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CHARACTERISTICS OF LIPIDS OF WOAD (ISATIS TINCTORIA L.) SEEDS

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Abstract:
The article presents the results of studies of Woad seeds and presents these characteristics of seed lipids, fatty acid composition of neutral lipids, glycolipids and phospholipids of seeds. The qualitative composition of unsaponifiable substances was determined by the TLC method. The qualitative composition of unsaponifiable substances was determined by the TLC method. The Folch method was used to extract a polar lipid concentrate consisting of residues of neutral lipids, glycolipids and phospholipids. The carotenoid content in unsaponifiable substances was determined by the spectrophotometric method. Biologically active components such as hydrocarbons, carotenoids, free fatty acids, free phytosterols and triterpenols have been found among unsaponifiable substances. Sterols were the main component of unsaponifiable substances. Fatty acids were analyzed as methyl esters by the GC method. They were identified by comparing the retention times of their peaks on chromatograms with the retention times of peaks of a standard sample of a mixture of 37 methyl esters of fatty acids.
The results of the analysis showed that the seeds contain more than 50% of phospholipids, which are unsaturated fatty acids, which are of great nutritional value, and also have a powerful antioxidant effect: Omega-3, Omega-6 and Omega-9 fatty acids.

Keywords: Seeds of Isatis tinctoria, moisture content, unsaponifiable substances, carotenoids, fatty acid composition, neutral lipids, phospholipids, glycolipids.

Introduction

Isatis tinctoria L. (Brassicaceae), commonly known as Woad, is a species with an ancient and well-documented history of using indigo dye and a medicinal plant. Currently, I. tinctoria is more often used as a medicinal product, as well as as a cosmetic ingredient (1). The root of I. tinctoria has been accepted into official European phytotherapy by including its monograph in the European Pharmacopoeia. Also, this plant is included in the pharmacopoeia of China and is widely used in Chinese folk medicine as an anti-inflammatory and antiviral agent. Modern scientific studies in vitro and in vivo have proven anti-inflammatory, antitumor, antimicrobial, antiviral, antidiabetic and antioxidant activities (2).

It is known that plant seeds are a source of fatty acids necessary for the normal functioning of the body. In addition to fiber, almost all seeds contain omega-fatty acids, vitamins, amino acids and other components that are considered essential "building materials" of our body. From scientific articles, we learned that the oil obtained from the seeds of Woad is similar in properties to sea buckthorn and linseed oils. For this reason, we considered it very important to obtain an oil rich in fatty acids, vitamins and polyphenols from the raw materials of a local widespread plant, in order to reduce the cost and import substitution of pharmaceutical products.

The aim of the study is to determine the content of moisture, volatile substances, carotenoids, fatty acids in the composition of neutral, phospho- and glycolipids in the seeds of Woad growing in Uzbekistan.

Materials and methods of research

The plant samples were collected in the Parkent district of the Tashkent region of the Republic of Uzbekistan in September, after the seeds had fully matured. The objects of the study were dried seeds that were dried in a well-ventilated room at room temperature in the shade. Neutral lipids (NL) were isolated from air-dry crushed seeds in a Soxlet apparatus using extraction gasoline (t. kip. 72-800C) [2] [8]. Unsaponifiable substances (HB) were extracted from the oil by hydrolysis with a 10% KOH solution in methanol and their content was determined [3]. The qualitative composition of unsaponifiable substances was established by the TLC method on silica gel and Silufol plates. The identification of compounds was carried out on the basis of qualitative reactions, chromatographic mobility of spots in a thin layer of sorbent in a system of hexane solvents: ether (7:3); (6:4) and in comparison with the literature data [4] and lipid substances isolated from other natural sources.

After NL extraction, the meal was dried in air and then with a mixture of chloroform and methanol (2:1) using the Folch method [5], a concentrate of polar lipids (PL) consisting of NL residues, glycolipids (GL) and phospholipids (PhL) was extracted from it. The crude PL extract was treated with 0.04% aqueous CaCl2 solution to remove non-lipid components. Further, PL was fractionated by column chromatography (CC) on silica gel into separate groups of lipids, while NL was eluted with chloroform, GL – acetone, and PhL – methanol. The yield of the lipid groups was determined gravimetrically.

The content of carotenoids in unsaponifiable substances was determined by the spectrophotometric method.

Also, the content of moisture and volatile substances in the seeds was determined. The moisture content of the seeds was determined according to the well-known method [1] by drying the sample sample in a drying cabinet to a constant weight at a temperature no higher than 105 ° C.

The method of determining the lipid composition of the seeds of Woad.

Thin-layer chromatography (TLC) of lipids was performed on silica gel of the Chemapol brand (Czechoslovakia) with a particle size of 5/40 μ and on Silufol plates (Czechoslovakia). NL spots were manifested in J_2 vapors and by spraying the plates with 50% aqueous H2SO4 solution followed by heating. Solvent systems hexane: ether were used to separate NL 1) 8:2; 2) 6:4.

The composition of GL was established in the solvent system chloroform: acetone: methanol: acetic acid: water (65:20:10:10:3, v/v).

A solvent system chloroform: methanol: 25% ammonia (65:35:5, v/v) was used for the analysis of FL. The spots of the FL components were manifested by Vaskovsky and Dragendorf reagents [6].

According to the results of TLC analysis, the NL of Isatis seeds consisted of paraffin hydrocarbons, fatty acid esters (LC) with sterols and fatty alcohols, triacylglycerides (the main component), free LC, free phytosterols and triterpenols.

Biologically active components such as hydrocarbons, carotenoids, free fatty acids, free phytosterols and triterpenols have been found among unsaponifiable substances. Sterols were the main component of unsaponifiable substances.

The composition of GL was established by TLC on silica gel using a system of solvents chloroform – acetone – methanol – acetic acid – water 65:20:10:10:3, The components were developed with a solution of α – naphthol and a 50% aqueous solution of H2SO4. The predominant components of glycolipids (GL) were sterylglycosides, in addition, monohalactosyl- and digalactosyldiacylglycerides were found.

For the analysis of FL, a system of solvents chloroform – methanol – concentrated ammonia 65:35:5 was used, the components of the amounts of PhL were manifested by Vaskovsky and Dragendorf reagents. Phosphatidylcholines, phosphatidylethanolamines and phosphatidylinosites were found in the composition of PhL.

To determine the composition of fatty acids (FA), NL, GL and PhL of the studied samples were hydrolyzed with an alcoholic alkali solution, the isolated fatty acids were methylated with freshly prepared diazomethane [4]. LC in the form of methyl esters was analyzed by GC method on an Agilent 8860 GC device (USA), Supelco 100m x 0.25 mm capillary column, column programming temperature from 1000C to 2250C, flame ionization detector, nitrogen carrier gas, stationary phase SRtm-2560.

Identification was performed by comparing the retention times of their peaks on chromatograms with the retention times of peaks of a standard sample of a mixture of 37 methyl esters of fatty acids (Supelco® 37 component FAME mix, Sigma-Aldrich, USA).

Results and discussion.

Table 1 shows the results of the determination of moisture, volatile substances, carotenoids, etc.

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Table 1 Characteristics of lipids of Isatis seeds

Indicator	Content
Moisture and volatile substances, % of the seed weight	6,32
Neutral lipid yield (oil content) at actual humidity, % of the seed weight	14,93
NL output to a completely dry substance, % of the seed weight	15,93
Carotenoids in NL, mg%	42,23
The content of unsaponifiable substances, % of the NL weight	4,82
Carotenoids in unsaponifiable substances, mg%	109,40
Polar lipids (PL), % of the seed weight, including: glycolipids phospholipids	1,44 0,64 0,80

The results of the analysis of the composition of fatty acids NL, GL and PhL are presented in Table 2.

Table 2 The composition of fatty acids of neutral lipids, glycolipids and phospholipids, of Isatis seeds, % by weight of acids

Fatty acids	Content		
	NL	GL	PhL
10:0, Capric acid	traces	0,61	-
12:0, Lauric acid	traces	1,83	0,07
14:0, Myristic acid	0,06	2,02	0,31
15:0, Pentadecanoic acid	0,03	0,76	0,28
16:0, Palmitic acid	3,85	45,08	23,42
16:1, Palmitoleic acid	0,21	0,95	0,10
17:0, Margarine acid	0,06	0,86	0,08
18:0, Stearic acid	1,09	5,67	1,87
18:1ω9, Oleic acid *Omega 9*	17,17	10,00	31,04
18:2ω6, Linoleic acid *Omega 6*	9,79	11,16	16,52
18:3ω3, Linolenic acid *Omega 3*	28,98	9,74	5,44
20:0, Arachinic acid	1,20	0,57	0,27
20:1ω11, Eicosenic acid *Omega 11*	10,02	8,74	17,57
20:2ω6, Eicosadienic acid	0,64	-	0,17
20:3ω3, Eicosatrienoic acid	0,23	-	-
20:4ω6, Arachidonic acid	traces	-	0,04
22:0, Behenic acid	0,46	0,51	0,11
22:1ω9, Erucic acid	23,18	1,50	1,35
22:2 ω6, Docosadienoic acid	-	-	0,14
24:0, Lignoceric acid	0,40	Сл.	0,43
24:1ω9, Nervonic acid	2,63	Сл.	0,57
∑ saturated fatty acids	7,15	57,91	26,84
∑ unsaturated fatty acids	92,85	42,09	73,16

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Figures 1,2 and 3 show the GC chromatograms of the fatty acids NL, GL and PhL.

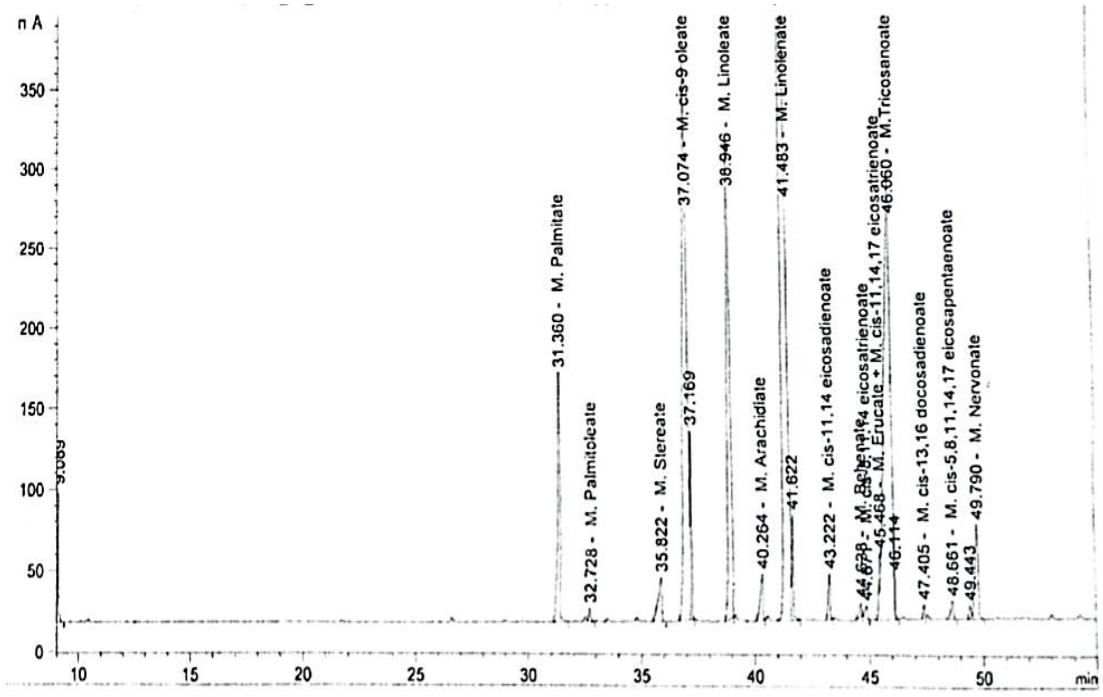

Fig.1 Chromatogram of neutral lipids

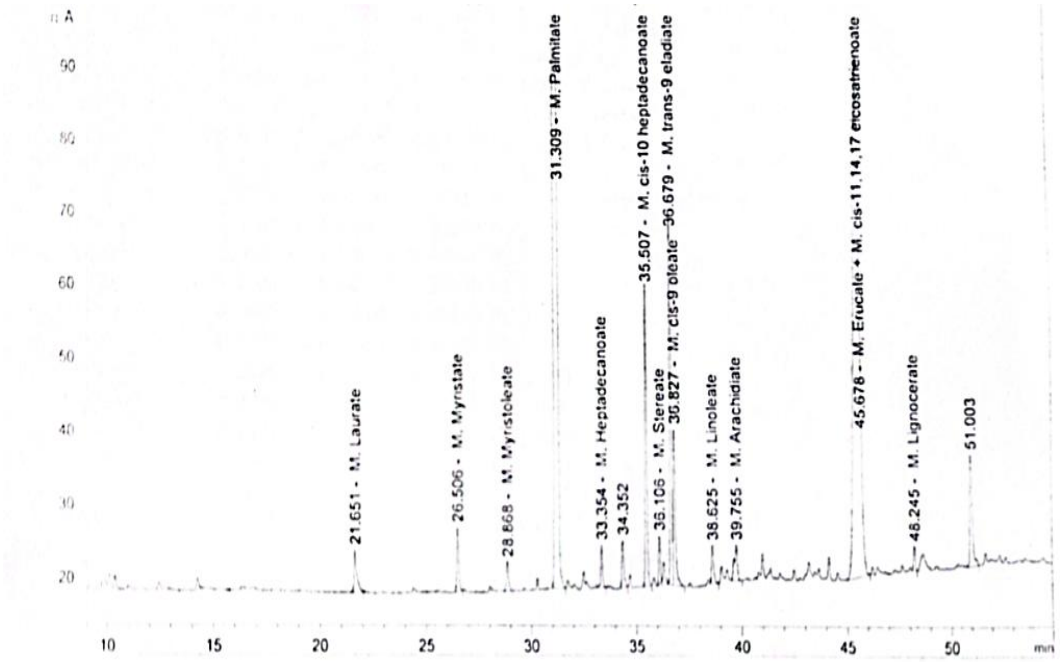

Fig.2 Chromatogram of glycolipids

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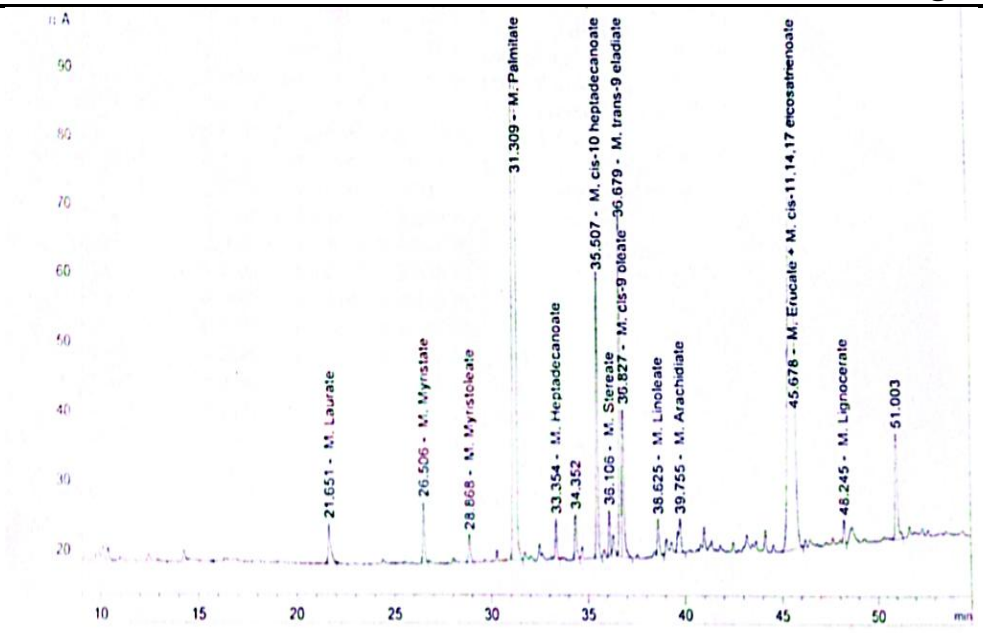

Fig.3 Chromatogram of phospholipids

Although, according to Table 2, the main fatty acids in the composition of the three types of lipid components of Isatis tinctoria seeds are: linolenic, erucic, oleic, palmitic, linoleic, and eicosenic acids, their quantitative content in the composition of lipid components varies greatly. In particular, 28.98% of neutral lipids are linolenic acid (omega 3), 45.08% of glycolipids are palmitic acid, 31.04% of phospholipids are oleic acid (omega 9). Phospholipids are considered to be the most important from a pharmacological point of view among lipids. According to the results of the analysis, it became known that more than 50% of phospholipids are unsaturated fatty acids, which have great nutritional value and have a powerful antioxidant effect: Omega-3, Omega-6 and Omega-9 fatty acids.

Conclusion

The qualitative and quantitative composition of fatty acids of the seed oil of the Isatis tinctoria plant has been studied. It has been established that the main components of the oil are unsaturated fatty acids- oleic, linolenic and palmitic acids.

These unsaturated fatty acids have great nutritional value and have a powerful antioxidant effect: Omega-3, Omega-6 and Omega-9, which cause an indirect immune response by enhancing the body's protective functions.

The research results show that the seeds of Isatis can be used to produce oil with healing properties and introduced into domestic pharmaceutical practice. It is also possible to use seeds and oil obtained from seeds to develop biologically active additives.

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