SOME RESULTS OF THE STUDY OF LIPIDS OF RED ALGAE OF THE CASPIAN SEA

Soyegov G.A.[1], Gurbanov I.[2], Berdiyev A.A.[3], Durdyev T.Sh.[4], Annanurova Sh.M.[5]

[1]Soyegov Gadam Akmuhammedovich – student;
[2]Gurbanov Ilmyrat – Candidate of Chemical Sciences, head of the department;
[3]Berdiyev Atamyrat Amangeldiyevich – lecturer;
[4]Durdyev Tachmurat Shamuhammedovich – lecturer;
[5]Annanurova Shirin Myradovna – lecturer,
DEPARTMENT OF PHARMACY,
MYRAT GARRYYEV STATE MEDICAL UNIVERSITY OF TURKMENISTAN,
ASHGABAT, TURKMENISTAN

Abstract: *when selecting the objects of study, first of all, preference was given to those species of red algae, which are quite often found on the eastern coast of the Caspian Sea and may be of some interest for their industrial harvesting or cultivation. By methods of thin-layer chromatography and spectrophotometry it was established that the lipid fraction of the studied algae includes 11 different limited and unsaturated fatty acids with the number of carbon atoms in the linear unbranched chain from 14 to 22. In all red algae the predominant components are unsaturated fatty acids. The results of preliminary preclinical studies have shown the prospective use of lipid extracts of seaweeds for the development of medicinal preparations.*
Keywords: *red algae, Caspian Sea, thin-layer chromatography and spectrophotometry, fatty acids, preclinical studies.*

НЕКОТОРЫЕ РЕЗУЛЬТАТЫ ИССЛЕДОВАНИЕ ЛИПИДОВ КРАСНЫХ ВОДОРОСЛЕЙ КАСПИЙСКОГО МОРЯ

Соегов Г.А.[1], Гурбанов И.[2], Бердиев А.А.[3], Дурдыев Т.Ш.[4], Аннанурова Ш.М.[5]

[1]Соегов Гадам Акмухаммедович – студент;
[2]Гурбанов Илмырат – кандидат химических наук, заведующий кафедры;
[3]Бердиев Атамырат Амангелдиевич – преподаватель;
[4]Дурдыев Тачмырат Шамухаммедович – преподаватель;
[5]Аннанурова Ширин Мырадовна – преподаватель,
кафедра Фармации,
Государственный медицинский университет Туркменистана имени Мырата Гаррыева,
г. Ашхабад, Туркменистан

Аннотация: *при выборе объектов исследования в первую очередь отдано предпочтение тем видам красных водорослей, которые достаточно часто встречаются на восточном побережье Каспийского моря и могут представлять определенный интерес для их промышленной заготовки или культивирования. Методами тонкослойной хроматографии и спектрофотометрии установлено, что в состав липидной фракции изученных водорослей входят 11 различных предельных и непредельных жирных кислот с числом углеродных атомов в линейной неразветвленной цепи от 14 до 22. При этом во всех красных водорослях преобладающими компонентами являются жирные кислоты непредельного ряда. Результаты предварительных доклинических исследований показали перспективность использования липидных экстрактов морских водорослей для создания лекарственных препаратов.*
Ключевые слова: *красные водоросли, Каспийское море, тонкослойная хроматография и спектрофотометрия, жирные кислоты, доклинические исследование.*

УДК 615

The search for sources of biologically active substances and their precursors is still an actual task of chemistry of natural and physiologically active compounds.

A first acquaintance with modern works on the chemistry of seaweeds shows that these plants represent a promising raw material group, especially since their reserves can be replenished by further cultivation [6].

The main bioproducts of seaweeds are carbohydrates, proteins and lipids. At the same time, seaweed lipids, containing a significant percentage of highly unsaturated higher fatty acids, which have valuable properties and can serve as precursors in the biosynthesis of prostaglandins, deserve great attention [1].

Relevance: the above-mentioned confirms the relevance of the study of qualitative and quantitative fatty acid

composition of lipids of 4 species of red algae of the Caspian Sea: Lourensia caspica A.Zin et Zaberzh (I), Polysiphonia caspica Kütz (II), Polysiphonia denudata (Dillw) Kiitz (III), Polysiphonia violacea (Roth) Grev (IV) - to create highly effective drugs on their basis.

Materials and methods: when selecting objects of research, first of all, preference was given to those species of red algae, which are often enough found on the eastern coast of the Caspian Sea and may be of some interest for their industrial harvesting or cultivation.

The fatty acid composition of algal lipids (I-IV) was studied by thin-layer chromatography (TLC) and spectrophotometry (SP).

Identification of lipid fatty acids by TLC was carried out on plates with silica gel firmly fixed with silicic acid sol and microsilica gel fixed with gypsum or florisil. Experiments have shown that the quality of separation of fatty acids is improved when using a silica gel plate. Such plates can be repeatedly regenerated with hot chromium mixture and reused for TCA.

In search of a suitable solvent system for TCA, mixtures of different polar and non-polar solvents were used. After testing numerous solvent combinations, clear separation of fatty acids was obtained by chromatography in the system hexane-ethyl ether- formic acid-methanol-water (100:40:20:20:20:8).

The lipids of each of the algae were chromatographed several times in the presence of standard 'witnesses'.

The TLC results showed that the lipid fraction of the algae contained 11 different fatty acids: tetradecanoic acid - C14:0(1), hexadecanoic acid - C16:0(2), octadecanoic acid - C18:0(3), octadecenoic acid - C18:1(4), octadecadienoic acid - C18: 2(5), octadecatrienoic - C18:3 α linolenic(6) and a mixture of α- and β-eleostearic acids(7), eicosatetraenoic - C20:4(8), eicosapentaenoic - C20:5(9), docosaenoic - C22:1(10) and docosahexaenoic - C22:6(11) acids.

In the lipid chromatogram of the studied algae lipids, 7 spots are the main ones: C16:0, C18:0 C18:1 C18:2 C18:3 (linolenic acid), C20:4 and C20:5 while the other 4 can be seen only as weak spots. Rf values were calculated for each individual acid (Table 1) [5].

Table 1. Rf values of fatty acids of the alga Polysiphonia demidate.

Solvent system and their ratio	Fatty acids										
	$C_{14:0}$	$C_{16:0}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$	$C_{18:3*}$	$C_{20:4}$	$C_{20:5}$	$C_{22:1}$	$C_{22:6}$
Hexane-ethyl ether-methanol-water (1:1:0.5:0.5)	0,74	0,70	0,64	0,57	-	0,53	0,37	0.27	-	0,20	0,12
Chloroform-petroleum ether-ethanol (1:1:0.5)	0,77	0,68	-	0,55	-	-	0,41	0,23	-	-	0,09
Hexane-ethyl ether-muravic acid-ethanol-water (1:1:0.5:0.5:0.5:0.5)	0,65	0,56	0,50	0,43	0,39	0,33	0,29	0,24	0,20	0,16	0,13
Hexane-ethyl ether- formic acid-methanol-water (1:0.4:0.2:0.2:0.2:0.08)	0,91	0,80	0,75	0,65	0,57	0,48	0,42	0,32	0,26	0,18	0,10
Hexane-chloroform-acetic acid-ethyl ether-water (1:0.4:0.2:0.2:0.2:0.08)	0,74	0,68	0,64	0,59	0,51	0,47	0,38	0,35	0,23	0,20	0,13
Hexane-chlorobenzene-acetic acid-ethyl ether-water (1:0.4:0.2:0.2:0.2:0.08)	-	0,70	0,66	0,61	0,57	0,48	0,44	0,39	0,31	0,22	0,18

Note. * - mixture of α- and β-oleostearic acids.

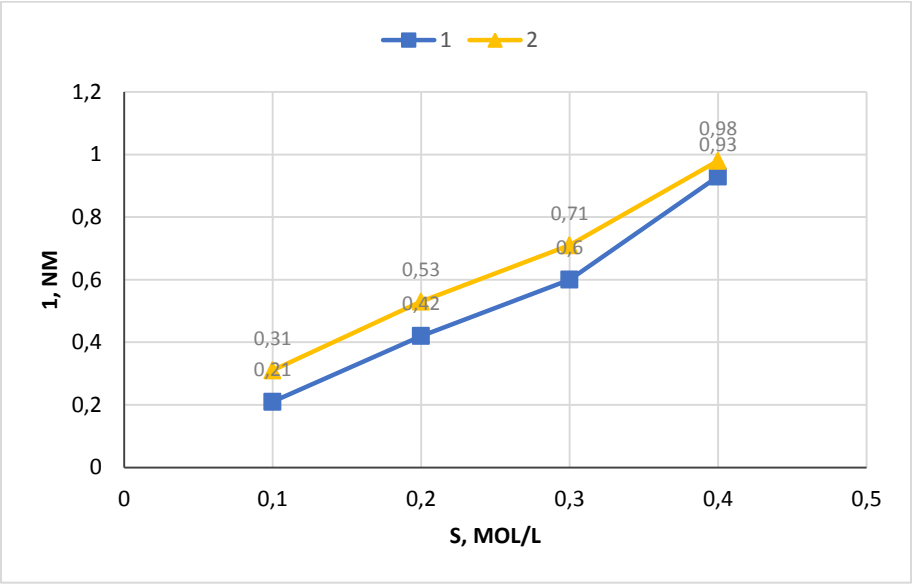
The quantitative content of fatty acids identified by TLC was determined by bichromate and SF methods [3, 4]. In the SF method, a calibration graph was constructed for the corresponding concentration of fatty acids at a known wavelength, by which the content of these acids was found for the optical density of the sample under study (Table 2, Fig. 1).

Table 2. Dependence of optical density of hexadecanoic (C16:0) and octadienoic (C18:0) acids on their concentration, mol/L.

D	Concentration
$C_{16:0}$	
0,93	0,50
0,60	0,35
0,42	0,20
0,21	0,15
$C_{18:0}$	

0,98	0,50
0,71	0,35
0,53	0,20
0,31	0,15

Fig. 1. Calibration plot of hexadecanoic (I) and octadecadienoic (2) acids for their quantification in the lipid fraction of algae (I-IV).



The absorption signals were measured at wavelengths equal at C14:0 - 239 NM, C16:0 - 268, C18:0 - 299, C18:1 - 258, C18:2 - 248, C18:3 - 284, C18:3 (mixtures of α- and β- eleostearic acids) - 282, C20:4 - 315, C20:5 - 346, C22:1 - 220, C22:6 - 375. The results of the SF method for determination of the amount of fatty acids in the algal lipid composition (I-IV) are shown in Table 3.

Table 3. Results of the NF method for determination of the amount (mol/l) of fatty acids in algal lipids (1-IV) of the Caspian Sea.

Fatty acids	***Lourensia caspica***	***Polysiphonia caspica***	***Polysiphonia denudata***	***Polysiphonia violacea***
$C_{14:0}$	0,08	0,34	0,24	0,41
$C_{16:0}$	0,36	0,56	0,28	0,46
$C_{18:0}$	0,17	0,35	0,29	0,22
$C_{18:1}$	0,10	0,70	0,06	0,65
$C_{18:2}$	0,18	0,44	0,16	0,54
$C_{18:3}$	0,17	0,28	0,12	0,19
$C_{18:3*}$	0,06	0,04	0,04	0,09
$C_{20:4}$	2,45	1,54	1,83	0,60
$C_{20:5}$	0,01	0,26	0,74	1,33
$C_{22:1}$	0,01	0,02	следы	следы
$C_{22:6}$	0,11	0,17	0,30	0,03

Note. * - mixture of α- and β-eleostearic acids.

All algae contain the same types of fatty acids but in different amounts. For red algae, the characteristic feature is a high concentration of unsaturated fatty acids, especially for C18:1, C18:3 (linolenic acid), C20:4 and C20:5. Thus, C20:4 in Lourensia caspica (I) accounts for almost 70% of the sum of unsaturated fatty acids. Quite significant differences in the content of saturated fatty acids are observed between individual representatives of red algae. Among them C16:0 prevails, the ratio of C14:0 and C18:0 is approximately the same.

Results and their discussion.

The data obtained indicate that TCA and SF methods are reliable for establishing the qualitative and quantitative

compositions, structure of fatty acids of algal lipids.

The results of the above preclinical tests of Caspian Sea algae extracts allowed us to recommend them as drugs for the treatment of 12-acid ulcer, stomach ulcer, burns and as hypolipoidemic agents. This indicates the promising potential of lipids of the studied algae as sources of highly effective drugs [4].

Lipid isolation and methodology for determining the qualitative content of higher fatty acids of algae (I-IV) by TLC have been published in scientific papers [2].

Quantitative determination of fatty acids separated in a thin layer was carried out by bichromate [3] and NF methods. In the latter, 4 standard solutions of fatty acids with different concentrations were prepared and after 30 min their absorbance (nm) was measured on a spectrophotometer 'Speetromom-203' at appropriate wavelengths. A calibration graph was plotted to determine the content of fatty acids in the lipid fraction of algae (I-V).

Conclusions

1. The use of TLC on silica gel plates fixed with silicic acid sol has a number of advantages over plates with silica gel-gypsum or silica gel-florisil layer.
2. By TLC and SF methods it was found that the lipid fraction of the studied algae includes 11 different limited and unsaturated fatty acids with the number of carbon atoms in the linear unbranched chain from 14 to 22. In all red algae the predominant components are unsaturated fatty acids.
3. The results of preliminary preclinical studies have shown the promising use of lipid extracts of seaweeds for drug development.

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