RESEARCH PAPER

CHROMATO-MASS SPECTROMETRY OF DRY EXTRACTS OF THE ROOTS OF ISATIS TINCTORIA GROWING IN UZBEKISTAN AND THE DEVELOPMENT OF THE BIOLOGICALLY ACTIVE ADDITIVE "VAIDIX-GRANULES"

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Annotation. As a result of the conducted research, diagnostic signs have been identified that can be used in the standardization and development of a draft of relevant regulatory documentation, and the localization of biologically active substances in raw materials of I. tinctoria has been established, when viewed under a binocular laboratory microscope. Due to the unique chemical composition of the Woad, its wide pharmacological effect is manifested. In order to effectively development of the new medicines with the maximum concentration of biologically active substances, certain biologically active substances must be isolated for the treatment of a particular disease. In this work, biologically active substances contained in the composition of dry extracts obtained with various solvents under optimal extraction conditions were studied by chromatomass spectrometry [1]. It was performed chromatography-mass spectrometry of aqueous and ethanol extracts of Isatis tinctoria (roots), which made it possible to identify 33 compounds for which mass spectra and structural formulas were obtained.

The basis of sample No. 1 is made up of high molecular weight aliphatic hydrocarbons - *tetracontane* and *dotriacontane;* a steroid compound (lanostane derivative) *-7,8-Epoxylanostan-11-ol, 3-acetoxy;* derivatives of carboxylic acids - *Docosanoic acid 1,2,3-propanetriyl ester (docosanoic acid ester), Dodecanoic acid, 1a,2,5,5a,6,9,10,10a- octahydro-5a-hydroxy-4-(hydroxymethyl)-1,1,7,9,-tetramethyl-6,11-dioxo* and *hexadecanoic acid, (2-phenyl-1,3-dioxolan-4yl) methyl ester, cis;* a forbol derivative, which is an element of tiglian- a part of the diterpene family - *Phorbol 12,13-dihexanoate;* an organosulfur compound classified as an oxazolidine derivative – *goitrin.*

Sample No. 2 contains biologically active substances such as: mono- and di- and polysaccharide derivatives - - *L-Glucose, α-d-6,3-Furanose, methyl-β-d-glucohexodialdo-1,4-furanoside, Glucopyranose, 4-O- β-D-galactopyranosyl, d-Glycero-d-ido-heptose, D-Allose, Sucrose, 1,5-Anhydroglucitol, Ethyl α-d-glucopyranoside (α-EG)* is found in sake (Japanese rice wine), which has a moisturizing and conditioning effect on the skin), *1,3-Propanediol, 2-ethyl-2 (hydroxymethyl), Polygalygatol* is a type of polyol (sugar alcohol). Glycosides are represented only by: *β-D-glucopyranoside, methyl; phytosterols – γ - Sitosterol* and *β-Sitosterol (γ-sitosterol* can be used to create a protein antidiabetic drug). It has also been reported that γ-sitosterol can affect the number and activity of components of the external pathway of apoptosis in human lung and breast adenocarcinoma cells. *β-Sitosterol* is being investigated as part of its potential to reduce benign prostatic hyperplasia and blood cholesterol levels) and the triphenylmethane dye Phenolphtalein (before the discovery of certain procancerogenic properties, phenolphthalein was used in medicine as a laxative (purgen) for more than a century and a half, although it has cumulative properties and can have an irritating effect on the kidneys and heart).

The basis of sample No. 3 consists of such BAS as: carbohydrates- *Sucrose, d-glycero-d-tallo-heptose, d-Mannose, 1,5-Anhydroglucitol* (a medium-term indicator of glycemic status reflecting fluctuations in the range of hyperglycemia); glycosides - *α-D-Glucopyranoside, O- α-D-glucopyranosyl-(1.fwdarw.3)-β-D-fructofuranosyl, Ethyl α-d-glucopyranoside, β-B-Glucopyranoside; Polygalygatol*

(crystalline sorbitol anhydride C6H12O5 contained in common milkweed (Polygala amara)); *Desulfosinigrin* is an organic substance, glucosylate, O-thioglycoside, sulfur-containing aminoglycoside, one of the components of black (up to 4%) and Sarepta mustard seeds, horseradish and rapeseed roots, gives them a burning taste and a specific smell [2,3]. Sinigrin was first discovered in extracts of essential oils obtained from black mustard seeds (Sinapis nigra), from which it got its name [4], as well as phytosterols - *γ - Sitosterol and β-Sitosterol.*

Also, the pharmacological activity of the roots was investigated, the technology of a biologically active additive based on a dry extract of the roots of Woad, with antiviral and anti–inflammatory effects was developed, and together with “SULTONMED PHARM” LLC the technological instruction for the production of a biologically active food additive - soluble granules “Vaidix” was approved.

Keywords: Isatis tinctoria roots, diagnostic signs, xylem, radial rays, chromato-mass spectrometry, ethanol, Isatis tinctoria root extracts, biologically active additive.

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Graphical abstract.

Introduction. The genus Isatis belonging to the Brassicaceae or Cruciferae family, according to various estimates includes about 79 species (1,2) and is mainly distributed in the Northern Hemisphere, especially in the Iran-Turan region, where almost 90% of its species grow [5,6].

Isatis species sometimes exhibit high morphological polymorphism, and morphological differences are often not obvious, even in fruits that provide the most valuable diagnostic features [7,8,9].

Hamid Moazzeni, Shahin Zarre, Ihsan A. Al. Shahbaz et al. (2007) studied a systematic study of the microscopy of the seed coat in representatives of 23 Isatis species (41 populations) and in four genera of the Isatideae tribe were studied using scanning electron microscopy (SEM). Among the studied samples, eight types of basic ornamentation patterns were identified. Çıtak B.Y., Şirin E., Ertuğrul K. (2021) studied the morphological and anatomical features of the root, stem and leaf of the Isatis quadrialata species and determined the anatomical characteristics and taxonomic significance.

I. tinctoria is a biennial plant, glabrous, glaucous. The stem and leaves are often colored purple. The stem is 30-100 cm high, glabrous, branched, the basal leaves are oblong, petiolate, gnawed-toothed along the edge. The petals are yellow, about 3 mm long. The stems are about 7 mm long, curved at the very bottom, thickened at the top. It blooms and bears fruit from the end of April to the beginning of June. It is cultivated by the local population to produce eyebrow and eyelash dye, and sometimes goes wild. General distribution: Central Asia (north), the south of the European part of the USSR, the Caucasus, Central Europe, the Mediterranean [10,11,12].

Isatis tinctoria has been widely used in folk medicine and cosmetology since ancient times. Due to the unique variety of chemical composition of the Woad, popularly called usma, its wide pharmacological action is manifested. In order to effectively develop new medicines with the maximum concentration of biologically active substances, it is necessary to isolate certain biologically active substances for the treatment of a specific disease [13,14,15].

Isatis tinctoria has been widely used in folk medicine and cosmetology since ancient times. Due to the unique diversity of the chemical composition of the Woad, popularly called Usma, its wide pharmacological effect is manifested. In order to effectively develop new medicines with the maximum concentration of biologically active substances, certain biologically active substances must be isolated for the treatment of a particular disease.

Scientific studies have shown that Isatis tinctoria root has anti-inflammatory, antimicrobial, antibacterial, and antiviral effects. Studies are also underway on the use of this plant as a remedy for the treatment of oncology. During a thorough study of this plant, it was revealed that the Isatis tinctoria root contains thioglycosides, which give it a bitter taste [16]. They have an antimicrobial effect in large doses and are able to stimulate appetite in small doses. Woad root contains substances that help to eliminate toxins. In Chinese folk medicine, its root is used in the form of decoctions and teas for colds and inflammatory processes [17]. Dried root (processed into granules and dissolved in hot water or tea) is popular throughout China, where it is used to eliminate toxic heat, relieve sore throats and treat flu, measles, mumps, syphilis and scarlet fever. It is also used for pharyngitis, laryngitis, erysipelas, carbuncle, for the prevention of hepatitis A, epidemic meningitis and inflammation.

Some compounds in the roots have anti—cancer activity, and root extracts have been used to treat patients with solid tumors and leukemia - a traditional application that led to the release of the indirubin component. Several published studies from China have shown that oral administration of 150-200 mg of purified indirubin per day leads to remission in 60 percent of patients with chronic myelocytic leukemia [18].

The roots of I. tinctoria, which grows in Uzbekistan, and its chemical composition have not been studied before. This determines the relevance and novelty of our research.

Chromato-mass spectrometric analysis was performed in the forensic laboratory and “Vaidix granules” supplements were developed in collaboration with “Sultonmed pharm”.

The aim of the study is to determine diagnostic signs, localization of biologically active substances in the raw materials of this species, as well as to study the composition of dry extracts of the roots of vida dye obtained by various extractants, mass spectroscopic method.

Materials and methods of research. The initial material of the study was the roots of the dye plant, harvested after the collection of raw materials (2024) in the Parkent district of the Tashkent region. The climatic conditions here are typical for the foothills. The roots were harvested after the fruits were fully ripe, in November. The roots were well washed and dried after harvesting. Drying was carried out in natural conditions, in ventilated rooms without access to direct sunlight.

Simultaneously with the morphological description, the raw materials were fixed in 70^0 ethanol for anatomical examination. Cross sections of the fetus are made serially [12,13,14]. Descriptions of the main tissues and cells are given by Metcalfe C.R., Chalk L. (1950). Microscopic examination was carried out in accordance with the Russian Federation State pharmacopeia XIV ed. GPhA 1.5.3.0003.15 "Technique of microscopic and microchemical examination of medicinal plant raw materials and medicinal herbal preparations" on a BioBlue S/N – EC-2209333 microscope consisting of a binocular nozzle and wide-field WF10x/18 eyepieces, four microscope lenses are mounted on a position-locking turret.

The initial material of the study was the roots of Isatis tinctoria. In the laboratory of the Department of Pharmaceutical Production and Quality Management of the Tashkent Pharmaceutical Institute, were obtained dry extracts of the roots. There were used following extractants: **purified water (sample 1), ethyl alcohol 70% (sample 2), ethyl alcohol 80% (sample 3)**. The extraction was carried out by ultrasonic extraction, the extract was freed from the extractant on a rotary evaporator – DLAB RE 100-Pro at a temperature of 60^0C and P =0.09 MPa, then dried on a BIOBASE BK-FD12P freeze dryer under vacuum at a temperature of -58^0C. Further, for the first time, a chromato-mass spectrometric study of dry extracts was performed, which made it possible to identify a wide range of compounds of various classes in its composition, for which mass spectra and structural formulas were obtained [7].

Chromatography-mass spectrometric analyses were performed on a chromatograph M. Agilent technologies 7890b, GSMS System with a mass-selective detector, using AcqMethod Drug_Scan_A G_40_60_Auto, 5977a MDS, software - MassHunter drugs_scanag-3666.

Results and discussion.

The root is taper-shaped, cylindrical with many root hairs, light brown in color, and has a bitter taste due to the presence of alkaloids and thioglycosides. In the second year of life, the root can reach up to 200-300 mm in length and 15-25 mm in diameter (Fig.1).

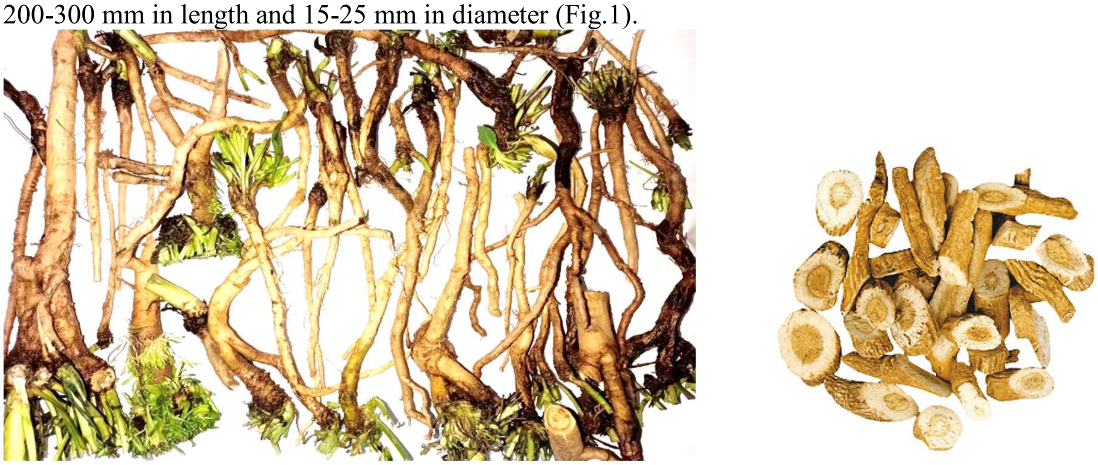

Figure 1. The appearance of the root of Isatis tinctoria

The anatomical structure of the root on the cross-section is rounded, non-bulky type, which consists of a periderm (plug), a secondary bark and a central cylinder. The cork consists of several rows of radially arranged cells of dark brown color, thick-walled and tightly closed.

Thus, for the first time in Uzbekistan, the anatomical structure of the root of I. tinctoria was studied. The following diagnostic features were identified: non-tufted root structure and more developed terminal and diffuse parenchyma, vasicentric parenchyma forms an incomplete lining of bundles; the presence of periderms; thin-walled numerous cortical parenchyma and localized biologically active substances; wood is diffuse-vascular; the presence of large and small xylem central cylinder provides efficient transport of water and minerals; The large, elongated and multi-rowed radial rays are filled with tannins, which leads to the isolation of individual root sites. These identified diagnostic signs can be used in the identification of plant raw materials.

The biologically active substances of the extracts were determined by mass spectroscopic method by pre-dissolving the samples in methanol. Identification and quantification of the compounds were carried out under the following chromatography conditions: sample entry with flow division (1:10), column ZB-5MS (30 m×0.25 mm×0.25 µm), injector temperature 280 0 C, carrier gas - helium, gas velocity through the column 29 ml/min [7].

In the mass spectrum of the 1st sample obtained by using purified water as an extractant, biologically active substances such as tetracontane (Fig. 2) and dotriacontane (Fig. 3) (alkanes); a derivative of lanostane, a polycyclic hydrocarbon, in particular triterpene (Fig.4); forbol (a chemical compound of natural, of plant origin, which is an element of tiglian- a part of the diterpene family) (Fig. 5); begenic (docosan) (Fig. 6), lauric (dodecanoic) (Fig. 7) and palmitic (hexadecanoic) (Fig. 8) acids (related to monobasic marginal carboxylic acids); goitrin (Fig. 9), which is an organosulfur compound classified as an oxazolidine derivative and as a cyclic thiocarbamate were detected.

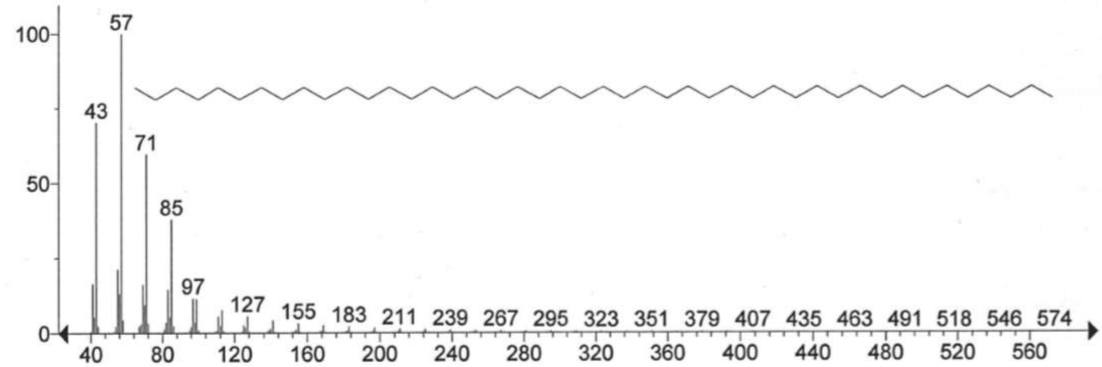

Figure 2. Tetracontane
$C_{44}H_{90}$; MF: 714; RMF: 791; Prob 13,5%.

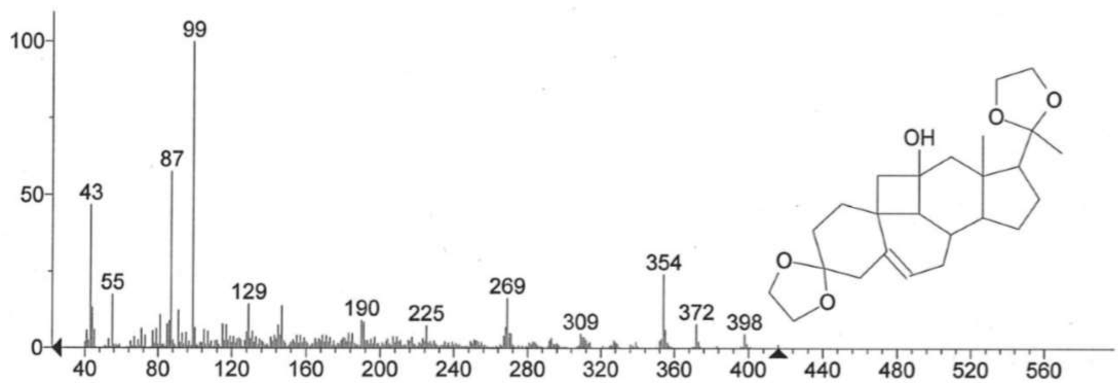

Figure 3. Dotriacontane $C_{32}H_{66}$, MF: 460; RMF: 615; Prob 11,3%

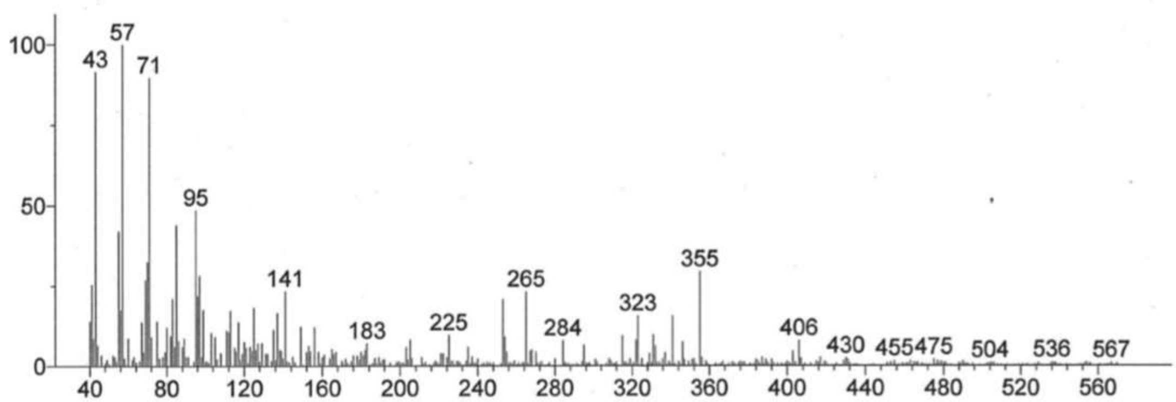

Figure 4. 7,8-Epoxylanostan-11-ol, 3-acetoxy
$C_{32}H_{54}O_4$;MF: 559; RMF: 583; Prob 15,9%.

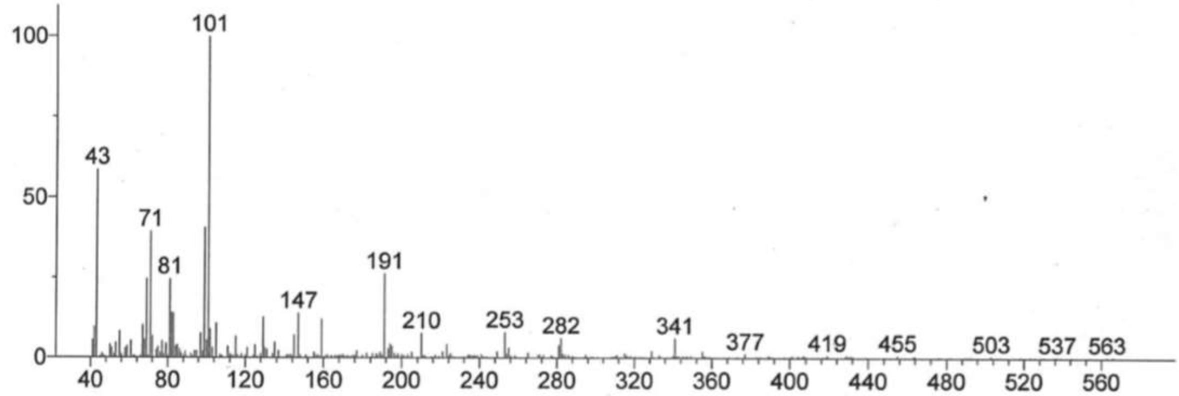

Figure 5. Phorbol 12,13-dihexanoate
$C_{32}H_{48}O_8$; MF: 522; RMF: 551; Prob. 11,1%

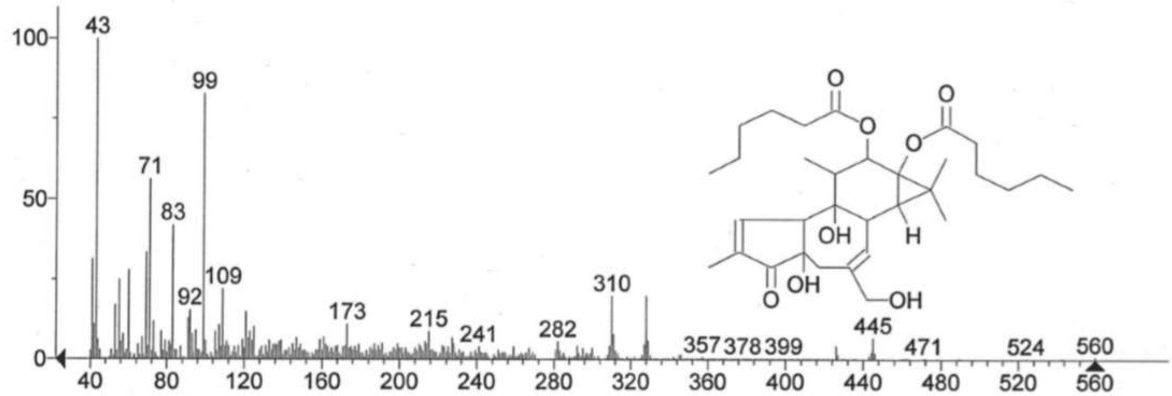

Figure 6. Docosanoic acid
1,2,3-propanetriyl ester
$C_{69}H_{134}O_6$; MF: 541; RMF: 595; Prob. 29,5%

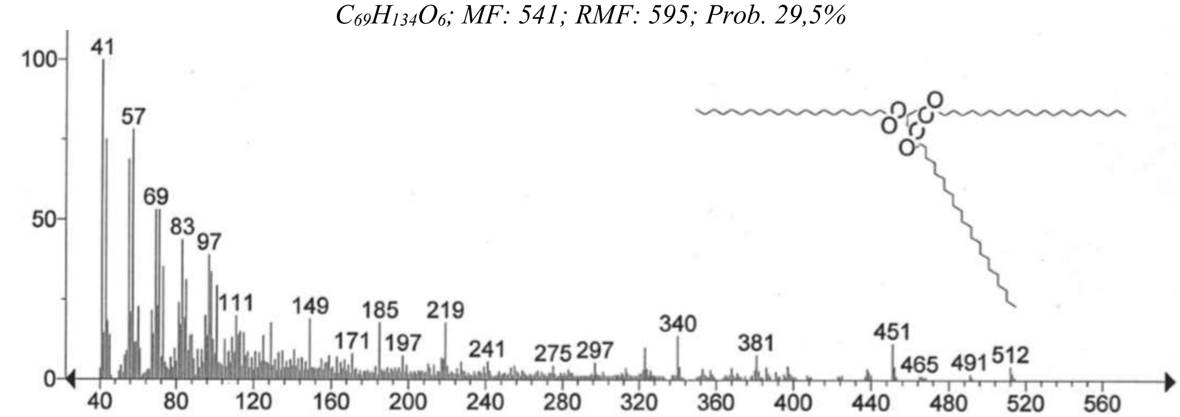

Figure 7. Dodecanoic acid, 1a,2,5,5a,6,9,10,10a- octahydro-5a-hydroxy-4-(hydroxymethyl)-1,1,7,9,-tetramethyl-6,11-dioxo
$C_{32}H_{48}O_6$; MF: 532; RMF: 542; Prob 21.4%

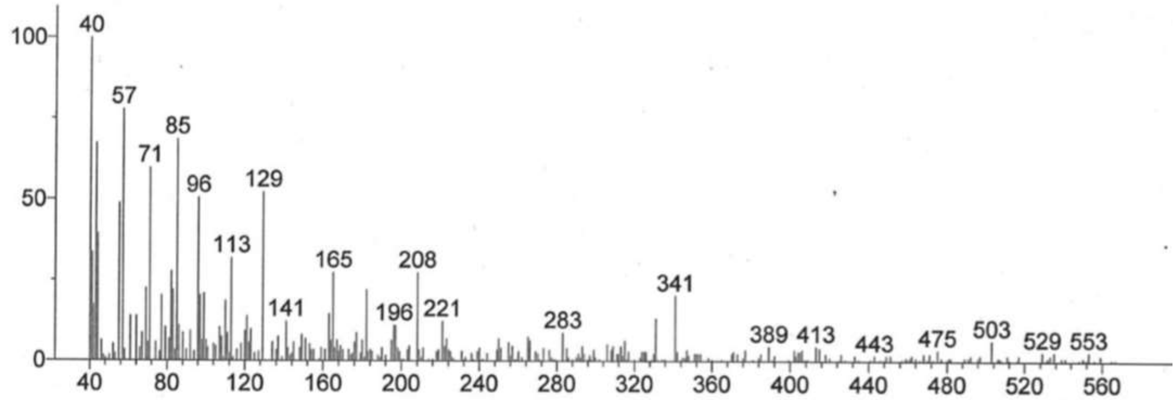

Figure 8. Hexadecanoic acid, (2-phenyl-1,3-dioxolan-4yl) methyl ester, cis
$C_{26}H_{42}O_4$; MF: 456; RMF: 540; Prob 28.3%

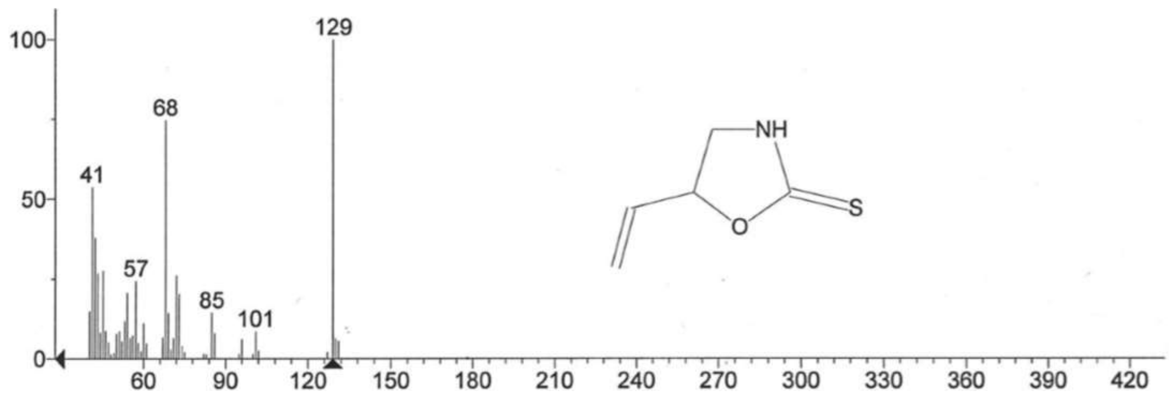

Figure 9. Goitrin C_5H_7NOS; MF: 815; RMF: 862; Prob 21,5%.

Biologically active substances such as carbohydrates - derivatives of glucose, furanoses, glucopyranoses, heptoses, allozes, sucrose; 1,5-anhydroglucitol, ethyl α–d-glucopyranoside, 2-ethyl-2-(hydroxymethyl)-1,3-propanediol; sugar alcohol - polyhalitol, phytosterols, phenolphthalein, etc. were found in the mass spectrum of the ***2-sample*** obtained when using 70%ethyl alcohol as an extractant (Fig. 10-14).

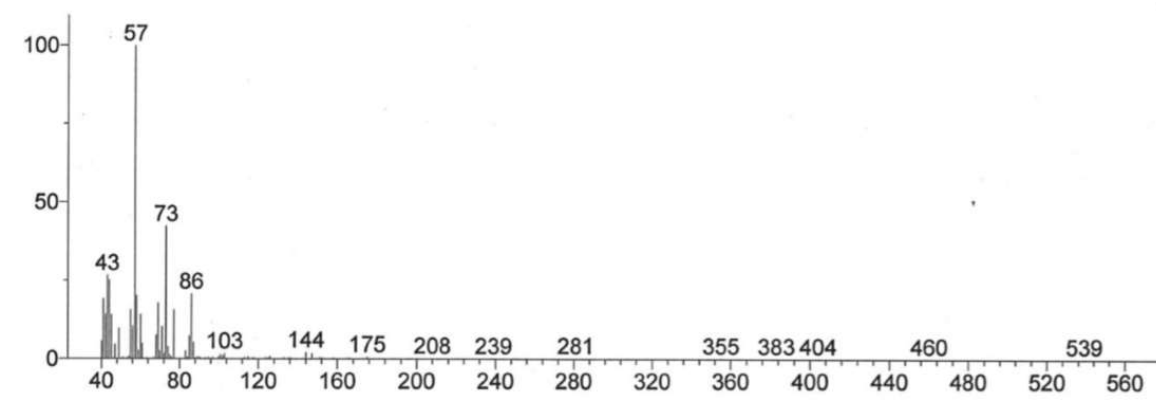

Figure 10. Sucrose $C_{12}H_{22}O_{11}$; MF: 752; RMF: 828; Prob 44,1%

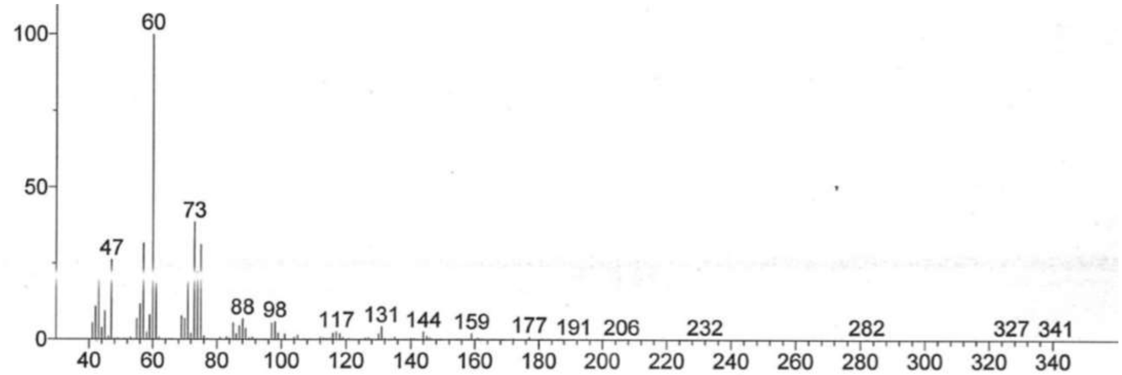

Figure 11. Ethyl α-d-glucopyranoside $C_8H_{16}O_6$; MF: 864; RMF: 906; Prob 76,4%

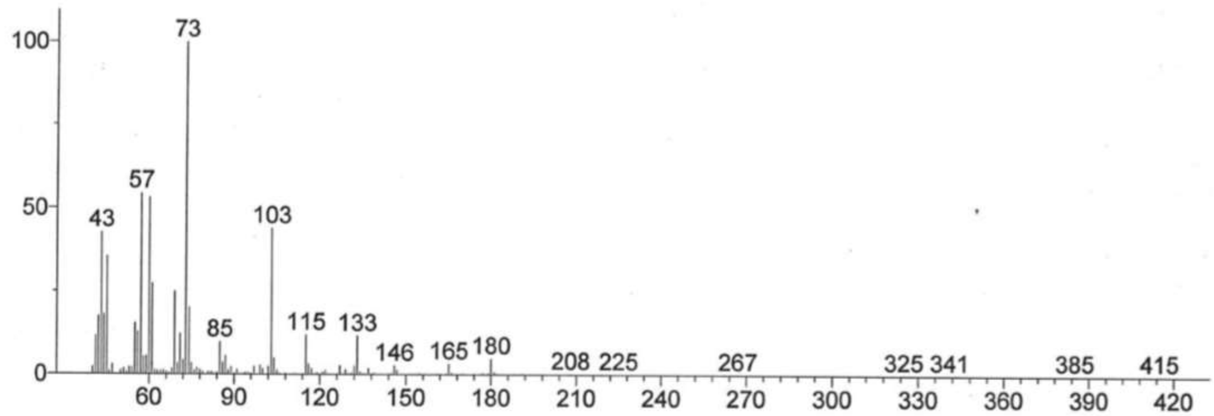

Figure 12. Polygalygatol $C_6H_{12}O_5$; MF: 831; RMF: 892; Prob 47,7%

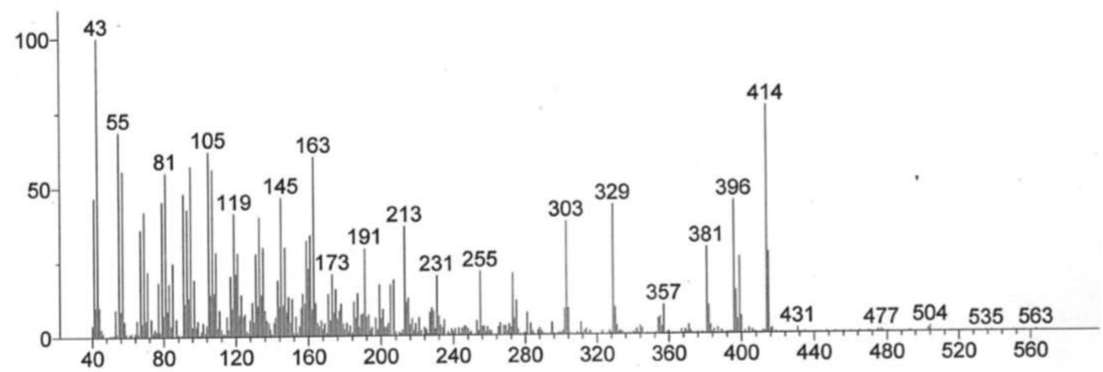

Figure 13. γ- Sitosterol $C_{29}H_{50}O$; MF: 832; RMF 862; Prob 50,8%

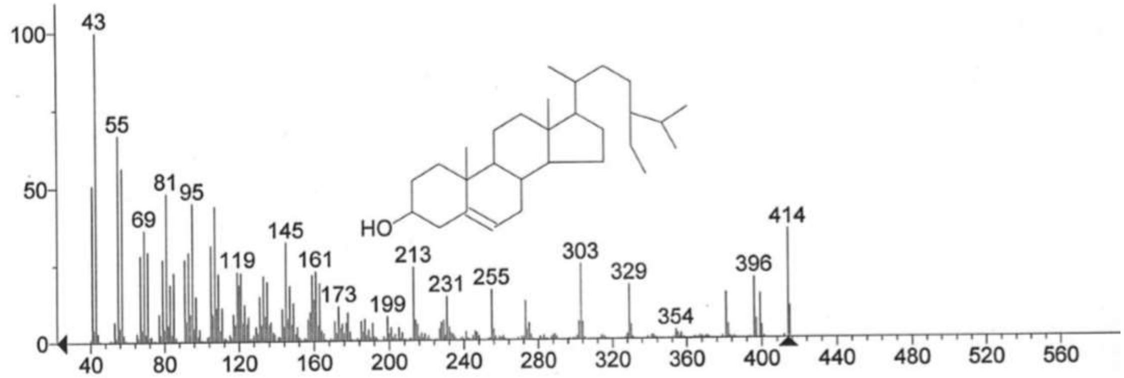

Figure 14. β- Sitosterol $C_{29}H_{50}O$; MF: 816; RMF: 857; Prob 29,3%

Biologically active substances such as sucrose, heptose, glucopyranoside, mannose, polyhalitol, anhydroglucitol, desulfosinigrin, sitosterols were found in the mass spectrum of the ***3-sample*** obtained using ethyl alcohol 80% as an extractant (Fig. 15-17).

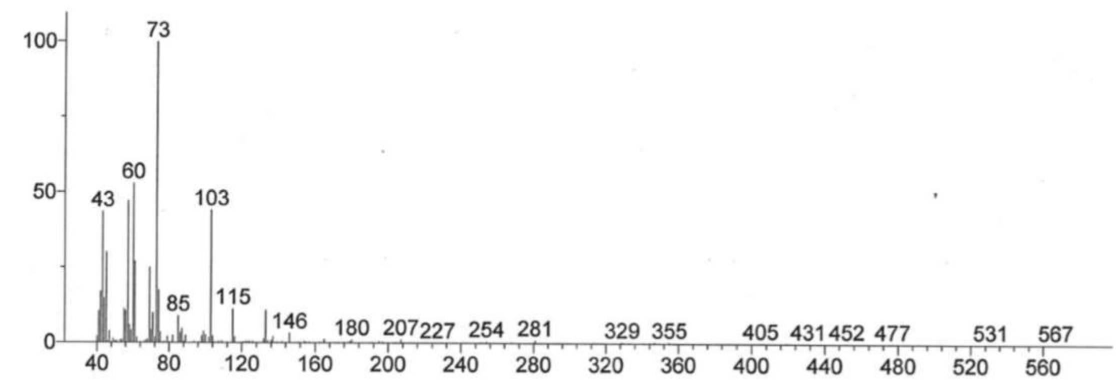

Figure 15. Polygalitol C6H12O5; MF: 810; RMF: 880; Prob 41,6%

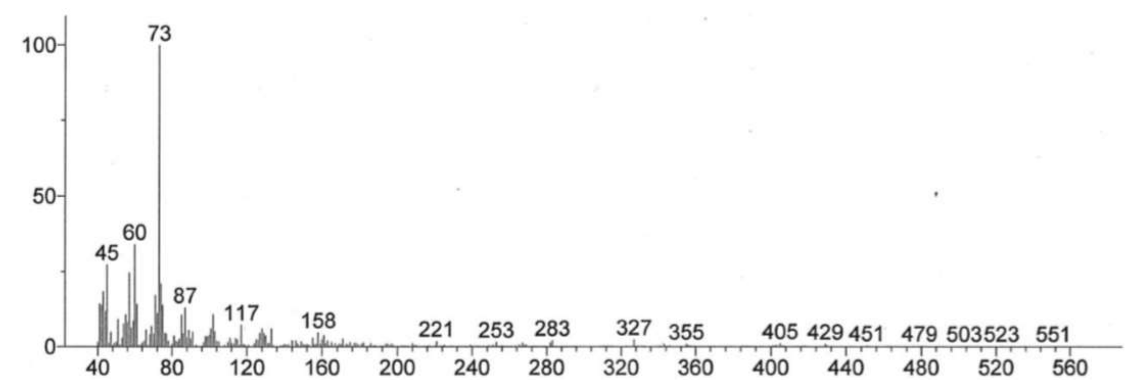

Figure 16. Desulphosinigrin $C_{10}H_{17}NO_6S$; MF: 652; RMF: 740; Prob 18,0%

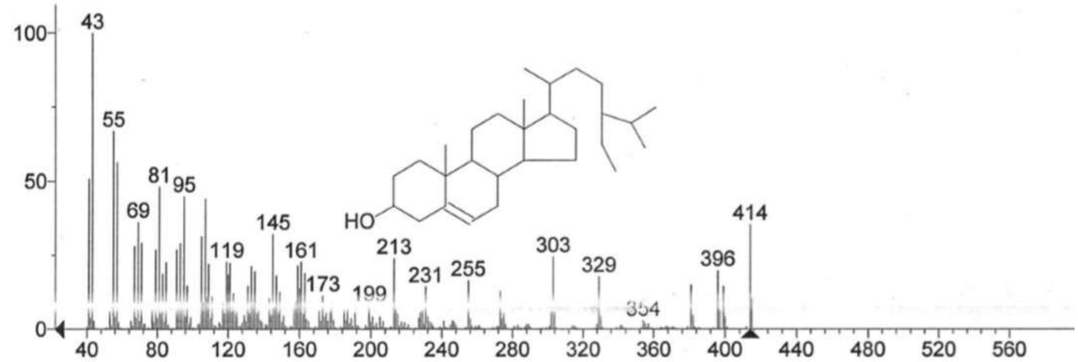

Figure 17. β-Sitosterol $C_{29}H_{50}O$; MF: 741; RMF: 813; Prob 32,9%

Table 1

Biologically active substances of dry extracts of Isatis tinctoria L. roots determined by chromato-mass spectroscopic method

Biologically active substances of dry extracts dissolved in methanol			
№	1-Sample	2-Sample	3-Sample
1	Tetracontane	glucose	sucrose
2	Dotriacontane	furanose derivatives	heptose
3	Lanostanum	Glucopyranose derivatives	glucopyranosides
4	Phorbol	heptose derivatives	mannose
5	Begenic acid	allose derivatives	polyhalitol
6	Lauric acid	sucrose derivatives	anhydroglucitol
7	Palmitic acid	1,5-anhydroglucitol	desulfosinigrin
8	Goitrin	ethyl α-d-glucopyranoside	γ – sitosterol
9		2-ethyl-2-(hydroxymethyl)-1,3-propanediol	β - sitosterol
10		polygalitol	
11		γ – sitosterol	
12		β - sitosterol	
13		phenolphthalein	

Preclinical studies have also been conducted to determine the specific activity, acute toxicity, and cumulative effect of Vida dye root extracts. It has been proven to have anti-inflammatory and antiviral activity. It should also be noted the effect of extracts of roots and leaves on blood plasma factors ( ).

Based on the conducted research, the technology of biologically active food additives in the form of instant granules in sachet bags – "Vaidix" was developed.

Instant granules – "Vaidix" is a herbal preparation based on the extract of the roots of vida dye. It has antiviral, anti-inflammatory, and antibacterial effects. The remedy is especially effective in the acute phase of respiratory diseases: high fever, sore throat and headache.Tea for colds and viruses can accelerate the process of restoring immunity, eliminate inflammation and microorganisms, improve lung function and reduce the risk of secondary bacterial infections. In addition, it has antiviral and antioxidant effects that can help fight various types of viruses.

1 sachet of soluble powder contains 7 g: Dry extract of the roots of Vida dye 5 g and sugar 2 g.

Vaidix cold and virus tea contains flavonoids, organic acids, glycosides, vitamins A, B1, B2, C, alkaloids, polysaccharides, saponins and other biologically active substances. Studies are currently underway that show the potential effectiveness of this extract against the coronavirus.

Indications for use:

• increased body temperature;
• sore throat;

• headache;
• laryngitis and laryngospasm with high fever;
• sore throat;
• aphthous stomatitis;
• colds (colds, flu, acute respiratory viral infections);
• symptomatic treatment of respiratory tract diseases;
• Prevention and treatment of viral infections;
• Strengthen the immune system.

The method of application. Mix half or the whole sachet in a glass of warm boiled water (200 ml), wait for the drink to cool down to room temperature. Take it 3 times a day with meals for 3 to 5 days. The use of antiviral antipyretic Vida extract during pregnancy and breastfeeding is possible only under the supervision of a specialist.

Dietary supplements in the form of soluble granules are packed: for single use in paper filter bags and 5 pieces packed in cardboard boxes (Fig. 19).

Figure 19. Secondary packaging of the "Vaidix granules" dietary supplement

Conclusion. Thus, for the first time, studies were conducted on the roots of the Dye Vida, which grows in the Tashkent region of the Republic of Uzbekistan. The obtained results allowed us to identify a number of morpho-anatomical and characteristic diagnostic features for this species.

The identified structural and diagnostic features make it possible to identify the raw materials of this plant when analyzing its authenticity and can be used in standardization and the development of a draft of relevant regulatory documentation.

According to the results of chromatography-mass spectrometric analysis, in extracts of the roots of Isatis tinctoria, it can be assumed that the pharmacological effect of sample No. 1 is mainly determined by the content of esters of carboxylic acids, high molecular weight alkanes, steroid compounds, diterpenes and organosulfur compounds.

Sample No. 2 showed the greatest variety of BAS. The pharmacological effect of sample No. 2 is mainly determined by the content of derivatives of mono-, di- and polysaccharides, phytosterols and glycosides.

The pharmacological effect of sample No. 3 is also due to biologically active substances such as carbohydrates, phytosterols, and O-thioglycoside.

The dye paste, which has a unique chemical composition, exhibits a wide range of pharmacological effects. There is an interest in further research on the isolation of dominant active elements – substances for obtaining the active substance of a medicinal product for introduction into domestic pharmacy. Also, for the effective development of new medicines from Isatis roots with the maximum concentration of biologically active substances for the treatment of a specific disease, it is necessary to select a rational extractant and optimal extraction conditions [21,22,23].

Based on the conducted research, a technology of biologically active food additives in the form of instant granules in sachet bags – "Vaidix" of antiviral and anti-inflammatory action has been developed [24].

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