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Study of sapropel extracts from Prybych natural deposits

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ABSTRACT

In recent years the specialists pay attention to the application of various natural substances as remedies. The use of native medicines and separate components of different peloids has shown its effectiveness, accessibility, convenience and safety for many years. Sapropel is a renewable natural resource and an unique organic material. Its deposits are typical only for freshwater lakes. The processes of the accumulation are still going on now and most lakes are progressive. The chemical composition of sapropel of different locations differs significantly and is determined by the conditions of its formation as well as the variety of flora and fauna [7,10,11]. Considering that the formation of deposits is inextricably related to an underwater vegetation and a vital activity of microorganisms, sapropel consists of 80% substance of organic origin. [6] We got liquid extracts of sapropel, using purified water, 40% ethanol, chloroform, hexane, corn oil as the extractant. At this stage we have investigated the antibacterial activity of aqueous extract and the carbonic acid content of alcohol extract of sapropel from Prybych deposits in Volyn region. As a result of research of the content of carbonic acids in the alcohol extract of sapropel by the method chromato-mass spectrometry were identified 19 compounds including 11 fatty acids and 8 organic that play an important role in human life. It points at the prospect of the use of sapropel in medicine, veterinary science and cosmetology. Studies of the antibacterial activity of the aqueous extract of sapropel from Prybych deposits indicate that it exhibits the antibacterial activity, which is a determining factor in the development of medicinal, cosmetic and veterinary preparations.

Key words: sapropel, chromato-mass spectrometry, fatty acids, organic acids.

INTRODUCTION

Despite the achievements of modern pharmacy, existing means of medicated therapy don't solve many problems of the treatment. It promotes the search for new medicines of natural origin. These remedies shouldn't yield to the traditional means of their efficiency, quality and safety. They shouldn't have contraindications or cause complications.

In recent years the specialists are attracted to the application of various natural substances as remedies. The use of native medicines and separate components of different peloids has shown its effectiveness, accessibility, convenience and safety for many years.

Sapropel is a renewable natural resource and a unique organic material. Its deposits are typical only for freshwater lakes. The processes of the accumulation continue and now. Moreover, for most lakes they are progressive in nature. The chemical composition of sapropel of different locations differs significantly and is determined by the conditions of its formation as well as the variety of flora and fauna. [7,10,11]. Considering that the formation of deposits is inextricably related to an underwater vegetation and a vital activity of microorganisms, sapropel consists of 80% substance of organic origin [6].

Sapropels have a pronounced antibacterial effect; possess anti-inflammatory and antioxidant properties; restore the immunity, activate the processes of fibrinolysis; bind and rid the body of slags, toxins, heavy metals and radionuclides, etc. [5,7].

We have obtained liquid extracts of sapropel, using purified water, 40% ethanol, chloroform, hexane, corn oil as the extractant.

The aim of our work was to investigate the antibacterial activity of aqueous extract and the carbonic acid content of alcohol extract of sapropel from Prybych deposits in Volyn region.

The scientific novelty is to have been investigated for the first time the extracts of sapropel from Prybych deposits to establish the content of carbonic acids and the antibacterial activity.

EXPERIMENTAL SECTION

For identification and quantative analysis of carboxilic acids in liquid extract of sapropel, which was obtained by 40 % ethanol percolation with further evaporation by means of vacuum evaporator under 50-60 C to a required volume, the method of chromato mass spectrometry was applied [2,3,4]. The chromatograph Aglient Technologies (USA) equipped with the chromatographic column (inner diameter 0,25mm and length of 30 mm), 6890 series with mass spectrometer 5973 series was used. Thermostat temperature was programmed for 50 C for 1 min and then increased to 320 C with the speed of 4 C/ min, the latter temperature was held for 9 minutes. Helium was used as a transporting gas, the speed of which was 1,2 ml/min. Inner standard, tridecane, was infused in quantity of 50 mg of substance per certain quantity of a herbal sample. The catalogue (filing system) of mass spectrums Nist 05 and Wiley 138 was used for identification of components [2,3].

Antibacterial properties of sapropel extract, which was obtained by means of processing of dehydrated sapropel 0,1 н with potassium hydroxide solution in liquid medium with 1:10 correlation of water and sapropel, pH <10 with simultaneous homogenization of the obtained mixture under 90°C over 2 hours with further influence of hydrochloric acid with mass fraction of 10% to pH 6,8-7,0 followed by processing in vacuum evaporator (50-60°C) [4], the study was conducted in the laboratory of biochemistry of microorganisms and growth medium of the Institute of Microbiology and Immunology named after Mechnikov of NAMS by a diffusion method.

In accordance with MOH recommendations, test-strains of Staphylococcus aureus ATCC 25923, Escherichia coli ATCC 25922, Pseudomonas aeruginosa ATCC 27853, Basillus subtilis ATCC 6633, Proteus vulgaris ATCC 4636, Candida albicans ATCC 885/653 were used for evaluation of substance effectiveness. Microbial suspension was prepared by means of Densi-La-Meter (PLIVA-Lachema, Checz Republic, wave length 540 нм). The suspension was made in accordance with the device user manual and guidelines of MOH on updates in "Standard of microbial suspensions" # 163-2006, Kyiv. Synchronization of cultures was carried out using a low temperature (4°C). Microbial load was 107 microbial cells per 1 ml of medium and accordingly to McFarland standard. We exploited a 18-24 hour culture of microorganisms. Mueller-Hinton agar was used in the course of the research (manufacturer - HIMedia Laboratories Pvt. Ltd. 23, Vadhami ind Est., LBS Marg. Mumbai - 400086, India. Customer Care No: 022-4095 1919, Email: techhelp@himedialabs.com. Medium expiry date is XI 2016). Agar Saburo was used for Candida albicans (manufacturer - HIMedia Laboratories Pvt. Ltd. 23, Vadhami ind Est., LBS Marg. Mumbai - 400086, India. Customer Care No: 022-4095 1919, Email: techhelp@himedialabs.com., medium expiry date is XI 2016) [1].

Determination of the effectiveness of antimicrobial medications/substances was performed on two layers of a dense growth medium placed into a Petri dish. The lower layer was used for "hungry", non- inoculated medium (agar-agar, water, salt). The lower layer was an underlay, 10-mm high which was horizontally mounted on 3-6 thin stainless steel cylinders with a diameter of 8 mm and a height of 10 mm. The space around the cylinders was filled with the upper layer consisting of an agar medium, melted and cooled to 40 C with the relevant standard of one-day culture of test bacteria added to it. Prior to that, the upper layer was well stirred until a homogeneous mass. After solidification the cylinders were removed with sterile forceps and the lunules formed were filled with the test substance taking its volume into account (0.3 ml).

The volume of medium used for the upper layer ranged from 14 to 16 ml. The plates were dried for 30-40 minutes at room temperature and placed in a thermostat for 18-24 hours.

When assessing new antibacterial agents we used the following criteria:

- absence of microbial growth inhibition zones around the lunules or such zones with the diameter up to 10 mm indicates that the organism is not sensitive to the substance in the lunule;
-growth inhibition zones with the diameter of 10-15 mm indicate low sensitivity of the culture to the test concentration of antibacterial agent;
- growth inhibition zones with the diameter of 15-25 mm are regarded as an indicator of sensitivity of the microorganism to the test drug;
- growth inhibition zones with the diameter of more than 25 mm indicate a high sensitivity of microorganisms to tested substances [9].

All research findings were processed by Statistica 5,0 for Windows PC. Statistical analysis of the findings was carried out using Student's (t) and Wilcoxon-Mann-Whitney (w) ratios [8].

RESULTS AND DISCUSSION

The defined content of carboxylic acid (> 0.1% of the total peak area) in the alcoholic extract of sapropel is given in the Table. 1 and Fig. 1.

Table 1:The results of the qualitative and quantitative content of carboxylic acids in the extract of sapropel from Prybych natural deposits, mg / kg

Sapropel		
Name of identifiable carboxylic acids	**Retention time, s**	**Content mg/kg**
1	**2**	**3**
Fatty acids		
Saturated fatty acids		
Arachic acid	35.345	14.04.
Behenic acid	38.351	57.05
Cerotic acid	44.982	13.62
Capronic acid	5.988	02.07.
Myristic acid	25.658	20.62
Palmitoleic acid	28.92	97.33
Stearic acid	32.211	18.32
Lignoceric acid	41.223	30.85
Unsaturated fatty acids		
Linolenic acid	34.358	20.14
1	**2**	**3**
Oleic acid	32.517	23.73
Organic acids		
Azelaic acid	27.476	15.24
Vanillic acid	34.519	4.49.
Gentisic acid	40.66	4.33.
ρ -coumaric	38.457	76.27
Citric acid	31.597	12.05.
2- pyruvic acid	8.67.	83.83
Ferulic acid	42.729	8.48.
Malic acid	24.425	25.52

The study of carboxylic acid content in the extract of sapropel by the method of chromatography-mass spectrometry identified 19 compounds, including 11 fatty acids. The largest amount of saturated fatty acids is represented by palmitic (97.33 mg/kg) and behenic (57.05mg/kg) acids.

Three unsaturated fatty acids were identified in the sapropel extract: linoleic, linolenic and oleic, linoleic acid predominated (29.17 mg/kg).

Among the carboxylic acids of the alcoholic extract of sapropel we identified eight organic acids (Table 1). The largest number is of pyruvic (83.83) and ρ -coumaric acid (76.27 mg/kg). Also the presence of azelaic, vanillic, gentisic, lemon, apple/malic and ferulic acids was spotted.

The results of the study of antibacterial characteristics of aqueous extract of sapropel are presented in Table 2.

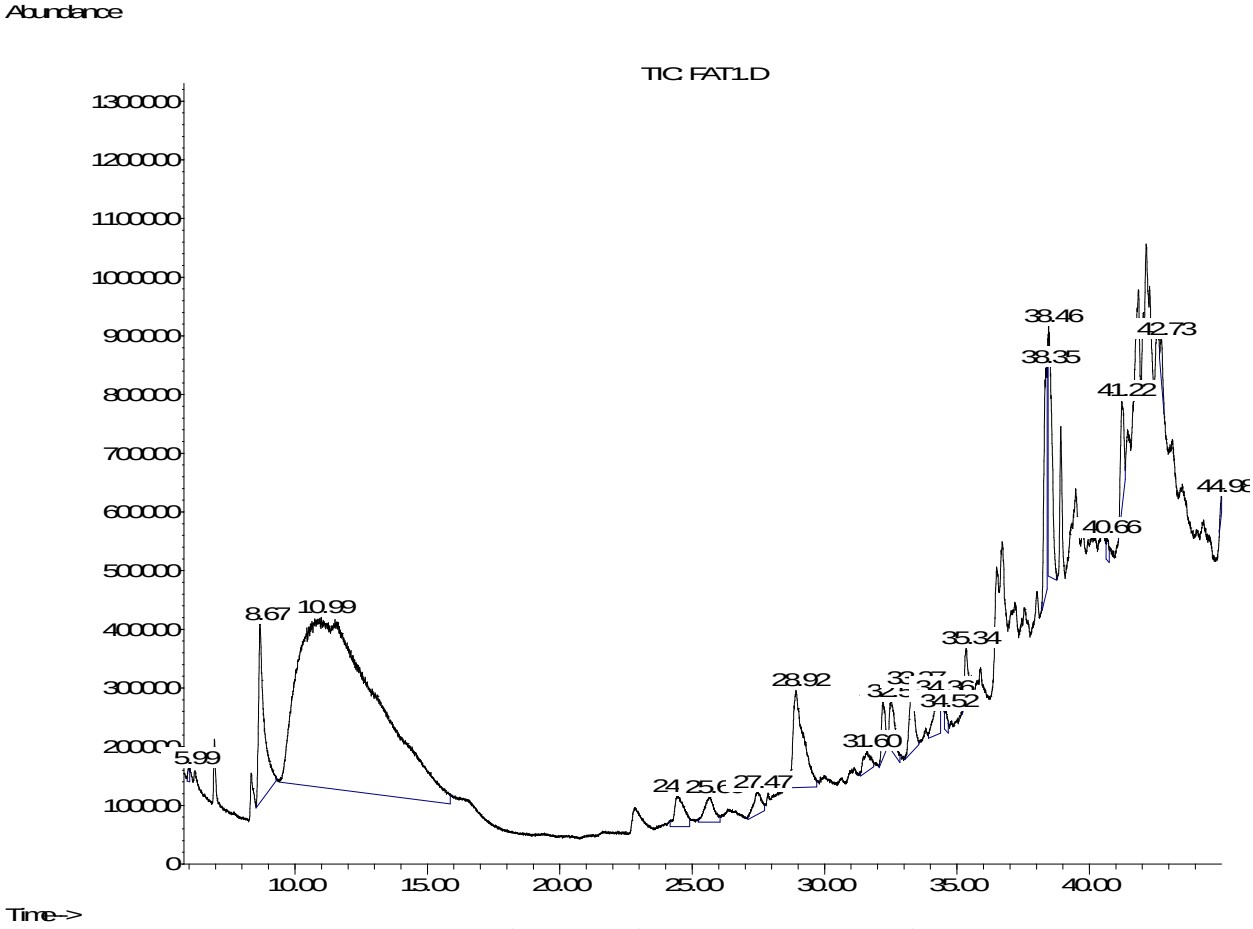

Figure 1: **Chromatogram of organic and fatty acids in alcohol extract of sapropel**

Table 2: Antibacterial characteristics of aqueous extract of sapropel

Sapropel extract	The diameters of the growth inhibition zones, in mm; n = 5					
	Staphylococcus aureus ATCC 25923	*Escherichia coli ATCC 25922*	*Proteus vulgaris ATCC 4636*	*Pseudomonas aeruginosa ATCC 27853*	*Bacillus subtilis ATCC 6633*	*Candida albicans ATCC 653/885*
1	**15,0±0,5**	**16,0±0,1**	**12,6±0,7**	**13,8±0,5**	**18,7±0,6**	**17,4±0,6**

It was experimentally determined that the extract under study possess certain antibacterial characteristics against *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Bacillus subtilis* ATCC 6633, Candida aibicans ATCC 653/885; and has minor antibacterial effect against Proteus vulgaris ATCC 4636 and Pseudomonas aeruginosa ATCC 27853.

CONCLUSION

The study of content of carboxylic acids in the alcoholic extract of sapropel by means of chromatography-mass spectrometry identified 19 compounds, 11 of them are fatty acids and 8 are organic. The largest number of saturated fatty acids are represented by palmitic (97.33 mg/kg) and behenic (57.05mg/kg) acids, including unsaturated (linoleic, oleic) acids, linoleic acid predominates (29.17 mg/kg). We identified that among the organic acids such as azelaic, vanillic, gentisic, ρ -coumaric, lemon, pyruvic, ferulic, malicpyruvic (83.83) and ρ -coumaric acids (76.27 mg/kg) predominate.

The research of antibacterial characteristics of aqueous extract of sapropel in Prybych natural deposits indicates its antibacterial effectiveness against Staphylococcus aureus, Escherichia coli, Basillus subtilis, Candida aibicans; and minor antibacterial effect against Proteus vulgaris and Pseudomonas aeruginosa.

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