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QUANTITATIVE RESEARCH ON THE ACTIVE COMPONENT OF SILYMARIN IN *SILYBUM MARIANUM* SEED POWDER FROM VARIOUS GEOGRAPHICAL AREAS

***Maria POROCH - SERIŢAN[1], Mihaela JARCĂU[1], Narcisa Mihaela CORNEA[2]**
[1]*Faculty of Food Engineering, Stefan cel Mare University,* Suceava, Romania*;* mariap@fia.usv.ro*,*
[2]*SC Laropharm SRL, Sos. Alexandriei 145 A, Bragadiru, Ilfov, Romania;*
*Corresponding author
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Abstract: *Many studies have proved that bioactive components of Silybum marianum powder have excellent hepatoprotective action. The silymarin is a mixture of mainly three flavonolignans, for example, silybin, silydianin and silychristin, with silybin being the most active. The active substances work upon the membrane of the liver cell preventing its destruction and favoring the reconstruction of the cell which is already in the process of destruction. In this study, we measured the quantitative of total silymarin in the powder of Silybum marianum from various geographical areas. Results showed that silymarin content varied according to the geographical location. The quantitative dosing of the silymarin extract existing in the Silybum marianum powder samples from the analyzed geographical areas was performed by the UV spectrophotometric method on soxhlet extraction and they fall within the parameters described in the European Pharmacopoeia.*

Keywords*: bioactive components, hepatoprotection, UV spectrophotometric method*

1. Introduction

According to the most recent statistics on medicinal and aromatic plants, statistical analysis shows a significant increase in the consumer interest of natural products in recent years, although, units that make pharmaceutical, cosmetic, and non-toxic products are excluded [1].
According to studies, people prefer natural products because they provide health benefits. Natural products, in general, are more widely available, have fewer side effects, are not addictive, are easily assimilated, can be combined with other drugs and diets, and are much more affordable [2].
People are increasingly turning to herbal treatments, teas, and other herbal products and by-products. In recent years, the cultivation of medicinal plants has become a good business opportunity due to the low costs, high yield, and easily bearable initial costs. Natural product demand is constantly increasing, both in the domestic and international markets [3].
The Romanian flora includes approximately 3.700 species recognized as having properties on a national level, with approximately 370 species having pharmacodynamic effects. Recently, the production and marketing of medicinal plants derived from natural vegetation have emerged as a major economic concern, providing income not only to producers but also to processors [4].
The *Cardui Mariae Fructus* is used to treat hepatitis, cirrhosis, pancreatitis, diabetes, and chronic digestive diseases [5, 6]. For *Silybum marianum Gaerth* plants, staggered fruit ripening is characteristic, the most valuable being in the central anthodes compared to those on the lateral sprouts [7].
The plant contains saponosides, volatile oil. The fruits contain silymarin (consisting

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of flavonolignans silychristin, silydianin, silybin, isosilybin), betaine hydrochloric acid, amino acids (1-cysteine, glycine, 1-glutamic acid, d-1-2-amino-butyric acid, d-1-leucine, tyramine), lipids (3-4%), polyhydroxyphenylchromone, fumaric acid, etc [8].

Silymarin, a mixture of flavonolignans (figure 1), is the active component isolated from the seeds and fruits of the plant (silychristin, silydianin, silybin A, silybin B, isosilybin A, isosilybin B, taxifolin) [9, 10].

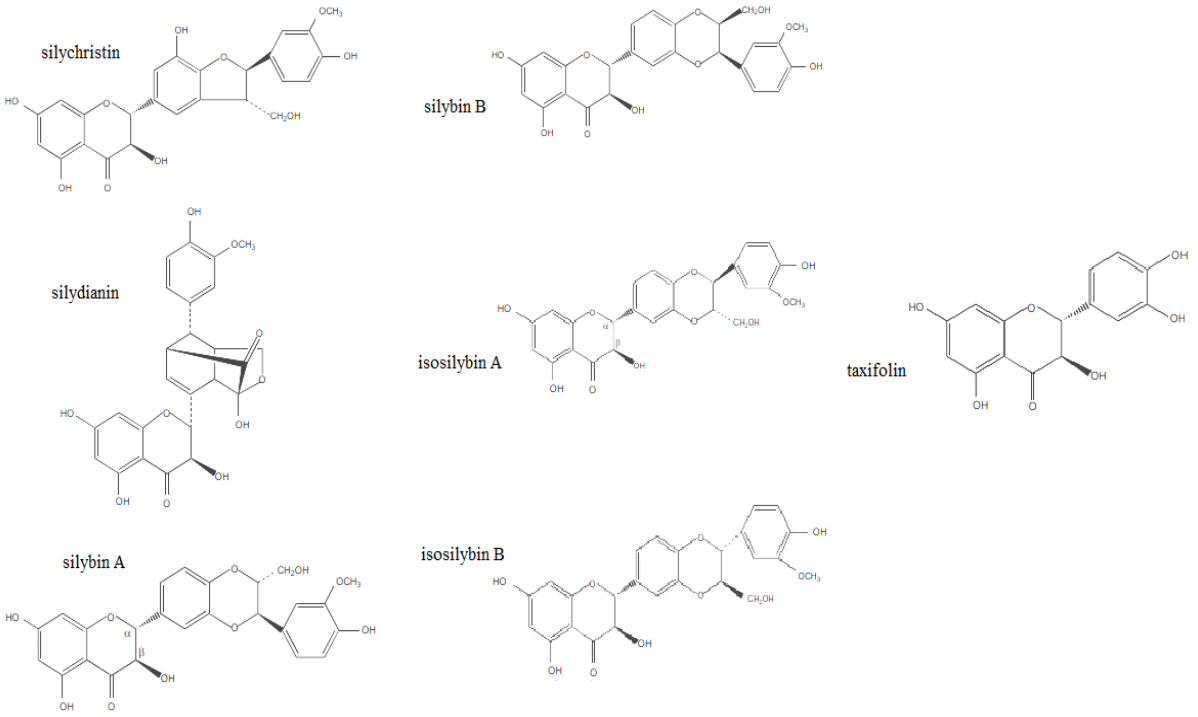

Fig. 1. Chemical structures of the main active constituents in *Silybum marianum*, the flavonolignans

The fruits have therapeutic value in both human and veterinary medicine. The active ingredients are eupeptic, tonic-bitter and hepatoprotective. The anti-hepatotoxic, protective, and curative effects on the liver have been confirmed [11]. The active substances act on the liver membrane, preventing the cell from destroying itself. It encourages the regeneration of liver cells, which are already being destroyed. Silymarin is non-toxic and does not have any side effects. Recommended in liver diseases (hepatitis, cirrhosis, liver failure), poisoning with α-amanitin and phalloidin in poisonous fungi, in indigestion [12, 13, 14].

Mechanism of Action

Silymarin hepato protective effects are purportedly accomplished via several mechanisms; these include:

- antioxidation;
- inhibition of lipidperoxidation;
- stimulation of ribosomal RNA polymerase and subsequent protein synthesis, leading to enhanced hepatocyte regeneration;
- enhanced liver detoxification via inhibition of phase I detoxification;
- enhanced glucuronidation and protection from glutathione depletion;
- anti-inflammatory effects, including inhibition of leukotriene and prostaglandin

synthesis, Kupffer cell inhibition, mast cell stabilization and inhibition of neutrophil migration;

- slowing or even reversing of fibrosis by reduction of the conversion of hepatic stellate cells into myofibroblasts;
- anticarcinogenesis by inhibition of cyclin-dependent kinases and discontinuance of cancer cell growth;
- silymarin is also found to have immunomodulatory effects on the diseased liver [15].

Adverse effects

- silymarin has very low toxicity and has been shown to possess a good safety profile. At high doses, a laxative effect is observed due to increased bile flow.
- adverse effects related to the intestinal gastrointestinal tract such as: dyspepsia, bloating, nausea and diarrhea.
- serious adverse effects, which are rare, include gastoenteritis associated with collape and allergy [15].

The goal of this study was to perform the quality control on the finished product, milk thistle (*Silybum marianum)* seed powder from various geographical areas, using analysis to verify the product conformity in terms of silymarin content dosing parameters.

2. Matherials and methods

Silybum marianum seed powder from various geographical areas (Southeast Europe, South Asia, Central Europe, and East Asia) was purchased in the domestic market from specialty stores. Each of the six powder samples was subjected to three determinations.

The extraction of silymarin from weaving seed powder is a two-step process: first, the weaving seed powder is degreased with n-hexane for 4 hours, followed by the actual extraction with the Soxhlet extractor for 8 hours with ethyl acetate, from which a fine yellow powder is obtained after evaporation at a maximum temperature of 50°C.

The drying loss is carried out in weighing vials, placed in the oven at 105°C for 3 hours until a constant mass and is calculated according to equation 1:

Loss on drying,

$$\% = \frac{m_{fp} - m_{fu}}{m_p} \cdot 100 \quad (1)$$

where: m_{fp} - ampoule mass containing the sample, expressed in g; m_{fu} - dry ampoule mass, expressed in g; m_p - sample mass, expressed in g [16].

The dosage of silymarin extracted from milk thistle (*Silybum marianum*) seed powder, by UV spectrometric method, consists in the following procedure: in a 100 ml bale weigh the equivalent of 0.1 g of silymarin (according to Ph. Eur. add 50 ml of methanol, ultrasound for 30 minutes, then make up to volume with the same solvent. 1 ml of the solution obtained is diluted to 100 ml with the same solvent. The absorbance was read at a wavelength of 287 nm, and the reference is methanol.

The calculation formula used for silymarin dosing is according to equation 2:

Silymarin, % (relative to the dry matter) =

$$\frac{A_P}{A_{sp}} \cdot \frac{100}{m_p} \cdot \frac{100}{100 - U} \cdot 100 \quad (2)$$

where: A_p– the value of the sample solution at an absorbance of 287 nm;

A_{SP}– 402.53 (specific absorbance);

m_p – the amount of substance to be analyzed in g; U – loss on drying [16].

3. Results and discussion

The rich chemical composition of the plant depends on a large number of factors: the type and conditions of cultivation, harvest time, climate, species and the method used for harvesting [17, 18].

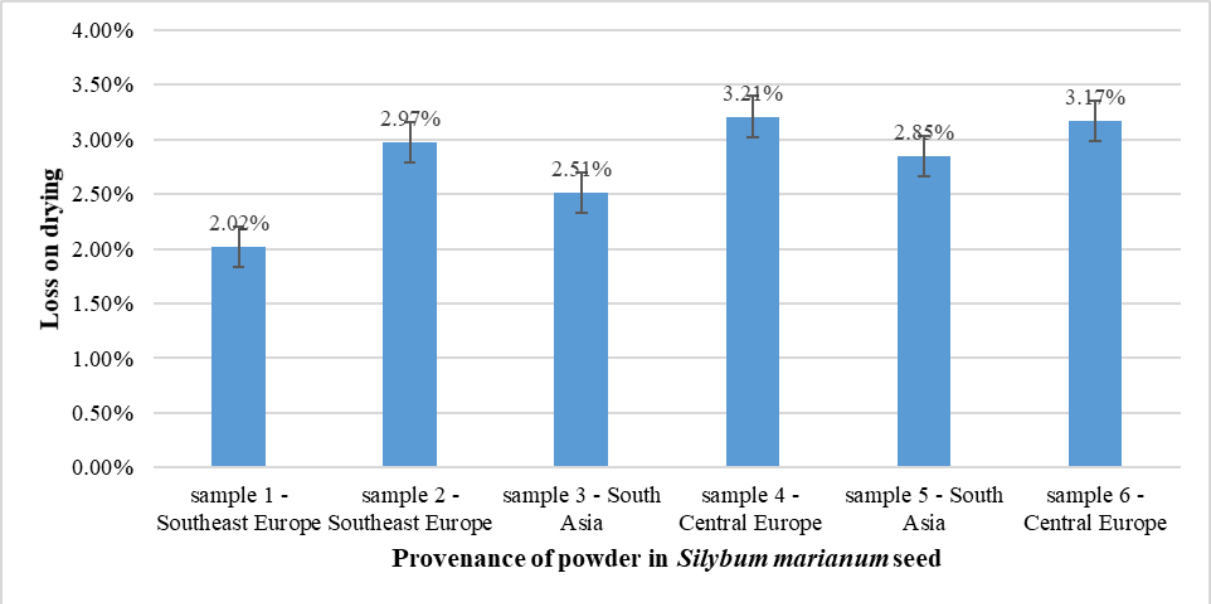

Fig. 2. Loss on drying of silymarin extract

Following the determination of the drying loss, figure 2, visible differences were found between the determined samples. Eligibility conditions: Loss on drying [%], - Maximum 7% [19].

For sample **4** and sample **6** in *Central Europe*, the highest values of drying loss were obtained, respectively 3.17% for sample **6** and 3. 21% for sample **4**.

This is directly related to the results from the parameter dosage. The amount of silymarin (%) being the lowest, figure 3, respectively 89.5% for the silymarin extract obtained for sample **6** and 85.62% for the silymarin extract obtained from sample **4**. Eligibility conditions: Silymarin dosage [%] (relative to dry matter) = Minimum 80% [19].

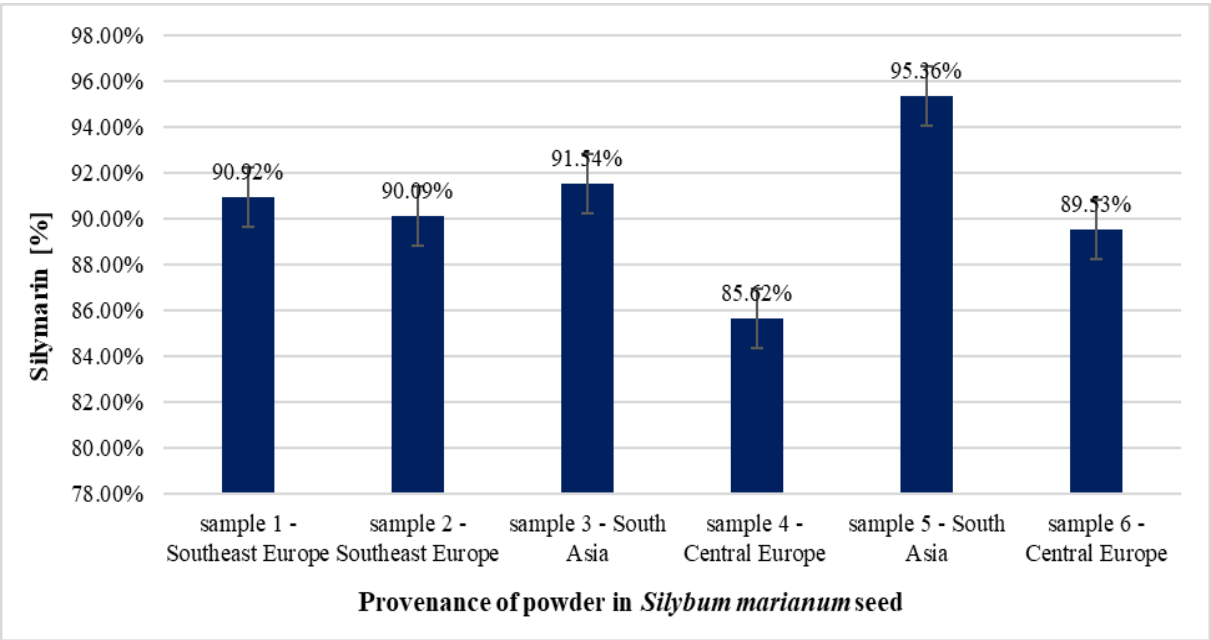

Fig. 3. Dosing of silymarin from powder of *Silybum marianum* seed

From this, we can conclude that in countries where the climate is richer in rainfall, it directly influences the amount of active substance in plant material.
For this reason, when we talk about cultivated medicinal plants, the interaction between vegetation factors must be taken into account, so as to ensure an optimal ratio between the pedoclimatic conditions and the biological requirements of the medicinal plants.
Situations where climatic conditions generally lead to an increase in the production of plant biomass, which is not important for the cultivation of medicinal plants, to the detriment of the content of active substances, should be avoided. Of interest to medicinal plants is the amount of active substances.

4. Conclusion

The flavonolignans silybin, silydianin and silychristin are the most active of the three flavonolignans that make up silymarin. We measured the quantity of total silymarin in Silybum marianum powder from diverse geographical areas in this study. The content of silymarin varied depending on the geographic area, according to the findings.
Quantitative dosing by UV method of the silymarin extract existing in the samples from the geographical areas analyzed falls within the parameters described in the European Pharmacopoeia (admissibility conditions > 80%).
Silymarin extracts obtained from areas richer in precipitation are quantitatively poorer in silymarin compared to areas with higher temperatures and less precipitation.

5. References

[1] ROMAN GH. V., TOADER M., EPURE L.I., ION V., BASA A. GH., *Cultivarea plantelor medicinal și aromatice în condiţiile agriculturii ecologice***,** Editura Ceres, București, (2009);
[2] BOJOR O., POPESCU O., *Fitoterapie tradițională și modernă*, Ediția a III-a, Editura Fiat Lux, București, (2005);
[3] ILEA (ZĂVOI) S. V., *Caracterizarea unor plante medicinale autohtone şi a unor bioproduse derivate cu acţiune hepatoprotectoare*, Rezumat al tezei de doctorat, Cluj-Napoca, (2011);
[4] ILIE T., MINOIU M., *Plante medicinale miraculoase din flora României*, Editura ARTMED, București, (2004);
[5] SHAKER E., MAHMOUD H., MNA S., Silymarin the antioxidant component and *Silybum marianum* extracts prevent liver damage, *Food and Chemical Toxicology*, 48, 803–806, (2010);
[6] STOICA V., CĂPRARU S., PIŢIGOI D., IONICĂ C., DICULESCU M., Evaluation of clinical and biological effects of silymarin versus silymarin +astragalus in chronic liver disease, *Practica farmaceutic*, 7(2), (2014);
[7] MUSTEAŢĂ G., Armurariu – *Silybum marianum* (*L.*) *Gaerth*, *Plante medicinale valoroase*, Chişinău, p. 6-11, (2002);
[8] AZIZ M., SAEED F., AHMAD N., AHMAD A., AFZAAL M., HUSSAIN S., MOHAMED A. A., ALAMRI M. S., ANJUM F. M., Biochemical profile of milk thistle (*Silybum Marianum* L.) with special reference to silymarin content, *Food Science&Nutrition*, 9 (1), 244-250, (2021), https://doi.org/10.1002/fsn3.1990;
[9] OMAR A.A., HADAD G.M., BAD RJ.M., First Detailed Quantification of Silymarin Components in the Leaves of *Silybum marianum* Cultivated in Egypt during Different Growth Stages, *Acta Chromatographica*, 2(43), 463–474, (2012), https://doi: 10.1556/AChrom.24.2012.3.9;
[10] KURKIN V. A., Antioxidant Activity of Flavolignans from *Silybum marianum (L). Gaertn*. Fruits, *Research and Reviews: Journal of Pharmacognosy and Phytochemistry*, 4(1), (2016);
[11] KSHIRSAGAR A., INGAWALE D., ASHOK P., VYAWAHARE N., Silymarin: A Comprehensive Review, *Phcog Rev*., 3(5), 126-134, (2009);
[12] SURAI P. F., Silymarin as a Natural Antioxidant: An Overview of the Current Evidence and Perspectives, *Antioxidants*, 4, 204-247, (2015), https://doi:10.3390/antiox4010204;
[13] ISHII Y., OKADA Y, MATSUOKA S., SHEN M., YUI K., YAGI M., YONEI Y., Effect of supplement containing *Silybum marianum* extract, soy extract, collagen peptide, bifidobacteria and apple extract on skin: A randomized placebo-controlled, double-blind, parallel group comparative clinical study, *Glycative Stress Research*; 3(3): 156-171, (2016);
[14] ZHU S. Y., JIANG N., TU J., YANG J., ZHOU Y., Antioxidant and Antiaging Activities of *Silybum Marianum* Protein Hydrolysate in Mice

Treated with D-galactose, *Biomed Environ Sci*, 30(9): 623-631, (2017);
[15] DIXIT N., BABOOTA S., KOHLI K., AHMAD S., ALI J., Silymarin: A review of pharmacological aspects and bioavailability enhancement approaches, *Indian J. Pharmacol*, 39(4), 172-179, (2007);
[16] Metodă dezvoltată și validată de Departamentul de Controlul Calității, Laropharm ce se desfășoară la standardele cerute de Regulile de Bună Practică de Fabricație și a Regulilor de Bună Practică de Laborator, - Method developed and validated by the Quality Control Department, Laropharm, which is carried out to the standards required by the Rules of Good Manufacturing Practice and the Rules of Good Laboratory Practice (2018), https://www.laropharm.ro/servicii/controlul-calitatii
[17] ELWEKEEL A., ELFISHAWY A., ZIDS. A., Silymarin content in *Silybum marianum* fruits at different maturity stages, *Journal of Medicinal Plants Research*, 7(23), 1665-1669, (2013);
[18] GIANNAKOUDAKIS D.A., HOSSEINI-BANDEGHARAEI A., TSAFRAKIDOU P., TRIANTAFYLLIDIS K.S., KORNAROS M., ANASTOPOULOS I., Aloe vera waste biomass-based adsorbents for the removal of aquatic pollutants: a review. *J Environ Manag*, 227:354–364, (2018), https://doi.org/10.1016/j.jenvman.2018.08.064;
[19] European Pharmacopoeia (Ph. Eur.) 10th Edition. https://www.edqm.eu/en/european-pharmacopoeia-ph-eur-10th-edition (accessed on 1.09.2021).