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Extraction of Biologically Active Components from Freshwater Sapropel

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***Abstract*—Sapropel has been used for different purposes - in agriculture as fertilizer, in construction as building material, in cosmetic products, in balneology also in medicine and pharmaceuticals as bioactive component. Previously sapropel has been commonly used in raw form and there is no general accepted method or standard method for obtaining sapropel extract. However, most extraction methods follow the same path. Currently, there are few extraction methods using several extractants for obtaining bioactive components from raw sapropel.**

The most commonly used extractant is alkaline solution. When sapropel is subjected to alkaline environment, the humic and fulvic acids, together with some lipids, vitamins and sugar, present in the raw sapropel become soluble, however other organic and mineral content present in the sapropel remain solid. Alkaline extraction is followed by filtration and water present in the aqueous mixture is evaporated off.

Latvian freshwater sapropel can be used as raw material for obtaining sapropel extract and use it as remedy. But the main question for sapropel usage in medicine, balneology and pharmacy is to develop quality criteria for raw sapropel and its extracts. The quality criteria should include minimum requirements for biologically active substance concentration, pH values, antioxidants as well as physical characteristics.

In future studies the differences in extract characteristics of the various deposit sites, as well as the stability of the extracts under different storage conditions should be defined; also, there is need for a common approach to develop method of extraction process for active substances from sapropel and analysis procedures of its extract.

***Keywords*—antioxidants, extraction, freshwater sapropel, fulvic acid, humic acid, sapropel**

I. Introduction

Sapropel has long been used as a remedy in medicine and veterinary medicine, having a positive effect on the health [1], [2]

Sapropel biological and biochemical structure and composition varies strongly depending on its origin. Its characteristics are determined by organic, mineral and biological compounds that can have a multitude of effects on skin [3]. Sapropel has a high heating capacity that makes it useful for topical applications in medicine and rehabilitation. It is proposed that the medical effects are due to its high heating capacity and a mixture of chemical elements, hormones, various organic acids and vitamins (C, B1, B2, B5, B6, B9, B12, E, D and P) found in sapropel [2], [4]being included in most of ancient Mediterranean/European medical texts and currently used to prepare therapeutic hot-muds (peloids.

Previously sapropel has been used in raw form and there is no general accepted method or standard method for obtaining extracts of its active components. There are number of extraction methods using several extractants for obtaining bioactive components from raw sapropel and most of the extraction methods follow the same path [5].

Extraction is a principal process for recovery and separation of biologically active compounds from nature materials. Extraction converts complex matrix into suitable ingredients for pharmaceuticals, medicine, cosmetics and analytical procedures [6].

Literature suggest different extraction methods for obtaining biological active substances from sapropel. Most popular is solid-liquid extraction (SLE) with alkaline solution [7]. There are recent reports for sapropel extract using microwave-assisted extraction (MAE), ultrasound-assisted extraction (UAE), supercritical fluid extraction (SFE) and hydrostatic pressure extraction (HHPE) [6]–[8]. All these techniques have proven effectiveness in extraction form natural matrices and could be used as extraction methods for raw sapropel. Also all methods have followed principles of maximizing the yield of extraction, can be adapted for industry and have procedures to avoid impurities [6].

The first step of extraction process is isolation of active components from cells by using physical and chemical processes [9]. Choice for appropriate cell disruption process depends on sapropel sediments, that consist of

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crystalline skeleton like sand and clay, and residues of water organisms – flora, fauna; it all makes colloidal mud solution, which has complex cellular matrix [10]. In case of sapropel extract, cell disruption usually is done by drying samples before solid-liquid extraction process.

II. Materials and methods

A. Sapropel samples

In this work the sapropel samples were extracted from 5 lakes in eastern Latvia. Lakes were selected by analysing the Latvian lake database (*ezeri.lv*) [11], containing official geological survey of Latvia lakes. The sapropel deposits depth, lakes coordinates, history of agriculture next to lake were considered in the selection of the lakes. The sapropel was obtained from 5 lakes: Audzelu lake (*Audzeļu ezers*), Dunaklu lake (*Dūnākļu ezers*), Ivusku lake (*Ivušku ezers*), Zeilu lake (*Zeiļu ezers*), and Little Kivdalova lake (*Mazais Kivdalovas ezers*) in Latgale region of Latvia.

The extraction of sapropel from the lakes was performed during the winter time when the surface of the lakes is frozen. Prior to the sample collection the thickness of the proper sediment layer was determined and the depth of sapropel deposit was established for each of the lakes as well as within each of the lakes by taking probes in several locations. To select a well-composed sapropel layer for further laboratory analyses the samples were taken from three different depths of sapropel sediment at each extraction point and up to eleven different points through lake coordinates (fig.1). During the sample collection procedure 21 samples were obtained from each for the lakes that resulted in 105 sapropel samples in total.

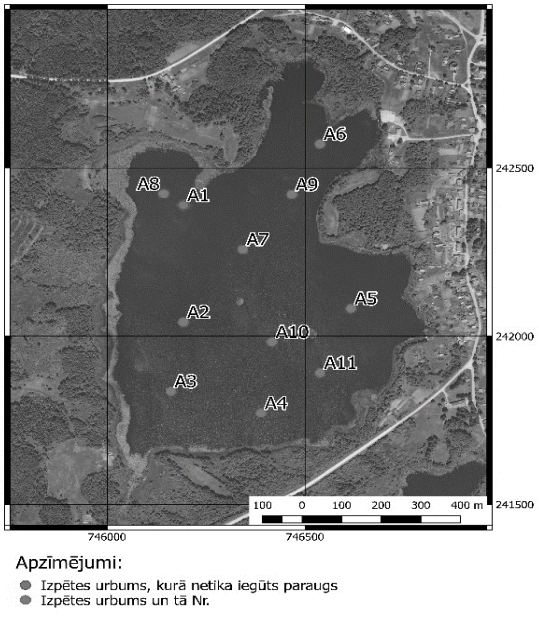

Fig. 1. Sapropel sample coordinates in Audzelu lake (A1 – A11 sample taking points)

Preservation of sapropel samples. All sapropel samples were kept in closed plastic containers without oxygen access to oxygen in order to prevent oxidation of the sapropel and its active components. The sediments were refrigerated and kept at **4°**C; in these conditions' samples were stored from 4 to 8 months before extraction process and analysis.

The storage temperature of 4°C was selected as it most closely resembled the natural water temperature at the bottom of the lake during the winter time.

B. Extraction of active componenets from Sapropel

For the extraction of active components from the sapropel samples the alkaline method was selected. Extract was obtained from each of the samples (n=56). Solid-liquid extraction process with 2% NaOH solution was used for the extraction of humic and fulvic acids from the sapropel samples. Sapropel sample with NaOH solution was stirred for 24 h, and then mixture was centrifuged at 5000 rmp for 30 min, and then filtrated. Filtrate was acidified with 5 N H_2SO_4 solution till pH 2 and centrifuged again. Filtrate was separated from solid particles, and both liquid extract and solid extract were stored at 4°C before use.

After sodium hydroxide solution was added pH level rises from neutral to pH 10, all chemical cell disruption processes began, stirring helps with mixing base alkaline solution with sapropel; colloidal mixture is formed. After centrifugation, sand particles and insoluble matter precipitates and is discarded. When acid was added humic acids molecules precipitated from the solution and stay in solid form; fulvic acid remains in the solution. The extraction process results in two forms of extract: solid, crystalline phase; main part of which is humic acids, and the liquid that contains high concentration fulvic acid solution.

C. Characterisation of the sapropel extract

For the characterisation of the sapropel extracts there are no generally accepted guidelines as it is usually the case for many plant extracts. In general the minimal quality indicators for plant extracts are the concentration of active substances, pH values, visual inspection, and raw material quality. The same principles were applied to the characterisation of the sapropel extracts.

Sapropel extracts were characterised by organic carbon content (TOC), humic acid (HA) and Fulvic acid (FA) concentration, pH level, and antioxidant level.

Total organic carbon, HA and FA were determined using spectrometric method.

Sapropel pH level was determined using distillated water (volumetric ration sample: water - 1:2.5).

To determine antioxidants the following methods were used: DPPH radical method, Folin-Ciocalteu method for determination of the total phenolic content and total antioxidants status were calculated.

The total phenolic content in sapropel extract was determined spectrophotometrically according to Folin-Ciocalteu method [12]we will determine the antioxidant properties of methanolic extract of propolis from Ghardaia and Khanchla provinces of Algeria and will correlate the values with total levels of polyphenolic compounds. Methods: The total polyphenol contents of methanolic extract of propolis were measured by using Folin-Ciocalteu spectrophotometric method. Thereafter, the antioxidant properties of these polyphenols were

determined by using the 2,2-diphenyl-1-picrylhydrazyl (DPPH. This method is based on the reaction of phenol in sapropel extract with Folin-Ciocalteu reagent. The content of phenolic compounds of the extract was expressed as gallic acid equivalents. The gallic acid was used to set up a standard curve. All the samples were analyzed in triplicates.

The total free radical scavenging capacity of sapropel extract was determined using the stable DPPH radical, which has absorption maximum at 515 nm. The radical solution was prepared by dissolving 2.4 mg DPPH in 100 ml methanol, a test sample (5µl) was added to methanolic DPPH. Also, absorption of blank sample (without antioxidant) was measured. A calibration curve was plotted with DPPH scavenged versus concentration of Trolox equivalent (TE mmol/L) [13], [14]. All samples were determined as triplicates.

Total antioxidant status (TAS) in samples was measured using Randox Total Antioxidant status kit (Randox Laboratories Ltd.) adapted to the RX Daytona automated chemistry analyzer (Randox Laboratories Ltd) [15]

ABTS® [2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)] incubated with H_2O_2 and peroxidase (metmyoglobin), generated the ABTS® radical cation. It has a relatively stable colour of green and blue, which absorbs at 600 nm. The antioxidants present in the sample prevent the formation of the cation; therefore the colour is proportional to its concentration. The result was expressed in milimoles of Trolox equivalent (TE mmol/L) of the sample solution.

III. Results and discussion

A. Sapropel samples

The location of balneologically usable sapropel layer in the studied lakes was found to be from 2.0 to 9.0 m from the surface of the sediment layer exact depth depending on the lake and the position of the measurement point in the lake. Actual thickness and location of the balneologically usable sapropel layer varied depending on the depth of the lake and the degree of the decomposition of organic matter in the lakes. If the depth is less than 1.5 m from the surface of the sediment layer, sapropel sediments are not fully developed and thus were not used in this study. Organoleptically testing the colour of samples it was found that sapropel colour varies from greenish yellow till black. Green and yellow coloured sapropel usually relates to high silica content and is found in moraine landscape lakes; black coloured sapropel has high organic matter and is found in lakes with low mineral content; brown and dark green sapropel is mixed type and its origin comes from lakes plankton, higher plants and sometimes its connected with peat layers [1]. Sapropel sample pH level is around 7 – 8 it means that these sapropel sediments has high mineral content [16]. The characteristics of the research areas are shown in table I.

TABLE I. THE CHARACTERISTICS OF THE RESEARCH AREAS

Lake name	Characteristics			
	Lake surroundings	Sapropel colour	The depth of the sapropel layer, m	pH
Audzelu	Small village forest	Black	2.65 – 11.4	7.14
Ivusku	Agricultural land	Brown	2.2 – 10.4	7.96
Dunaklu	Towns suburb, has an island	Greenish yellow	0.9 – 9.5	7.56
Zeilu	Forest, agricultural land, cemetry	Dark green	4.0 – 9.5	7.82
Little Kivdalova	Agricultural land, farmstead	Dark brown	1.7 – 11.2	7.27

B. Sapropel extract

All 105 sapropel samples were tested for the presence of heavy metal residues and pesticides. Almost all of the samples tested had the level of heavy metal and pesticides below the level accepted for medical use, 56 samples were selected for the extraction of humic and fulvic acids.

In literature it is reported that the concentration of humic and fulvic acids in the sapropel extract varies due to differences in the chemical structure of humic substances (HS) and physical availability of the organic matter associated with mineral in sapropel [17].

Extraction performed with sodium hydroxide the yielded approximately is 22 -28 g of humic acids and 5 - 9 g fulvic acids from one-kilogram dried sapropel. Outcome of acids is calculated in dried extract form, for fulvic acids excess liquid was evaporated. Humic acids, fulvic acids and total organic carbon, extracted from one g sapropel from each of the lakes are shown in fig. 2; median values of HA, FA and TOC were calculated to show average values of each lake. The highest difference between the sapropel from different lakes is in the yield of the fulvic acids where the highest and lowest values differs by more than 80%, while the total organic carbon is more uniform with the difference between lowest and highest values less being less than 30%. Results show that the highest organic acid concentration is in Audzelu lake and Little Kivdalova lake. The high organic acid concentration in these lakes can be related to the way sapropel forms in these lakes. In both lakes the sapropel sediments are organic sapropel with lower mineral content and with lover pH values.

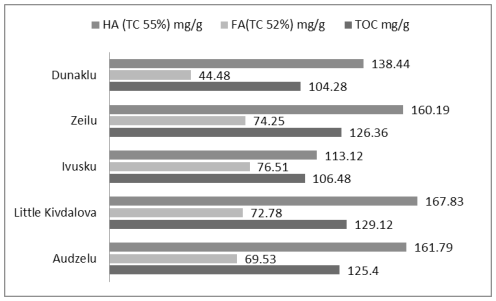

Fig. 2. Concentration of humic acid (HA), fulvic acid (FA) and total organic carbon (TOC) in each lake, mg/g.

It is reported in [18], [19] that not only the color of sapropel sediments can vary, but also the color of extracted HS can be different, indicating the degree of humification and HA and FA concentration in the extracts. Color of extracts was from light yellow to dark brow. Extracts from Dunaklu and Ivusku lake were yellow but extracts from Audzelu and Little Kivdolova lakes were darker almost black and it correlates with HA and FA concentration.

The concentration of humic and fulvic acids in sapropel extract are higher in organic sapropel, it also is related to the age of sapropel layers as the formation of bioactive substances, mineralisation of lakes and degradation of organic matter all influences the concentration of HA and FA in sapropel [3], [20].

In the analysis of the extracts the antioxidant level was measured. Antioxidant levels were calculated for each sapropel layer, there was no significant difference in antioxidant concentration of each sapropel extract from various layer, so median concentration of antioxidants was calculated to show average findings from each lake. It was found that antioxidant level is considerably higher in organic sapropel extracts from the lakes Audzelu, Little Kivdalovu and Zeilu. The difference between the highest and the lowest values is almost threefold for the total antioxidant level. The reason for so drastic differences in the antioxidant levels between different lakes is not fully understood. It seems that the antioxidant level does not correlate with the level of humic and fulvic acids

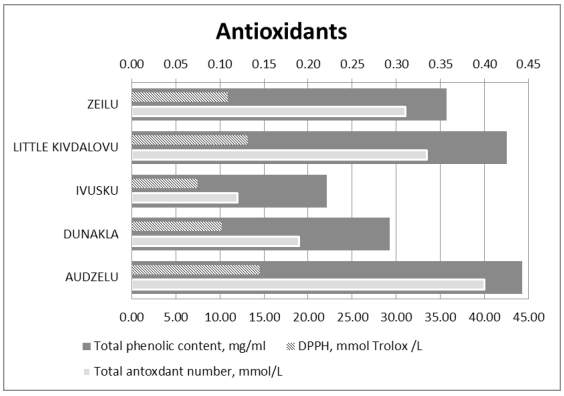

Fig. 3. Antioxidant level in sapropel extract from each lake.

One trend is that one of the lakes – Dunaklu lake gives considerably lower both antioxidant and humic and fulvic acids levels. However, Ivusku lake with the lowest antioxidant levels is high in fulvic acid level. More studies of different samples from the sediments in the same lake might be needed to better understand the variations of these parameters in the sapropel extracts obtained from different sources. The antioxidant measurement results are shown in fig.3 for each lake.

IV. CONCLUSION

Balneologically usable sapropel was found in all studied lakes. The most suitable lake as a source of sapropel was found to be Audzelu lake. It is easy reachable, because of the small village next to it, it has high humic and fulvic acids concentration and it shows the highest antioxidant level.

The concentration of humic and fulvic acids and the antioxidant levels varies strongly between different lakes. In the studied samples the concentration of humic and fulvic acids do not correlate with the antioxidant level.

The difference in humic acid levels between different lakes is much less pronounced than the difference in the fulvic acid and antioxidant levels.

In cases where higher fulvic acids and or antioxidant level are desirable it is important to select correct lake for the raw sapropel extraction since the fulvic acid content and antioxidant level varies strongly between the lakes.

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