NOVATEUR PUBLICATIONS
JournalNX- A Multidisciplinary Peer Reviewed Journal
ISSN No: 2581 - 4230
VOLUME 6, ISSUE 9, Sep. -2020

WATER-SOLUBLE VITAMINS OF DRY EXTRACT OF MILK THISTLE (SILYBUM MARIANUM) SEEDS, GROWN IN UZBEKISTAN. HPLC METHOD

QAYUMOV FERUZ SOBIR OGLI
Assistant of the Department of Biotechnology, Tashkent Pharmaceutical Institute

TUXTAYEV FARHOD HAKIMOVICH
Tashkent Pharmaceutical Institute
The Department of Biotechnology is a Senior Lecturer

ABSTRACT:

In order to reflect on the characteristics of any plant, especially its biophysiological or technological importance to the human body, it is necessary to initially turn to the chemical composition of this plant. That is, a systematic study of its chemical composition will serve as the main issue. The purpose of this research work and the results obtained are also about the water soluble vitamins while the vitamins contained in the seeds of the silybum marianum plant are exactly.

KEYWORDS: HPLC method, pharmaceutical, silybum marianum, vitamin.

INTRODUCTION:

At the same time, most of the pharmaceuticals, that is, the drugs used and taken by humans, are derived from plants and the products of their processing. To fully open and expand the scope of application, it is necessary to determine its content. The study of this selected and studied plant, and in terms of its chemical composition, began much earlier and yielded great results [1]. In this study, on the other hand, the study of the composition of species grown in the environment in a small circle: in the context of vitamins.

MAIN PART:

HPLC analysis of water-soluble vitamins is carried out on an Agilent Technologies 1200 chromatograph on an ExlipseXDBC18 column (reversed-phase), 5µm, 4.6x150mm. Diode-matrix detector (DBP), 254nm, 290nm. Solution A: 0.5% acetic acid, pH 1.7: B: CH3CN (acetonitrile). Flow rate 1 ml / min. Gradient% B / min: 0-5min / 96: 4%, 6-8min / 90: 30%, 9-15min / 80: 20%, 15-17min / 96: 4% Thermostat 250C.

1. Analytical analysis amount of vitamins

Vitamins	Thistle mg / kg
B_1	1,089224
B_2	11,70472
B_3	8,569052
B_6	4,380287
C	0,435456
PP	0

The chemical composition of milk thistle fruit besides flavonolignans also include other flavonoids (such as taxifolin, quercetin, dihydrokaempferol, kaempferol, apigenin, naringin, eriodyctiol, and chrysoeriol), 5,7-dihydroxy chromone, dehydroconiferyl alcohol, fixed oil (60% linoleic acid; 30%, oleic acid; 9% palmitic acid), tocopherol, sterols (cholesterol, campesterol, stigmasterol, and sitosterol), sugars (arabinose, rhamnose, xylose, and glucose), and proteins[2]. However, the highest concentration, comprising approximately 50–70% of the extract, is silybin, which is the major bioactive component of extract, which has been confirmed in various studies. The silybin concentrations typically found in common

pharmaceutical products containing a silymarin range of 20–40%[3]. The chemical structure of silybin was first established by Pelter and Hansel in 1968, by careful examination of 1H-NMR (100 MHz, DMSO-d_6) and MS spectra[4]. Silybin, which is also called flavobin, silliver, silybine, silymarin I, silybina, and silybine, has a molecular formula of C25H22O10 and a molecular weight of 482.441, CAS No. 22888-70-6 (data obtained from the pubchem website). The silybin structure consists in two main units. The first is based on a taxifolin, which is a flavononol group in flavonoids. The second is a phenyllpropanoid unit, which in this case is conyferil alcohol. These two units are linked together into one structure by an oxeran ring[5].

In silybin's structure, we can recognize five hydroxyl groups, which are the primary targets of the derivatization process. Three of these hydroxyl groups (5-OH, 7-OH, and 20-OH) possess a phenolic nature. The 5-OH group has a very strong hydrogen bonding to the adjacent oxo group, which is in the conjugation with the aromatic ring and acts as a free electron pair donor to the hydrogen bond with the 5-OH group. The 7-OH and 20-OH have similar properties, although the C-7 OH group is more reactive than the 20-OH group due to its lower steric hindrance and the presence of a hydrogen bond. The C-23 OH group have properties leading to the esterization or the oxidation of carboxylic groups. The C-3 OH group can easily be oxidized (even with atmospheric oxygen) to a ketone, which is responsible for the creation of a 2,3-dehydrosilybin. Silybin is poorly soluble in polar protic solvents (EtOH and MeOH), and insoluble in non-polar solvents (chloroform and petroleum ether), but highly soluble in polar aprotic solvents such as DMSO, acetone, DMF, and THF[6].

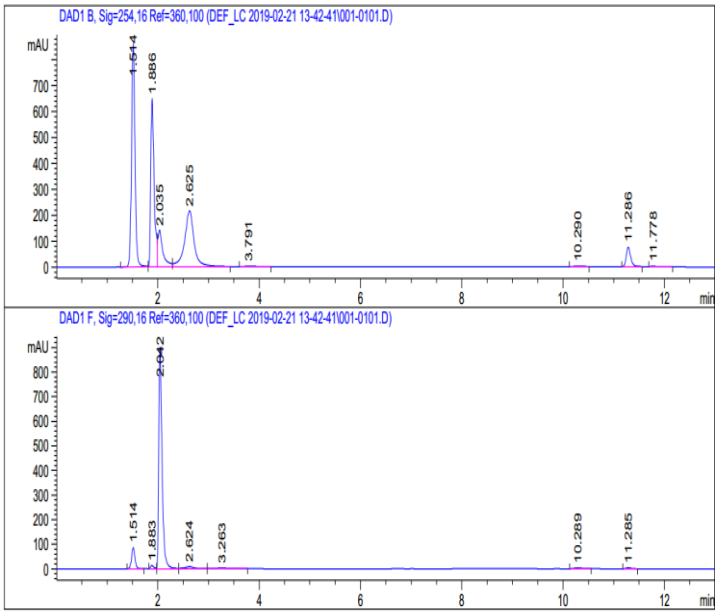

Fig.2. Sum of Vitamin.

Acq. Operator : Seq. Line : 1; Acq. Instrument : Instrument 1 Location : Vial 1; Injection Date : 2/21/2019 1:42:59 PM Inj : 1; Inj Volume : 10.0 µl; Acq. Method : C:\CHEM32\1\DATA\DEF_LC 2019-02-21 13-42-41\VITAMINS_LC.M; Last changed : 2/21/2019 1:42:40 PM; Analysis Method : C:\CHEM32\1\METHODS\ALLAPININ_LC.M; Last changed : 4/24/2019 4:18:27 PM(modified after loading); Method Info : Allapinin. Bufer: 70% 0.1 TFU. 30% MeCN

3. Area Percent Report1

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	1.514	VV	0.0735	4074.65137	856.35236	35.3532
2	1.886	VV	0.0732	3073.97046	649.88629	26.6709
3	2.035	VV	0.0943	977.22162	141.92021	8.4787
4	2.625	VB	0.1904	2866.46509	216.59613	24.8705
5	3.791	BB	0.1959	30.45904	2.30877	0.2643
6	10.290	BB	0.1484	11.91264	1.19436	0.1034
7	11.286	BB	0.0926	471.34912	75.78448	4.0896
8	11.778	BB	0.1447	19.52029	1.85679	0.1694

Totals : 1.15255e4 1945.89939

Area Percent Report: Sorted By : Signal; Multiplier: 1.0000; Dilution: 1.0000; Use Multiplier & Dilution Factor with ISTDs; Signal 1: DAD1 B, Sig=254,16 Ref=360,100

4. Area Percent Report2

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	1.514	BB	0.0735	405.36401	85.28299	8.4615
2	1.883	BV	0.0697	60.48137	13.64295	1.2625
3	2.042	VB	0.0709	4084.17847	900.68549	85.2522
4	2.624	BB	0.1997	116.91922	8.43589	2.4405
5	3.263	BB	0.3181	54.33157	2.38210	1.1341
6	10.289	BB	0.1482	39.24804	4.01025	0.8193
7	11.285	BB	0.0952	30.17669	4.81144	0.6299

Totals : 4790.69938 1019.25111

Signal 2: DAD1 F, Sig=290,16 Ref=360,100

CONCLUSION:

This practical work was conducted by the Research Institute of Bioorganic Chemistry named after Sodikov, Proteins and peptides were tested in the chemistry laboratory and conclusions were drawn. All other relevant literature was analyzed and some data were used

REFERENCES:

1) Michal Bijak Molecules. 2017 Nov; 22(11): 1942. Published online 2017 Nov 10. doi: 10.3390/molecules22111942
2) Milk thistle in liver diseases: past, present, future. Abenavoli L, Capasso R, Milic N, Capasso F Phytother Res. 2010 Oct; 24(10):1423-32.
3) Analysis and comparison of active constituents in commercial standardized silymarin extracts by liquid chromatography-electrospray ionization mass spectrometry. Lee JI, Narayan M, Barrett JSJ Chromatogr B Analyt Technol Biomed Life Sci. 2007 Jan 1; 845(1):95-103.
4) Pelter A., Hansel R. The structure of silybin (silybum substance E6), the first flavonolignan. Tetrahedron Lett. 1968;9:2911–2916. doi: 10.1016/S0040-4039(00)89610-0.
5) Mechanistic study of the biomimetic synthesis of flavonolignan diastereoisomers in milk thistle. Althagafy HS, Meza-Aviña ME, Oberlies NH, Croatt MP J Org Chem. 2013 Aug 2; 78(15):7594-600. And Kurkin V.A. Phenylpropanoids from medicinal plants: Distribution, classification, structural analysis, and biological activity. Chem. Nat. Compd. 2003;39:123–153. doi: 10.1023/A:1024876810579.
6) Chemistry of silybin. Biedermann D, Vavříková E, Cvak L, Křen V Nat Prod Rep. 2014 Sep; 31(9):1138-57.
7) Molecular structure and stereochemistry of silybin A, silybin B, isosilybin A, and isosilybin B, Isolated from Silybum marianum (milk thistle). Lee DY, Liu Y J Nat Prod. 2003 Sep; 66(9):1171-4.
8) Stereoselective metabolism of silybin diastereoisomers in the glucuronidation process. Han YH, Lou HX, Ren DM, Sun LR, Ma B, Ji M J Pharm Biomed Anal. 2004 Mar 10; 34(5):1071-8.
9) New insight into the biosynthesis of flavanolignans in the white-flowered variant of Silybum marianum. Nyiredy S, Samu Z, Szücs Z, Gulácsi K, Kurtán T, Antus S J Chromatogr Sci. 2008 Feb; 46(2):93-6.
10) Silybin inactivates cytochromes P450 3A4 and 2C9 and inhibits major hepatic glucuronosyltransferases. Sridar C, Goosen TC, Kent UM, Williams JA, Hollenberg PF Drug Metab Dispos. 2004 Jun; 32(6):587-94.
11) Application of liquid chromatography-electrospray ionization-ion trap mass spectrometry to investigate the metabolism of silibinin in human liver microsomes. Gunaratna C, Zhang T J Chromatogr B Analyt Technol Biomed Life Sci. 2003 Sep 5; 794(2):303-10.
12) Lorenz D., Lucker P.W., Mennicke W.H., Wetzelsberger N. Pharmacokinetic studies with silymarin in human serum and bile. Methods Find. Exp. Clin. Pharmacol. 1984;6:655–661
13) Zuber R., Modriansky M., Dvorak Z., Rohovsky P., Ulrichova J., Simanek V., Anzenbacher P. Effect of silybin and its congeners on human liver microsomal cytochrome P450 activities. Phytother. Res. 2002;16:632–638. doi: 10.1002/ptr.1000.