscopy and structure determination. In Chlorophylls and bacteriochlorophylls (pp. 79-94). Dordrecht: Springer.

Kübler, A. C. (2005). Photodynamic therapy. Medical Laser Application, 20 (1), 37-45.

Melkozernov, A. N., & Blankenship, R. E. (2006). Photosynthetic functions of chlorophylls. In Chlorophylls and Bacteriochlorophylls (pp. 397-412). Dordrecht: Springer.

Pandey, R. K., & Zheng, G. (2000). Porphyrins as photosensitizers in photodynamic therapy. The Porphyrin Handbook, 6, 157-230.

Permentier, H. P., Neerken, S., Overmann, J., & Amesz, J. (2001). A bacteriochlorophyll *a* antenna complex from purple bacteria absorbing at 963 nm. Biochemistry, 40, 5573–5578.

Petrović, S., Zvezdanović, J., & Marković, D. (2017). Chlorophyll degradation in aqueous mediums induced by light and UV-B irradiation: An UHPLC-ESI-MS study. Radiation Physics and Chemistry, 141, 8-16.

Repetto, M. G., Ferrarotti, N. F., & Boveris, A. (2010). The involvement of transition metal ions on iron-dependent lipid peroxidation. Archives of Toxicology, 84 (4), 255-262.

Repetto, M., Semprine, J., & Boveris, A. (2012). Lipid peroxidation: chemical mechanism, biological implications and analytical determination. In Lipid Peroxidation. InTechOpen.

Rinco, O., Brenton, J., Douglas, A., Maxwell, A., Henderson, M., Indrelie, K., Wessels, J.,& Widin, J. (2009). The effect of porphyrin structure on binding to human serum albumin by fluorescence spectroscopy. Journal of Photochemistry and Photobiology A: Chemistry, 208 (2-3), 91-96.

Scheer, H. (1991).Structure and occurrence of chlorophylls. In Chlorophylls (pp. 1-30).Boca Raton: CRC Press.

Stanojević, J. S., Marković, D. Z., & Zvezdanović, J. B. (2013). Benzophenone suppression of quercetin antioxidant activity towards lipids under UV-B irradiation regime: Detection by HPLC chromatography. Journal of Chemistry, 2013, 1-9.

Sternberg, E. D., Dolphin, D., & Brückner, C. (1998). Porphyrin-based photosensitizers for use in photodynamic therapy. Tetrahedron, 54(17), 4151-4202.

Weiss, C. (1978). Electronic Absorption Spectra of Chlorophylls. In D. Dolphin (Ed.), The Porphyrins, Vol. III, Physical Chemistry, Part A (pp. 211-223). New York: Academic Press.

Yano, S., Hirohara, S., Obata, M., Hagiya, Y., Ogura, S.-i., Ikeda, A., Kataoka, H., Tanaka, M.,& Joh, T. (2011). Current states and future views in photodynamic therapy. Journal of Photochemistry and Photobiology C: Photochemistry Reviews, 12 (1), 46-67.

Yin, H., Xu, L., & Porter, N. A. (2011). Free radical lipid peroxidation: mechanisms and analysis. Chemical Reviews, 111 (10), 5944-5972.

Zvezdanović, J., Marković D., CvetkovićD. & Stanojević J. (2012). UV-induced change in quercetin antioxidant activity toward benzophenone initiated lipid peroxidation. Journal of the Serbian Chemical Society, 77 (11), 1571-1588.

Zvezdanović, J., & Marković, D. (2008). Bleaching of chlorophylls by UV irradiation *in vitro*: the effects on chlorophyll organization in acetone and *n*-hexane. Journal of the Serbian Chemical Society, 73 (3), 271-282.

Zvezdanović, J., Cvetić, T., Veljović-Jovanović, S., & Marković, D. (2009). Chlorophyll bleaching by UV-irradiation *in vitro* and *in situ*: Absorption and fluorescence studies. Radiation Physics and Chemistry, 78 (1), 25-32.

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Fotostabilnost bakteriohlorofila *a* i bakteriofeofitina *a* na tretmane UV-A, UV-B i vidljivom svetlošću u metanolnim rastvorima

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SAŽETAK

Bakteriohlorini, derivati porfirina, poznati su fotosenzibilizatori sa velikim potencijalom za primenu u različitim granama farmacije i medicine. Fotostabilnost izabranih bakteriohlorina, bakteriohlorofila *a* i bakteriofeofitina *a*, u rastvorima metanola (sa lipidima i bez lipida), tokom kontinualnog ozračivanja UV-A, UV-B i vidljivom svetlošću, praćena je UV-VIS spektrofotometrijski sa mogućnošću kinetičke analize. Primenjena ozračivanjadovode do ireverzibilne degradacije oba izabrana bakteriohlorina, prateći kinetiku prvog reda. Bakteriofeofitin *a* pokazao je znatno veću fotostabilnost u poređenju sa bakteriohlorofilom *a* prema svim primenjenim zračenjima–za oko jedan do tri reda veličine. Fotohemijska degradacija bakteriohlorina je energetski zavistan proces, određen energijom upadnih fotona. Lipidno okruženje ima zaštitnu ulogu za oba bakteriohlorina prema svim primenjenim tretmanima. Kod bakteriofeofitina *a* potvrđeno je da indukuje process lipidne peroksidacije tokom UV-A ozračivanja.

Ključne reči: fotostabilnost, bakteriofeofitin a, bakteriohlorofil a, zračenje, lipidi, lipidna peroksidacija

Photostabilité de la bactériochlorophylle *a* et de la bactériophéophytine *a* contre les traitements UV-A, UV-B et les traitements lumière visible dans des solutions de méthanol

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Résumé

Les bactériochlorines, en tant que dérivés des porphyrines, sont des photosensibilisateurs bien connus avec un grand potentiel pour une utilisation dans divers domaines de la pharmacie et de la médecine. La photostabilité de bactériochlorines sélectionnées, de bactériochlorophylle *a* et de bactériophéophytine *a*, dans les différentes solutions de méthanol (avec et sans lipides) pendant les traitements continus aux UV-A, UV-B et à la lumière visible, a été étudiée en utilisant la spectroscopie d'absorption UV-VIS fournissant une analyse cinétique. Les traitements d'irradiation appliqués ont entraîné une dégradation irréversible des deux bactériochlorines sélectionnées en obéissant au premier ordre de cinétique. La bactériophéophytine *a* a montré une photostabilité significativement plus élevée par rapport à la bactériochlorophylle *a* pour tous les traitements d'irradiation appliqués, pour environ d'un à trois ordres de grandeur. La dégradation photochimique des bactériochlorines est un processus dépendant de l'énergie, régi par l'apport d'énergie des photons. L'environnement lipidique joue un rôle de stabilité pour les deux bactériochlorines contre tous les traitements – UV-A, UV-B et lumière visible. La bactériophéophytine *a* est un processus de peroxydation lipidique induit lors du traitement d'irradiation UV-A.

Mots-clés: photostabilité, bactérioféophytine *a*, bactériochlorophylle *a*, irradiation, lipides, peroxydation lipidique.

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Фотостабильность бактериохлорофилла А и бактериофеофитина А в отношении воздействия УФ-А, УФ-В и видимого света в растворах метанола

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Резюме

Бактериохлорины в качестве производных порфиринов являются хорошо известными фотосенсибилизаторами с большим потенциалом для использования в различных областях фармации и медицины. Фотостабильность выбранных бактериохлоринов, бактериохлорофилла А и бактериофеофитина А в различных растворах метанола (с липидами и без них) во время непрерывной обработки УФ-А, УФ-В и видимого света изучали с использованием абсорбционной УФ-ВИС-спектроскопии, обеспечивающей кинетический анализ. Применяемая обработка облучением привела к необратимой деградации обоих выбранных бактериохлоринов, подчиняющихся первому порядку кинетики. Бактериофеофитин а показал значительно более высокую фотостабильность по сравнению с бактериохлорофиллом а для всех примененных обработок облучением, примерно на один-три порядка. Фотохимическая деградация бактериохлоринов-это энергозависимый процесс, который зависит от энергии фотонов. Липидная среда играет роль стабильности как бактериохлоринов, так и УФ-А, УФ-Б и видимого света. Бактериофеофитин-индуцированный процесс перекисного окисления липидов пре обработке УФ-А.

Ключевые слова: фотостабильность, бактериофеофотин а, бактериохлорофилл а, облучение, липиды, перекисное окисление липидов

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Photostabilität von Bakteriochlorophyll *a* und Bakteriophäophytin *a* gegen Behandlungen mit UV-A, UV-B und sichtbarem Licht in Methanollösungen

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ABSTRAKT

Bacteriochlorine als Porphyrinderivate sind bekannte Photosensibilisatoren mit großem Potenzial für den Einsatz in verschiedenen Bereichen der Pharmazie und Medizin. Die Photostabilität ausgewählter Bakteriochlorine, Bakteriochlorophylle *a* und Bakteriophäophytine *a* in verschiedenen Methanollösungen (mit und ohne Lipide) während kontinuierlicher Behandlungen mit UV-A-, UV-B- und sichtbarem Licht wurde unter Verwendung der UV-VIS-Spektroskopie untersucht, um eine kinetische Analyse zu erhalten. Angewandte Bestrahlungsbehandlungen führten zu einem irreversiblen Abbau beider ausgewählter Bakteriochlorine, der Kinetik erster Ordnung folgend. Das Bacteriophäophytin a zeigte für alle angewendeten Bestrahlungsbehandlungen eine im Vergleich zu Bacteriochlorophyll a signifikant höhere Photostabilität, und zwar um etwa ein bis drei Größenordnungen. Der photochemische Abbau von Bakteriochlorinen ist ein energieabhängiger Prozess, der vom Energieeintrag der Photonen abhängt. Die Lipidumgebung spielt für beide Bakteriochlorine eine Schutzolle gegenüber allen Behandlungen mit UV-A, UV-B und sichtbarem Licht. Das Bacteriophäophytin *a* induzierte den Lipidperoxidationsprozess während der UV-A-Bestrahlungsbehandlung.

Schlüsselwörter: Photostabilität, Bakteriophäophytin a, Bakteriochlorophyll a, Bestrahlung, Lipide, Lipidperoxidation

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It’s about time

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ABSTRACT

Time is an enigmatic dimension. People assign somewhat mystical properties to time, especially the prospect of time travel. Nearly everyone has considered the possibility of a time machine at some point in their life, and this isn’t just because of the author Jules Verne. When you consider wanting a time machine, do you want to travel forwards or backwards in time? Since we are always apparently moving forwards in time, I would assume that the obsession is going backwards. The reason for this is that all people make mistakes and have regrets, and they would like to do some things differently in hindsight. But these are all humanistic desires, and it is this humanistic desire that incites people to give a mystical and magical property to time. It is probable that most people would wish to correct a past mistake at some point in their lives. The truth is that people can atone for their mistakes, but they can never absolutely undo them as if they never happened. The desire and ability to reverse time is currently only scientific on a psychological level. In fact, in the absence of some as-yet undiscovered physics the arrow of time is decided by Bolzmann’s entropy, therefore if a person can appreciate the meaning of entropy then they will fundamentally understand the arrow of time. Hence, chemistry students are in a good position when it comes to understanding time.

Keywords: Boltzmann’s entropy, time, thermodynamics, chemical reactions, reversibility

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A brief introduction to time

Some people say that the Sumerians "invented" time around 5000 years ago (Bertman, 2003). At the same time, in England, Stonehenge was being built by pre-Celtic tribes. Stonehenge has features that that align with the Sun during the winter and summer solstices, so it demonstrates that these tribes had an appreciation of time and developed an astronomical clock (Cunliffe, 1997). The Sumerian concept of time was possibly more sophisticated based upon available records. However, it was still based upon astronomical observations. The Sumerians gave us our basic units of time, namely 60 minutes in an hour, 24 hours in a day, 360 days in a year (more correctly 365 days as later established). The reason for these divisions was that the Sumerian mathematical system operated in base 60 and 60 multiplied by 6 is equal to 360. The choice of 60 seconds in a minute and 60 minutes in an hour is convenient in base 60. 12 multiplied by 5 is equal to 60. The ancient Egyptians used shadow clocks to determine 10 daylight hours. They also added two twilight hours when it may not have been possible to use a shadow clock, but it was still possible to see. So, there were twelve hours when people could see. The 12 hours of nighttime were determined by observation of star constellations on the ecliptic, which is the circle in the celestial sphere which the Sun appears to move on during the passage of one year. This is where the 12 signs of the zodiac are found.

The Sumerians also divided a circle into 360^{o}, so they probably understood that the Earth was going around the Sun in a near circular orbit and that the Earth was round like the Sun and the Moon (actually spherical). Hence the divisions that describe a section of a circle are the same as the divisions of time.

The astronomical clock at Stonehenge is also built in a circle, so although the culture that built it left no written records, they were probably operating with a common set of understanding of the cyclic nature of seasons, night and day and astronomical motions. Similar stone circles of similar age can be found all over England, Scotland, Ireland and Scandinavia. Clearly, there was a need to understand seasons for religious and practical reasons. It is probably no coincidence that pastoral societies were developing at the same time so that knowing when to plant crops would have been helpful.

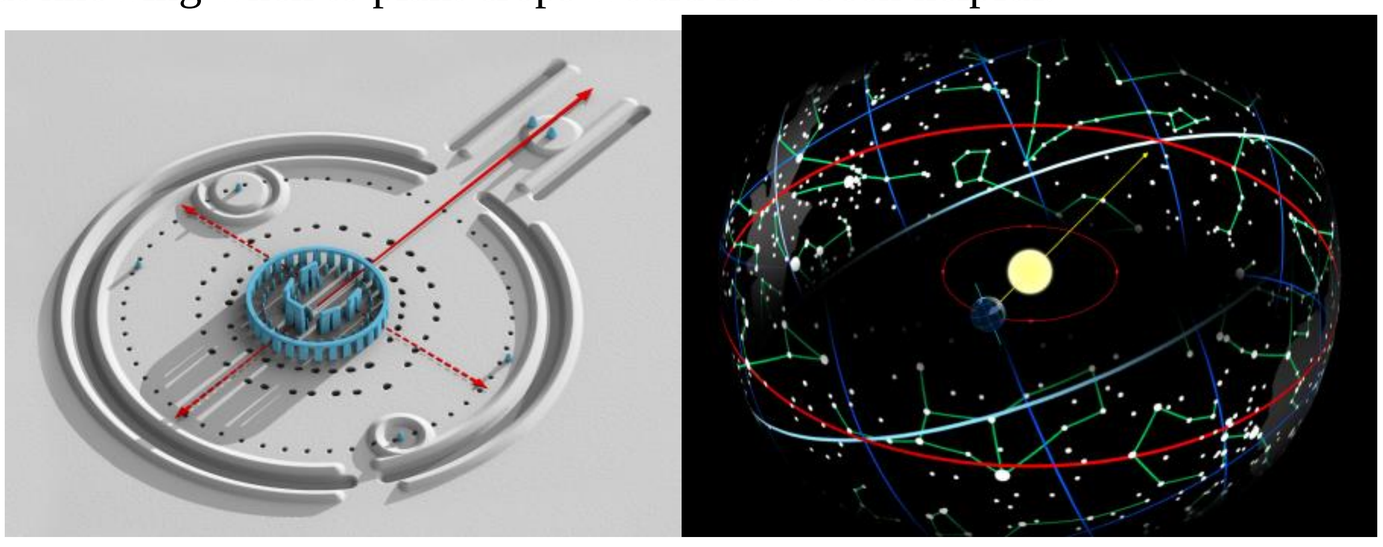

Figure 1. Left. A reconstruction of Stonehenge. Right. The Earth, Sun, Celestial Sphere and the ecliptic. (Public Domain)

For thousands of years celestial bodies have been used for navigation. It is easy to tell your latitude using stars *etc.* because their height in the sky is determined by latitude. Longitude presented a new problem, because the Earth's rotation affects the positions of the stars that are required to determine

the location east to west. This problem was solved by John Harrison early in the 18th Century (Sobel, 1996). He created the first workable ships clock, which had an accuracy that could keep time for the long periods that were typical during voyages of exploration at the time. Knowing the time, referenced to the Greenwich Meridian (longitude of zero) it was then possible for the navigator to derive their current longitude by correcting for the Earth's rotation using astronomical almanacs. This enabled voyages of discovery to be undertaken which led to the colonization of the Earth by seafaring western countries. Despite the controversy behind colonization it has to be recognized that an accurate knowledge of time significantly altered the history of the world.

More recently science uses atomic clocks as an accurate measurement of time. This involves analysis of the frequency of transitions between hyperfine energy levels of atoms (McCarthy and Speidelmann, 2009). Hyperfine structure is the small splitting of the energy levels of an atom due to the interaction between the state of the nucleus and the electron cloud. More precisely the hyperfine splitting is partly caused by the interaction of the nuclear magnetic dipole moment and the magnetic field caused by the electron cloud. The interaction of the nuclear electric quadrupole moment and the electric field gradients in the atom also contribute to the splitting. Since 1968 the SI definition of time has been based on the transition frequency between two energy levels in the ground state of the cesium 133 atom (^{133}Cs). 1 second is equal to the time it takes for 9192631770 transitions to occur.

The design of the atomic clock is based on a tunable microwave cavity or maser. The masing (equivalent to microwave lasing) medium is ^{133}Cs gas, at close to absolute zero temperature, that is in one hyperfine state that is excited within the microwave cavity. The number of atoms excited to the other hyperfine state is detected and the cavity is tuned to maximize the number of excited states. From this the frequency of the transition is measurable. The National Physics Laboratory in the UK has an atomic clock that would lose or gain less than 1s over a timescale of 138 million years. The accuracy of all other SI units such as amps, volts *etc.*, which have definitions that dimensionally contain seconds, are enhanced by an accurate measurement of the second.

1. The arrow of time and Boltzmann's entropy

In many processes the direction in which the process goes is determined by energy. In a sand-clock the sand falls from the upper to the lower chamber due to the decrease in potential energy as the sand enters the lower chamber. In order to reverse this, the user has to input some energy by turning the sand clock upside down again so that the sand is once again in the upper chamber. Actually this process needs to be done twice in order to get the sand back into the same up chamber as it was in at the start. However by turning the clock twice we did not turn back time. This is because the position of each particle of the sand in the upper chamber is completely different to the individual sand grain positions in the initial state. Furthermore, friction as each sand grain rubs against the others as it falls will alter the surface of each sand grain on the molecular level a tiny bit. This could never be compensated for by simply turning the sand clock twice. Consider this in terms of Gibbs free energy of the system.

$$\Delta G = \Delta H - T\Delta S \quad (1)$$

ΔG is the overall energy change of the process and it must become more negative for the entire process to be spontaneous. ΔH is the enthalpy change of the process and is linked to the reduction in

potential energy of the system. As the sand falls down it becomes more negative. When the user turns the clock energy is added that can overcome the entropy reduction and make the sand gain potential energy, which again reduces as the sand then falls. ΔS is the entropy of the system. It is a measure of the increase in disorder during the process. Probably the entropy of the process is rather unchanged as the sand falls and as the sand clock is turned, because it is always similarly random. However if the act of turning the glass was going to "turn back time" even on a local scale inside the sand clock then every atom worn off every grain of sand would need to return to the exact same position in the lattice upon turning the glass and every grain of sand would need to return to its original position oriented in exactly the same way. This is entropy. Moving forwards we can let the sands randomly fall into any of the possible positions that are available because going forwards is a random process. If we specified that only the original set of grain orientations is acceptable then that would increase the energy of the process because we are specifying the process to be non-random. This is an argument against pre-ordained futures.

Consider what entropy is based upon a simple model of socks. If you have socks of different colors and you put them into your sock drawer without first combining them into matching pairs then when you wake up late for class and go to the sock drawer to get a pair of your socks (lets make it harder by saying you must do it without looking) then the chance that you will retrieve a matching pair before the class is over is very small. However, if you took the time to match them then you would get a matching pair the first try every time. The reason for this is that the entropy of the unpaired socks is higher than the matched socks. However, in order to make the entropy decrease by matching up the socks we had to do a bit more work and that cost some energy. The point being that decreasing entropy in equation 1 takes quite a lot of energy in even a moderately complicated system.

People often say that time can go backwards in the quantum world. It is true that quantum processes can be very reversible, just like a videotape is reversible. But when we reverse a videotape we don't rewind the entire universe. We are just rewinding the cassette! Furthermore, just like the sand clock there would be slight differences in the state of the rewound tape compared to the initial state. In the quantum world an atom can quite easily slip between two states with near perfect reversibility due to the quantization of states, especially for a simple atom like hydrogen. However it would probably have changed its location a bit during the transition, because translational motion is not quantized, so it has not really gone backwards in time. Additionally going backwards in time should require the entire Universe to go back in time and a nearly reversible transition in an atom would certainly not influence the entire universe.

The reversibility of a process with respect to time does not mean that time goes backwards. An orbiting planet can move clockwise over time or it can move in the opposite anti-clockwise direction over time. Gravity and orbits can just work either way with equal ability. But just because one planet moves one way and the other another way does not mean people on one planet get older with time whilst people on the other planet get younger with time.

The "arrow of time" is clearly determined by the second law of thermodynamics. There is nothing mysterious. It's just that the entropy of the universe always increases, *i.e.* disorder increases. Going forwards in time by one unit of Planck time, $\sim 10^{-44}$ of a second, the Universe can move into any one of a enormous number of possible future permutations. The more permutations available the lower the energy required to proceed into any one of them. To go backwards by $\sim 10^{-44}$s to a point in which every atom and subatomic particle was in exactly the same state is to forbid the possibility of choosing any of the huge number of other available possibilities. The process of managing to forbid any of the other permutations would take an energy input approaching infinite. Hence, we cannot really travel back in time. This is

simple thermodynamics. Equally a pre-ordained future is also going to involve huge amounts of energy that is simply not available.

Some people claim that time is merely an illusion. The time clock of everything in our universe began with the Big Bang. The evidence I will state for this is the CMBR (cosmic microwave background) radiation. The CMBR gives us our closest picture of the distribution of energy in the universe just after the Big Bang. The radiation was not always microwave, once it was much shorter wavelength but as the universe expanded over time so did the space in which the light was traveling. Hence that light now has microwave frequency and wavelength. Hence, it is apparent that time is not an illusion and that things are changing over time since the Big Bang. We have to trust our everyday experiences and accept this. A person cannot become younger; a tomato left long enough will go rotten & you cannot make it become un-rotten, you cannot un-boil an egg; entropy increases.

From the chemistry point of view, chemical reactions can go forwards and backwards but we should not consider them as reversing in time. In fact the critical driving force is the need to establish a stable thermal equilibrium, which is governed by the following equation,

$$\Delta G = RT\ln K_e \qquad (2)$$

Where R is the gas constant and K_e is the equilibrium constant.

Reversible equilibrium is established from whichever direction is least stable. The method of flash photolysis in photochemistry often takes advantage of the possibility to shift a previously stable equilibrium to a higher energy state or to produce an excited state by adding photon energy in a light pulse from a flash-lamp or a laser. Rapidly, sometimes in femtoseconds the more energetic state returns to the ground state by dissipating its energy. However entropy will not allow every molecule to go into the same place and be in the same orientation as in the pre-pulsed original state. Similarly irreversible exothermic explosions can not suddenly reverse to form an in-tact bomb.

Without invoking some currently undiscovered physics it does seem that the arrow of time is fixed to going forwards by the huge energy of going perfectly backwards in time due to entropy. However, some unusual things do happen with time. Time dilation is one such phenomenon. However, time dilation is arguably only unusual because it does not noticeably occur under any conditions that we are used to in our everyday lives on Earth. There are two types of time dilation. One is derived from differences in velocity between two observers and the other is derived from differences in gravitational field (Einstein, 1905).

Velocity time dilation occurs because the speed of light is constant irrespective of the relative motion of two observers. If two observers separated by a distance L are stationary relative to each other and light travels from one observer to the other and is then reflected back then, using the formula Distance divided by Speed is equal to time, the time it takes for the round trip (Δt) is equal to 2L/C:

$$\Delta t = 2L/C \qquad (3)$$

However, if one observer is moving relative to the other, the length of the path of the light will be different D. D is longer L and the difference in length depends upon the relative speed of the observers and Pythagorean mathematics.

$$\Delta t' = 2D/C \tag{4}$$

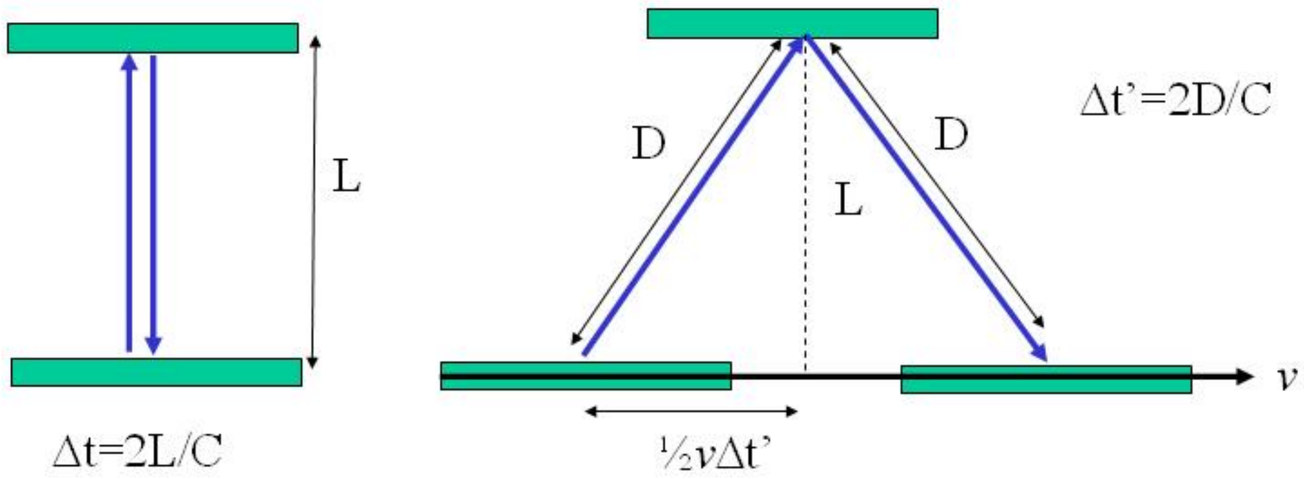

Figure 2. Schematic of the concept of time dilation for stationary observers and observers moving relative to each other.

From equations 4 and 5 we can derive:

$$D = \sqrt{\left(\frac{1}{2}v\Delta t'\right)^2 + L^2}$$

and by elimination we can get

$$\Delta t' = \frac{\Delta t}{\sqrt{1 - \frac{v^2}{c^2}}} \tag{5}$$

If each observer carried a clock then each would see the others clock ticking more slowly than their own.

Gravitational time dilation also occurs (not derived here) and results that the observers' clock is ticking faster in a reduced gravitational field compared to an observer in a stronger gravitational field. Hence, time would be slower on Jupiter compared to Earth.

From the equations for velocity dilation due to the v^2/C^2 relationship the dilation is miniscule at the speeds at which we normally travel. However for photons and light particles traveling at very close to light speed $\Delta t = \Delta t'/0$, the time for light speed objects has not got any consequence. Hence, for a photon there is no longer any time.

$$L = L_0\sqrt{1 - v^2/c^2} \tag{6}$$

Equally from the Fitzgerald Lorenz contraction (Lorentz, 1892) for a photon, space is no longer consequential. And this could go some way towards explaining how a single photon can pass through both slits in the Young's double slit experiment. As a rippling wave traveling through the Universe at light speed the photon does not experience the Universe in the "usual" way that we do on the Earth. If v^2/C^2 became greater than 1, then both the time dilation and the Fitzgerald Lorentz equations would involve the square root of -1, which is an imaginary number. This would give us some problems which is why it is said that light speed cannot be superseded and why ideas such as travelling faster than light in order to travel through time are equally problematic. After all we live in a real world, so that with our current understanding, although time dilations occur and time can be stopped. The direction of time travel

is always in a forward direction. Couple that with entropy and we will probably always struggle to make a time machine to turn back time to the good old days.

Conflict-of-Interest Statement

There are no conflicts of interest in this work

References

Bertman, S. (2003). Handbook to life in ancient Mesopotamia. Oxford University Press.

Cunliffe, B., & Renfrew, C. (1997). Science and Stonehenge, The British Academy 92. Oxford University Press.

Einstein, A. (1905). Special relativity. Physik, 322 (10), 891–921.

Lorentz, H. A. (1892). The relative motion of the Earth and the aether. Zittingsverlag Akad. V. Wet., 1, 74–79.

McCarthy, D., & Speidelmann, K. P. (2009) Time, from earth rotation to atomic physics. Weinheim Wiley-VCH, Chapters 10, 11.

Sobel, D. (1996). Longitude, London: Fourth Estate LtD.

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It’s about time

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SAŽETAK

Vreme je enigmatična dimenzija. Ljudi dodeljuju pomalo mistična svojstva vremenu, posebno perspektivi putovanja kroz vreme. Skoro svi su razmatrali mogućnost vremenske mašine u nekom trenutku svog života, a to nije samo zbog književnika Žila Verna. Kada razmišljate da želite vremensku mašinu, da li želite da putujete unapred ili unazad kroz vreme? S obzirom na to da se očigledno krećemo unapred kroz vreme, pretpostavljam da je opsesija putovanje u prošlost. Razlog za to je što svi ljudi prave greške i žale, a voleli bi da su stvari uradili drugačije u prošlosti. Ali sve su to ljudske želje, i upravo ta želja podstiče ljude da pripisuju vremenu mistično i magično svojstvo. Verovatno je da bi većina ljudi želela da ispravi grešku iz prošlosti u nekom trenutku svog života. Istina je da ljudi mogu popraviti svoje greške, ali ih nikada ne mogu apsolutno poništiti kao da se nikada nisu dogodile. Želja i sposobnost da se obrne vreme trenutno je naučno zasnovana samo na psihološkom nivou. Zapravo, u nedostatku još neotkrivene fizike, strelu vremena odlučuje Bolcmanova entropija, pa ako čovek može da proceni značenje entropije, tada će u osnovi razumeti strelicu vremena. Dakle, studenti hemije su u dobrom položaju kada je u pitanju razumevanje vremena.

Ključne reči: Bolcmanova entropija, vreme, termodinamika, hemijske reakcije, reverzibilnost

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Il est temps

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Résumé

Le temps est une dimension énigmatique. Les gens attribuent des propriétés quelque peu mystiques au temps, en particulier à la perspective d'un voyage dans le temps. Presque tout le monde a envisagé la possibilité d'une machine à remonter le temps à un moment donné de sa vie et ce n'est pas uniquement à cause de l'écrivain Jules Verne. Lorsque vous envisagez de vouloir une machine à voyager dans le temps, voulez-vous avancer ou reculer dans le temps? Puisque nous avançons toujours dans le temps, je suppose que notre obsession est de rentrer dans un moment du passé. La raison en est que tous les gens font des erreurs, ont des regrets et ils aimeraient faire certaines choses d'une manière différente dans le passé. Mais ce ne sont que des désirs de l'homme et c'est ce désir qui incite les gens à donner au temps une propriété mystique et magique. Il est probable que, dans un moment de leur vie, la plupart des gens souhaiteraient corriger une erreur du passé. La vérité est que les gens peuvent réparer leurs erreurs, mais ils ne peuvent jamais les défaire comme si elles ne s'étaient jamais produites. Aujourd'hui, le désir et la capacité de renverser le temps sont scientifiquement fondés uniquement au niveau psychologique. En fait, en l'absence de physique encore inconnue, la flèche du temps est définie par l'entropie de Boltzmann. Par conséquent, si l'homme est capable d'apprécier le sens de l'entropie, il comprendra le principe de la flèche du temps. Les étudiants de chimie sont, dès lors, bien placés pour comprendre le temps.

Mots-clés: entropie de Boltzmann, temps, thermodynamique, réactions chimiques, réversibilité.

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Это о времени

Джонатан Хобли

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Время - загадочное измерение. Люди приписывают мистическим свойствам время, особенно перспективу путешествий во времени. Почти каждый рассматривал возможность использования машины времени в какой-то момент своей жизни, и это не только из-за автора Жюля Верна. Если вы хотите получить машину времени, хотите ли вы путешествовать во времени вперед или назад? Поскольку мы, очевидно, всегда движемся вперед во времени, я бы предположил, что одержимость идет назад. Причина этого заключается в том, что все люди делают ошибки и сожалеют, и они хотели бы сделать некоторые вещи в ретроспективе. Но это все гуманистические желания, и именно это гуманистическое желание побуждает людей отдавать мистическое и магическое свойство времени. Вполне вероятно, что большинство людей захотят исправить прошлую ошибку в какой-то момент своей жизни. Правда состоит в том, что люди могут искупить свои ошибки, но они никогда не смогут полностью исправить их, как будто они никогда не случались. Желание и способность обратить вспять время в настоящее время являются только научными на психологическом уровне. На самом деле, в отсутствие какой-то пока еще не обнаруженной физики стрелка времени определяется энтропией Больцмана, поэтому, если человек может оценить значение энтропии, он в основном поймет стрелу времени. Следовательно, студенты, изучающие химию, находятся в хорошем положении, когда дело доходит до понимания времени.

Ключевые слова: энтропия Больцмана, время, термодинамика, химические реакции, обратимость

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Es ist Zeit

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ABSTRAKT

Zeit ist eine rätselhafte Dimension. Die Menschen weisen der Zeit etwas mystische Eigenschaften zu, insbesondere der Perspektive der Zeitreise. Fast jeder dachte irgendwann in seinem Leben die Möglichkeit einer Zeitmaschine nach, und dies ist nicht nur wegen des Schriftstellers Jules Verne. Wenn Sie eine Zeitmaschine wünschen, möchten Sie in der Zeit vorwärts oder rückwärts reisen? Da wir uns offensichtlich immer in der Zeit vorwärts bewegen, würde ich annehmen, dass die Besessenheit eine Reise in die Vergangenheit ist. Der Grund dafür ist, dass alle Menschen Fehler machen und etwas bereuen, und dass sie wünschten, sie hätten die Dinge in der Vergangenheit anders gemacht. Das sind aber alles menschliche Wünsche, und gerade diese Wünsche regen die Menschen dazu an, der Zeit eine mystische und magische Eigenschaft zuzuschreiben. Es ist wahrscheinlich, dass die meisten Menschen irgendwann in ihrem Leben einen Fehler aus der Vergangenheit korrigieren möchten. Die Wahrheit ist, dass Menschen ihre Fehler korrigieren können, aber sie können sie niemals absolut rückgängig machen, als ob sie niemals passiert wären. Der Wunsch und die Fähigkeit, die Zeit umzukehren, ist derzeit nur auf psychologischer Ebene wissenschaftlich begründet. In der Tat wird der Pfeil der Zeit in Ermangelung einer noch unentdeckten Physik von Bolzmanns Entropie bestimmt. Wenn also eine Person die Bedeutung der Entropie verstehen kann, wird sie den Pfeil der Zeit grundlegend verstehen. Daher sind Chemiestudenten in einer guten Position, wenn es darum geht, die Zeit zu verstehen.

Schlüsselwörter: Boltzmanns Entropie, Zeit, Thermodynamik, chemische Reaktionen, Reversibilität

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Tomato-the chemistry of “golden apple”

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ABSTRACT

Food is expected to meet basic physiological needs, but also to exert a positive influence on the health and protection of cells from negative environmental factors. Tomatoes are a good source of vitamins and minerals and also have antioxidant properties. The main antioxidants in tomatoes are carotenoids, ascorbic acid and phenolic compounds. Lycopene is responsible for the red color of tomatoes and is considered to be an antioxidant with high biological activity. Studies have shown that lycopene protects the skin from ultraviolet (UV) rays and thus provides some protection against skin cancer.

Keywords: Tomato, chemical composition, lycopene, polyphenolic compounds

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Introduction

Tomato (*Solanum lycopersicum*) is a vegetable, whose fruit is used for nutrition at botanical maturity but also as a green, after acidification (Madhavi and Salunkhe, 1998). Ripe fruit is used in the diet as a salad, a supplement to various dishes and raw material for various processing. Cultured tomato varieties originate from wild relatives, which are still part of spontaneous vegetation in the Andes region of South America. Migrations of Native American populations to the Central American and Mexican regions of the Andean region conveyed wild forms of tomatoes, where they were cultivated and used for eating in the early seventh century. In the cultivation of tomatoes, a significant role was played by the tribe Astecs who called it "xtomatl". The name “tomati“ derives from this name, which was generated by other Native American tribes of Central America and is the root of the word „tomato“ in many languages. At the beginning of the sixteenth century, it is believed that tomatoes were transferred to Europe, first to Spain, Italy and Portugal, and then to other parts. It was initially grown as an ornamental plant and was not used for eating since its fruits were thought to be poisonous. The first description of the tomato plant was given by the Italian botanist Mattioli in 1544, calling it "pomi d'oro" (golden apple) indicating that the first-born plants had yellow fruits. From that description, the current Italian name for tomato "pomodoro" also originates. New forms of larger red fruits are likely to have spread to France, which is why the French have called them "pomme d'amour" (apple of love). Newly arrived vegetables have been called "paradiesapfel" in Germany, which means paradise apple. The current scientific name for the Lycopersicon esculentum tomato was suggested by the English botanist Miller in 1768, which would translate into a wolf's edible apple (Matotan, 2008). Tomatoes for eating in Europe are thought to be first used in Italy in the mid-sixteenth century, and then spread to other Mediterranean countries and later to northern Europe.

Chemistry of tomato

The chemical compounds present in tomatoes greatly affect the biological properties and organoleptic properties. The chemical composition of tomato fruits depends on factors such as variety, maturity and environmental conditions in which they are grown (Abushita et al., 1997; Davies and Hobson, 1981; Giovanelli et al., 1999; Thompson et al., 2000). It has been shown that ripening processes and storage temperatures can greatly influence the final nutrient composition (Madhavi and Salunkhe, 1998). The average chemical composition of tomato fruits is: 94% water, 4% carbohydrates, 0.9% protein, 0.2% fat, 0.6% minerals and 0.2% pectin (Hernández Suárez et al., 2008). It has low calories and a high content of vitamins C and A, which have a beneficial effect on the immune system and break down fat. Nutritional composition of tomato is shown in Table 1.

Table 1. Nutritional composition of tomatoes

Nutritional composition per 100g	**Mass**
Water	93g
Proteins	1,3g
Fats	0,3g
Carbohydrates	5,8g
Cellulose	1,1g
Minerals	
K	353mg
P	35,8mg
Ca	14,9mg
Mg	16,4mg
Fe	0,4mg
Na	7,5mg
Zn	0,3mg
Mn	0,2mg
Vitamins	
Vitamine C	18,9mg
Vitamine E	0,8mg
Carotene (provitamine A)	0,7mg
Vitamine K	0,1mg
Vitamine PP	0,5mg
Vitamins B1, B2, B3, B5, B6	0,82mg
Folates (vitamine B9)	22,3µg
Essential aminoacids	0.9g

Tomatoes and its products are good sources of carotenoids (especially lycopene), ascorbic acid (vitamin C), vitamin E, folate, chavonoids and potassium (Beecher, 1998; Leonardi et al., 2000). Other ingredients are protein and dietary fiber (Davies and Hobson, 1981).

Lycopene, a plant pigment belonging to the carotenoid group, is responsible for tomato red color (Nguyen and Schwartz, 1999) and is considered an antioxidant with high biological activity. This pigment possesses the ability to inhibit the proliferation of breast, lung, and endometrial cancer cells (Heber and Lu, 2002). Studies have shown that lycopene protects the skin from ultraviolet (UV) rays and thus provides some protection against skin cancer. The content of lycopene in tomatoes varies considerably, so research has shown that grown tomatoes contain higher levels of lycopene (5.2 to 23.6 mg / 100 g fresh weight (SM)) (Abushita, 1997) than tomatoes in greenhouse (0.1 and 10.8 mg / 100 g SM) (Leonardi et al., 2000). In tomatoes with yellow fruits, lycopene is present in traces. The tomato fruit also contains other colored substances (β-carotene and xanthophylls), which are responsible for the color of the non-red tomato. From a chemical point of view, the carotenoids are tetraterpenic and belong to the lipid class in terms of physical and chemical properties (Lajšić and Grujić-Injac, 1998). They can be divided into two groups: carotene - dissolved in non-polar solvents and oxidizing products of carotenoids - xanthophylls - which dissolve in polar solvents and occur in the form of alcohols, aldehydes, ketones, esters (Lajšić and Grujić-Injac, 1998). These plant pigments participate in the process of photosynthesis, and they have a provitaminic role in the human body (Kadian and Garg, 2012) and an antioxidant role (Padovani and Amaya-Farfán, 2006). Another essential compound in tomatoes is flavonoid zeaxanthin. Zeaxanthin protects the eyes from the disease

of yellow spot degeneration (ARMD (Age-Related Macular Degeneration), which occurs in the elderly, caused by the action of harmful UV rays.

In addition to carotenoids, which play an antioxidant role, many other compounds that exhibit antioxidant activity, such as polyphenolic compounds and vitamin C, are also present in Table 2. Table 2 shows the average content of compounds showing antioxidant activity (Frusciante et al., 2007).

Table 2. Content of compounds exhibiting antioxidant activity in tomato samples

Compound	**Content (mg/100 g tomato)**
Lycopene	1.86-14.62
β-carotene	0.11-1.07
Luteine	0.08-0.34
Fenolic acids	2.85-4.68
Flavonoids	1.15-8.16
Vitamine C	2.20-21
Vitamine E	0.11-1.84

The content of these compounds varies in seeds, husks and meat. George et al. (2004) found that the content of polyphenolic compounds was higher in the shell than in the meat of tomatoes (10.4-40.0 mg / 100 g and 9.2-27.0 mg / 100 g). Naringenin, chalconaringenin, quercetin, kaempferol, myricetin, *p*-hydroxybenzoic, cinnamon, salicylic, protocatechin, coumaric, vanillin, caffeic, chlorogenic, ferulic and synaptic acid were isolated from tomato samples (Slimestad and Verheul, 2009).

Cis-3-hexenal, hexanal, *cis*-3-hexenol, β-ionone, β-damascenone, 1-penten-3-one, 3-methylbutanal, 2-isobutylthiazole are responsible for the aroma of this vegetable (Yilmaz, 2001).

Tomatoes have found a wealth of vitamins, minerals and other nutrients. It contains 17 times more iron than milk, twice as much as eggs and even three times as much as fish. Ripe fresh tomatoes contain 2-3 times more vitamin C (as much as 21% of the recommended dose per 100 grams) than green. It has very few calories (15 kcal per 100 grams of food) and is therefore an integral part of most diet menus. Cherry tomato is a variety of tomato cerasiforme that has been only used for decorative purposes until numerous positive effects on the organism are discovered. It is mostly small, of various shapes (across round and oval to pear-shaped) and colors (green, red, orange, black).

References

Abushita, A. A., Hebshi, E. A., Daood H. G., & Biacs, P. A. (1997). Determination of antioxidant vitamins in tomatoes. Food Chemistry, 60, 207-212.

Beecher, G. R. (1998). Nutrient content of tomatoes and tomato products. Proceedings of the Society for Experimental Biology and Medicine, 218, 98-100.

Davies, J. N., & Hobson, G. E. (1981). The constituents of tomato fruit—the influence of environment, nutrition, and genotype. CRC Critical Reviews in Food Science and Nutrition, 15, 205-280.

Frusciante, L., Carli, P., Ercolano, M. R., Pernice, R., Di Matteo, A., Fogliano, V., & Pellegrini, N. (2007). Antioxidant nutritional quality of tomato. Molecular Nutrition & Food Research, 51, 609-617.

George, B., Kaur, C., Khurdiya, D.S., & Kapoor, H.C. (2004). Antioxidants in tomato (*Lycopersium esculentum*) as a function of genotype. Food Chemistry, 84, 45-51.

Giovanelli, G., Lavelli,V., Peri, C., & Nobili, S. (1999). Variation in antioxidant components of tomato during vine and post-harvest ripening. Journal of the Science of Food and Agriculture, 79, 1583-1588.

Heber, D., & Lu, Q-Y. (2002). Overview of mechanisms of action of lycopene. Experimental Biology and Medicine, 227, 920–923.

Hernández Suárez, M., Rodríguez, E. M., & Díaz Romero, C. (2008). Chemical composition of tomato (*Lycopersicon esculentum*) from Tenerife, the Canary Islands. Food Chemistry, 106, 1046-1056.

Kadian, S. S., & Garg, M. (2012). Pharmacological effects of carotenoids: A review. International Journal of Research in Pharmaceutical Sciences, 3, 42-48.

Lajšić, S., & Grujić-Injac, B. (1998) Hemija prirodnih proizvoda, Univerzitet u Novom Sadu, Tehnološki fakultet.

Leonardi, C., Ambrosino, P., Esposito F., & Fogliano, V. (2000). Antioxidative activity and carotenoid and tomatine contents in different typologies of fresh consumption tomatoes. Journal of Agricultural and Food Chemistry, 48, 4723-4727.

Madhavi, D. L., & Salunkhe, D. K. (1998). Production, composition, storage, and processing. New York.

Matotan, Z. (2008). Plodovito povrće. Bjelovar: Neron.

Nguyen, M. L., & Schwartz, S. J. (1999). Lycopene: chemical and biological properties. Food Technology, 53, 38-45.

Padovani, R. M., &Amaya-Farfán, J. (2006). Procurement of β-carotene, lycopene, lutein and zeaxanthin in households of Brazil's urban areas. Segurança Alimentar e Nutricional, 13, 49-63.

Slimestad, R., & Verheul, M. (2009). Review of flavonoids and other phenolics from fruits of different tomato (*Lycopersicon esculentum* Mill.) cultivars. Journal of the Science of Food and Agriculture, 89, 1255-1270.

Takeoka, G. R., Dao, L., Flessa, S., Gillespie, D. M., Jewell, W. T., Huebner, B, Bertow, D., & Ebeler, S. E. (2001). Processing effects on lycopene content and antioxidant activity of tomatoes. Journal of Agricultural and Food Chemistry, 49, 3713–3717.

Thompson, K. A., Marshall, M. R., Sims, C. A., Wei, C. I., Sargent, S. A., & Scott, J. W. (2000). Cultivar, maturity, and heat treatment on lycopene content in tomatoes. Journal of Food Science, 65, 791–795.

Yilmaz, E. (2001). The chemistry of fresh tomato flavor. Turkish Journal of Agriculture and Forestry, 25, 149-155.

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Paradajz-hemija “zlatne jabuke”

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SAŽETAK

Od hrane se očekuje se da zadovolji osnovne fiziološke potrebe, ali i da ima pozitivan uticaj na zdravlje i zaštitu ćelija od negativnih factora iz životne sredine. Paradajz je dobar izvor vitamina i minerala, a takođe ima antioksidativna svojstva. Glavni antioksidanti u paradajzu su karotenoidi, askorbinska kiselina i fenolnajedinjenja. Likopen je odgovoran za crvenu boju paradajza i smatra se antioksidansom sa visokom antioksidativnom aktivnosti. Studije supokazale da likopen štiti kožu od ultraljubičastih (UV) zraka i tako pruža zaštitu od raka kože.

Keywords: Tomato, chemical composition, lycopene, polyphenolic compounds

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Tomate – la chimie de la « pomme d'or »

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Résumé

La nourriture devrait répondre aux besoins physiologiques de base, mais également exercer une influence positive sur la santé et la protection des cellules contre les facteurs environnementaux négatifs. La tomate est une bonne source de vitamines et de minéraux et elle possède également des propriétés antioxydantes. Les principaux antioxydants de la tomate sont les caroténoïdes, l'acide ascorbique et les composés phénoliques. Le lycopène est responsable de la couleur rouge de la tomate et il est considéré comme un antioxydant à haute activité biologique. Des études ont montré que le lycopène protège la peau des rayons ultraviolets (UV) et, de ce fait, il assure une protection contre le cancer de la peau.

Mots-clés: tomate, composition chimique, lycopène, composés polyphénoliques.

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Помидор-химия «золотого яблока»

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Резюме

Ожидается, что пища будет отвечать основным физиологическим потребностям, а также оказывать положительное влияние на здоровье и защиту клеток от негативных факторов окружающей среды. Помидоры являются хорошим источником витаминов и минералов, а также обладают антиоксидантными свойствами. Основными антиоксидантами в томатах являются каротиноиды, аскорбиновая кислота и фенольные соединения. Ликопен отвечает за красный цвет томатов и считается антиоксидантом с высокой биологической активностью. Исследования показали, что ликопин защищает кожу от ультрафиолетовых (УФ) лучей и, таким образом, обеспечивает некоторую защиту от рака кожи.

Ключевые слова: томат, химический состав, ликопен, полифенольные соединения

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Tomate - die Chemie des "goldenen Apfels“

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ABSTRAKT

Von der Nahrung wird erwartet, dass sie die physiologischen Grundbedürfnisse befriedigt, aber dass sie auch einen positiven Einfluss auf die Gesundheit und den Schutz der Zellen vor negativen Umweltfaktoren ausübt. Tomaten sind eine gute Quelle der Vitamine und Mineralien und haben auch antioxidative Eigenschaften. Die Hauptantioxidantien in Tomaten sind Carotinoide, Ascorbinsäure und Phenolverbindungen. Lycopin ist für die rote Farbe von Tomaten verantwortlich und gilt als Antioxidans mit hoher antioxidantiven Aktivität. Studien haben gezeigt, dass Lycopin die Haut vor ultravioletten (UV) Strahlen schützt und somit einen gewissen Schutz gegen Hautkrebs bietet.

Schlüsselwörter: Tomate, chemische Zusammensetzung, Lycopin, Polyphenolverbindungen

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Coffee - from plant to popular beverage

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ABSTRACT

Coffee, one of the most popular beverages in modern world, is obtained from the beans of perennial bush *Coffea*. The distinctive aroma and taste of coffee is obtained by thermal treatment (roasting) of raw coffee beans. There are different degrees of roasting that can be divided according to the color of the grain: easy, medium, dark and Italian. The most famous types of coffee used in the production of beverages are Arabica and Robusta. Raw coffee beans contain a certain amount of caffeine, sugar, essential oil, potassium, calcium and magnesium. Roasting the grains leads to the loss of dry matter through formation of carbon dioxide, water and volatile products of pyrolysis. Coffee also has a moderate healing effect, while in overdoses it causes rapid heartbeat, insomnia, even euphoria.

Keywords: coffee, Arabica, Robusta

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Introduction

Coffee is the most popular beverage in the world and is obtained by preparing grounded beans of the same name perennial shrub (Lat. *Coffea*). It belongs to the family Rubiaceae. The coffee plant can reach a height of 2 to 5 m, if left untreated it can reach a height of 10 m. It benefits from heavy rain and temperatures from 12 to 27^{o}C. It thrives in the lowlands, but it is of higher quality that grows at higher altitudes. Its flowers are needle-like, white in color. The fruit is green in color, and at the stage of ripening it turns a yellowish color which darkens and turns reddish, when the fruit is ready to be harvested (Figure 1). Within this fruit are two grains that are greenish-brown in color. After heat treatment, the beans turn dark brown, bursting and getting a distinctive coffee appearance.

Figure 1. The fruit of coffee (photo taken from Dritan Alsela instagram profile)

Coffee history

The plant coffee is named after town of Kaffa, located in Ethiopia, and it is believed that this plant originates from that region.

From the African plateau, coffee was transferred to Arabia in the ninth century, from where the beverage we know today as coffee, comes from. African natives used coffee beans as food. The coffee beans were ground and mixed with water, spices and animal fat, eaten especially before the battle to gain strength. Two Syrians first brought coffee to Constantinople in 1555. There were cafes where coffee was consumed exclusively. The Venetian merchants brought it to Venice in 1570 and coffee from there, started to spread throughout Europe. First it was luxury, consumed only by the wealthiest class of society. With the increase of coffee imports, it soon spread to all households and became part of the daily routine of common people. Coffee production is today the second largest industry in the world, after oil.

Coffee species

There are many types of coffee, but only two are the most used in the world - Arabica and Robusta. Among the other coffee species, known are: Arabusta, created by hybridization of Arabica and Robusta, Liberica and Excelsa (Liberian coffee), Stenophylla (Sierra Leone coffee), Gallienii, Mogenetii and Bonnieri (caffeine-free).

Arabica originates from the southwestern regions of Ethiopia. It is the first type of coffee used by humans that thrives in altitude from 1300 to 1500 m. Contains about 1.5% caffeine, has a rich and sophisticated taste and less grain acidity. Therefore, it is considered as superior and high-quality type of coffee.

Robusta comes from central Africa. It is better suited for cultivation, but of lower quality than Arabica. Contains about 2.7% caffeine, has strong spicy taste, most commonly used to make instant coffee. Due to its intense bitter-sour taste in coffee production, it is often mixed with Arabica. Coffee containing Robusta makes better foam.

The basic difference between Arabica and Robusta is shown in Figure 2.

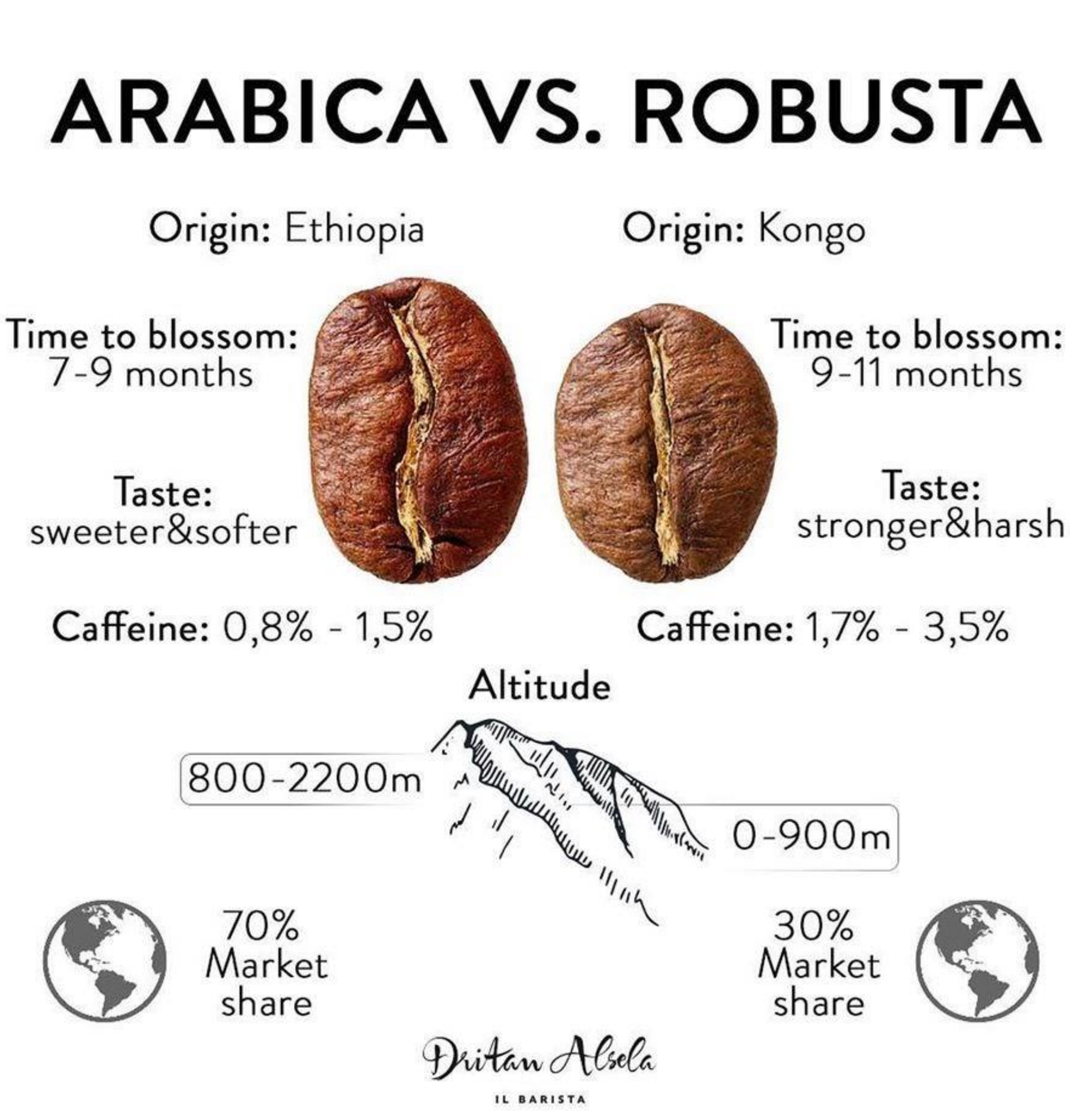

Figure 2. Differences between Arabica and Robusta coffee (photo taken from Dritan Alsela instagram profile)

Coffee chemistry

When roasting coffee beans, the loss of dry matter occurs, transforming in carbon dioxide, water and volatile pyrolysis products, including PAHs. This process degrades polysaccharides, amino and

chlorogenic acids, and increases the content of organic acids and lipid components. The presence of citric, lactic and acetic acid (less than 1%) is observed in roasted coffee, while chlorogenic acid makes up about 7% of the dry matter in Arabica coffee. Caffeine and quinic acid form chlorogenic acid, which is found in many types of fruits, and is found in coffee in the highest concentration. The average chemical composition of roasted coffee is shown in Table 1.

Table 1. Average chemical composition of roasted coffee.

Substances	%
Water	1.3
Proteins	14.0
Lipids	10.3
Carbohydrates	67.7
Ash	4.0

Raw coffee beans contain 0.9 to 2.4% caffeine alkaloids, 10 to 12% fat, up to 40% cellulose, up to 10% sugar, low essential oil, calcium, magnesium and potassium.

The most famous and important ingredient of coffee is caffeine (1,3,7-trimethyl-xanthine), derivative of purine. Caffeine diffuses easily into the cerebrospinal fluid, stimulates the central nervous system (CNS), and thus increases alertness and mood. Caffeine is retained in the blood for about 4 hours. It also acts as a diuretic, having the most active effect on the CNS and skeletal muscle, while it has the least activity on the smooth muscle and the heart (Bogdanović, 1963).

Numerous scientific studies have shown that regular coffee intake helps treat diabetes (type 2) and also reduces the risk of its appearance. Coffee has a positive effect on human health due to the presence of antioxidants such as: chlorogenic, ferulic, caffeic and coumaric acid (Nicoli et al., 1997). In addition, melanoidins (Steinhart et al., 2001), caffeine and phenylalanines (Farah and Donangelo, 2006) are thought to be responsible for the antioxidant activity of coffee.

Green coffee contains more chlorogenic acid than roasted coffee. The antioxidant activity of coffee depends on the polyphenolic compounds, dominantly due to the presence of chlorogenic acid, which exerts high antioxidant activity. Green coffee extracts have the effect of reducing fat and weight (Igho et al., 2011). In addition to chlorogenic acid and its derivatives, caffeine, theobromine, theophylline, caffeine and tocopherols (Stelmach et al., 2015) contribute to this property of coffee.

Because coffee also acts as an antioxidant, it protects cells from damage caused by antioxidant stress and helps prevent the onset of some degenerative diseases (*e.g.* liver cirrhosis, Alzheimer's and Parkinson's disease) (Žikić et al., 2014).

People who enjoy coffee, actually enjoy caffeine, which is a toxic herbal base and is classified as a drug. Decaffeinated coffee does not exist, it is only possible to reduce the level of caffeine in coffee in different ways. One way is to immerse the coffee in acid, reducing the percentage of caffeine, but increasing the acidity of the coffee. The plants did not develop caffeine to help us get through our morning meetings, enjoying coffee. Like many secondary metabolites of plants, caffeine, which is quite bitter, is actually a chemical weapon that can incapacitate or even kill insects that threaten plants.

There are many more molecules that give coffee a distinctive aroma and taste. Pyrazine, for example, gives earthy, and methyl propanol a fruity aroma to coffee. By roasting, bitter chlorogenic acid is converted into various derivatives, making coffee basis for a very pleasant and aromatic drink.

To obtain the true specific and characteristic aroma and taste of coffee, it is necessary to heat the raw beans. The aroma is created from a complex that is a mixture of volatile components, and non-volatile components determining the acidity, bitterness and taste.

The process of roasting coffee is divided into three stages. The first phase is drying of the coffee beans, where the moisture content of the beans is reduced. The aroma and color of raw grains change from green to golden yellow. In the second phase, the grain is twice as small, with the release of a large amount of carbon dioxide and several hundred substances that combine into a unique coffee aroma. This is where the grain gets a dark brown color. Initially, the process is exothermic. The pyrolysis reaction is the most intensive at 220-250^{o}C when the process becomes endothermic and evaporation of volatile components occurs. Reactions become exothermic again at 250^{o}C. During roasting, as a result of pyrolysis, the characteristic aroma and taste of coffee is obtained. Roasting time ranges from 90 seconds to 40 minutes, in dependence of the type of coffee. From the eleventh to twelfth minutes, hundreds of aromas develop, acidity and sugar decrease, and bitterness increases. The third phase is the final phase, and rapid cooling is performed to stop the exothermic reactions. Air or water is used for refrigeration, and the best coffee is considered to be the one, that is suddenly cooled with water.

In various parts of the world, the beverage is prepared from grounded coffee beans, roasted to different degrees, which can also be defined by the color of the beans:

- Easy (light) roasting - at 205^{o}C; the coffee bean color is reminiscent of cinnamon, so this type of roasting is also called "cinnamon" roasting. The coffee roasted in this way has a sweet and slightly sour taste, is coarsely ground and used for "filter coffee";
- medium roasting - at 219^{o}C; the grain is dark brown, used for so-called “Turkish coffee”. This kind of grain after roasting is oily and the aroma is pronounced and pleasant.
- Dark roasting - at 240^{o}C; the grain is dark brown to black with a glossy surface that comes from the liberated oil. This method of roasting produces "espresso" coffee, which is why it can be called "espresso" roasting or so called "French coffee". It contains significantly less caffeine than raw coffee, due to intense heat treatment.
- Italian roasting - at 245^{o}C; the grains turn almost black, and the acidity is almost removed.

Various hot drinks are made from coffee, the most popular of which are classic coffee or so-called “Turkish” coffee, espresso, macchiato, cappuccino, latte, affogato, flat white, Americano coffee, Coretto coffee, Ice coffee, Mexicano coffee, Irish coffee and many others.

In addition to coffee that is obtained only from pure coffee beans, there is a so-called surrogate coffee. The surrogate coffee consists of roasted fruits and roasted parts of plants rich in starch, sugars, inulin boiled or dissolved in water. The basic raw material for the production of surrogates, can be barley, rye, wheat, carob, soybeans, *etc.* Most coffee consumed is not entirely made from coffee beans but contains surrogates.

The most expensive and extremely rare coffee is Kopi luwak, originating from the Philippines and Indonesia. This coffee is obtained from the feces of a civet, a small animal from the mammalian family (Figure 3). In Indonesia, civet is called luwak, and coffee is called kopi. The civet is fed with coffee beans, then the beans are boiled and discarded. During brewing, the coffee beans remain intact because only the fleshy part of the fruit is removed. Enzymes secreted by civet during brewing improve the quality and taste of coffee. During the brewing process, the proteins and caffeine content of the coffee

bean is reduced, making the beans less bitter and having a specific aroma. Such coffee beans are dried in the sun and further processed.

Figure 3. Civet with coffee grains

References

Bogdanović, S., (1963). Farmakologija, Univerzitet u Beogradu 266-267.

Farah, A., & Donangelo C.M. (2006). Phenolic compounds in coffee. Brazilian Journal of Plant Physiology, 18, 23–36.

https://www.instagram.com/dritanalsela/?hl=en

Igho, O., Rohini, T., & Edzard, E. (2011). The use of green coffee extract as a weight loss supplement: a systematic review and meta-analysis of randomised clinical trials. Gastroenterology Research and Practice, 31, 1–6.

Nicoli, M.C., Anese, M., Manzocco, L., & Lerici, C.R. (1997). Antioxidant properties of coffee brews in relation to the roasting degree. Lebensmittel-Wissenschaft und –Technologie, 30, 292–297.

Steinhart, H., Luger, A., & Piost, J. (2001). Antioxidative effect of coffee melanoidins; Proceedings of the 19th International Scientific Collogue on Coffee; Trieste, Italy. 14–18 March, 2001.

Stelmach, E., Pohl, P., & Szymczycha-Madeja, A. (2015). The content of Ca, Cu, Fe, Mg and Mn and antioxidant activity of green coffee brews. Food Chemistry, 182, 302–308.

Žikić S., Tobić D., &Žikić G., (2014). Enciklopedija lekovitog bilja i pečuraka.PI PRESS, Pirot, 150 – 152.

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Kafa – od biljke do popularnog napitka

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Od zrna višegodišnje žbunaste biljke *Coffea* dobija se najpopularniji i mnogima omiljeni napitak – kafa. Prepoznatljiv miris i ukus kafe dobija se termičkom obradom (pečenjem) sirovog zrna kafe. Postoje različiti stepeni prženja koji se mogu podeliti prema boji zrna: lako, srednje, tamno i italijansko prženje. Najpoznatije vrste kafe koje se koriste u proizvodnji napitaka su Arabika i Robusta. Sirovo zrno kafe sadrži određenu količinu kofeina, šećera, etarskog ulja, kalijuma, kalcijuma i magnezijuma. Prženjem zrna, dolazi do gubitka suve materije, odnosno stvaranja ugljen-dioksida, vode i isparljivih proizvoda pirolize. Kafa ima i lekovito dejstvo u umerenim količinama, dok u prevelikim dozama izaziva ubrzan rad srca, nesanicu, čak euforiju.

Ključnereči: kafa, Arabika, Robusta

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Café – de la plante à la boisson populaire

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Résumé

Le café, l'une des boissons les plus populaires dans le monde moderne, est obtenu à partir des grains de la brousse vivace de café. L'arôme et le goût distinctifs du café sont obtenus par le traitement thermique (torréfaction) des grains de café crus. Il existe différents degrés de torréfaction qui peuvent être divisés en fonction de la couleur du grain : facile, moyen, foncé et italien. Les sortes de café les plus célèbres utilisées dans la production des boissons sont l'Arabica et le Robusta. Le grain de café cru contient une certaine quantité de caféine, de sucre, d'huile essentielle, de potassium, de calcium et de magnésium. La torréfaction des grains entraîne la perte de matière sèche, c'est-à-dire la formation du dioxyde de carbone, de l'eau et des produits volatils de pyrolyse. En quantité modérée, le café possède également un effet curatif, tandis qu'en cas de surdosage, il provoque les battements cardiaques rapides, l'insomnie, voire l'euphorie.

Mots-clés: *café, Arabica, Robusta.*

Кофе-от растения до популярного напитка

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Резюме

Кофе-один из самых популярных напитков в современном мире, получаемый из фасоли многолетнего куста кофе. Отличительный аромат и вкус кофе получают термической обработкой (обжаркой) сырых кофейных зерен. Существуют различные степени обжарки, которые можно разделить по цвету зерна: легкая, средняя, темная и итальянская. Наиболее известные виды кофе, используемые при производстве напитков, - это арабика и робуста. Сырые кофейные зерна содержат определенное количество кофеина, сахара, эфирного масла, калия, кальция и магния. Обжарка зерен приводит к потере сухого вещества за счет образования углекислого газа, воды и летучих продуктов пиролиза. Кофе также обладает умеренным заживляющим эффектом, в то время как при передозировке он вызывает учащенное сердцебиение, бессонницу и даже эйфорию.

Ключевые слова: кофе, арабика, робуста

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Kaffee - von der Pflanze zum beliebten Getränk

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ABSTRAKT

Kaffee, das populärste und beliebteste Getränk, wird aus den Bohnen der mehrjährigen Strauchpflanze *Coffea* gewonnen. Den erkennbaren Duft und Geschmack von Kaffee erhält man durch thermische Behandlung (Röstung) der rohen Kaffeebohnen. Es gibt verschiedene Röstgrade, die je nach Farbe der Kaffeebohnen, in leichte, mittlere, dunkle und italienische Röstung unterteilt werden können. Die bekanntesten Kaffeearten, die zur Herstellung von Getränken verwendet werden, sind Arabica und Robusta. Rohe Kaffeebohnen enthalten eine bestimmte Menge an Koffein, Zucker, ätherischem Öl, Kalium, Kalzium und Magnesium. Beim Rösten der Kaffeebohnen kommt es zum Verlust von Trockenmasse d.h. zur Bildung von Kohlendioxid, Wasser und flüchtigen Pyrolyseprodukten. Mäßiger Kaffeekonsum hat auch eine heilende Wirkung, während übermäßiger Herzrasen, Schlaflosigkeit und sogar Euphorie verursacht.

Schlüsselwörter: Kaffee, Arabica, Robusta

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