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Recent advances in the analysis of flavonolignans of *Silybum marianum*

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In memory of Prof. Dr. Szabolcs Nyiredy (1949–2006)

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ABSTRACT

Extracts of milk thistle (*Silybum marianum*, Asteraceae) have been recognized for centuries as remedies for liver and gallbladder disorders. The active constituents of milk thistle fruits are flavonolignans, collectively known as silymarin. Flavonolignans in *S. marianum* are structurally diverse, 23 constituents have been isolated from purple- and white-flowering variants. Flavonolignans have a broad spectrum of bioactivities and silymarin has been the subject of intensive research for its profound pharmacological activities. Silymarin is extracted from the seeds, commercialized in standardized form, and widely used in drugs and dietary supplements. The thorough analysis of silymarin, its constituents and silymarin-containing products has a key role in the quality control of milk thistle-based products. Due to the low concentration of analytes, especially pharmacological and pharmacokinetic studies require more and more selective and sensitive, advanced techniques.

The objective of the present review is to summarize the recent advances in the chemical analysis of *S. marianum* extracts, including the chemical composition, isolation and identification of flavonolignans, sample preparation, and methods used for qualitative and quantitative analysis. Various analytical approaches have been surveyed, and their respective advantages and limits are discussed.

1. Introduction

Due to its remarkable biological activities, milk thistle [*Silybum marianum* (L.) Gaertn., Asteraceae] is an important medicinal plant. The fruits have been used for over 2000 years as a remedy for several diseases, especially for liver and biliary tract disorders. The special milk thistle extract, silymarin, stimulates the liver regeneration, and its constituents act as antioxidant, anti-inflammatory and hepatoprotective agents, and therefore are effective in the treatment of mushroom (*Amanita* sp.) poisoning, hepatitis, cirrhosis and fibrosis of the liver. In addition, milk thistle fruit extracts have antiviral [1] and antitumor [2] activities and their constituents are under intense research in the clinical therapy of cancer for chemoprevention, treatment, and amelioration of chemotherapy-associated side effects. Milk thistle preparations are safe, well tolerated, and cause no serious side effects except mild gastrointestinal and allergic reactions [3].

Several clinical trials have been conducted with milk thistle extracts, standardized to flavonolignans, on patients with liver diseases of different origin. The majority of the studies showed improvement in clinical aspects of the disease but with no changes in laboratory parameters after treatment with silymarin [4–6]. The most recent Cochrane review, including 13 randomised clinical trials assessed milk thistle in 915 patients with alcoholic and/or hepatitis B or C virus liver diseases. Liver-related mortality was significantly reduced by milk thistle in all trials; however, the authors draw the attention to the lack of good quality clinical trials. The number of participants was usually low, only 23% of the trials reported adequate allocation concealment and only 46% were considered adequately double-blinded. Moreover, in none of the randomised clinical trials was an intention-to-treat method used to evaluate the data [7]. The importance of silymarin is stressed by the fact that it is a safe, effective and aspecific tool in the therapy of liver impairments of different origin. Recently there is a growing interest in the potential targeted application of silymarin (or its constituents) in patients with hepatitis C infection. In a clinical study, intravenously administered silybin was well tolerated and showed a substantial antiviral effect against hepatitis C virus, since the viral load decreased dose-dependently [8].

Abbreviations: CC, column chromatography; HPLC, high pressure liquid chromatography; IT, ion trap; LPLC, low pressure liquid chromatography; MPLC, medium pressure liquid chromatography; MRM, multiple reaction monitoring; NP, normal phase; Q-TOF, quadrupole-time-of-flight; RP, reversed phase; SQ, single quadrupole; TOF, time-of-flight; TLC, thin-layer chromatography; VLC, vacuum liquid chromatography.

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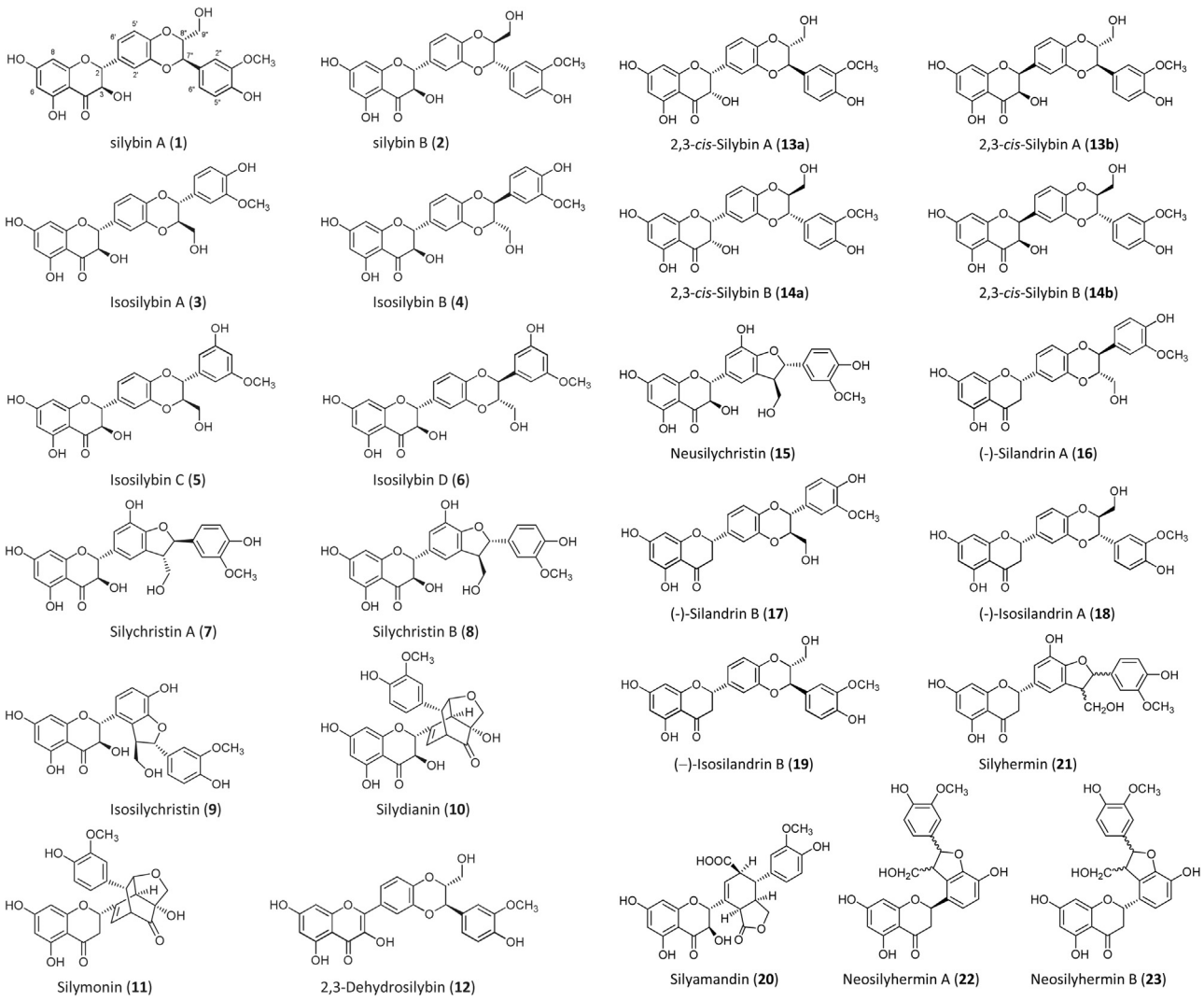

Fig. 1. Chemical structures of flavonolignans from *S. marianum*.

In modern therapy, standardized silymarin products are used. According to the European Pharmacopoeia, the nominal silymarin content of the refined and quantified milk thistle dry extract is within the range of 30–65%. The content of silymarin corresponds to: a) sum of the contents of silychristin and silydianin 20–45%, b) sum of the contents of silybin A and silybin B 40–65%, c) sum of the contents of isosilybin A and isosilybin B 10–20%. The extract should contain 90–110% of the normal content of silymarin, expressed as silybin [9]. An important challenge in the manufacturing of silymarin-based products is the poor aqueous solubility of flavonolignans. Attempts to improve bioavailability include the formulation of salts, glycosides, complexes, microspheres, nanoemulsions, nanosuspensions, nanocrystal and microemulsion systems, liposomes, emulsomes, cerasomes, dendrimers or carbon nanotubes [10].

Compounds of silymarin belong to the chemical class of flavonolignans. This widely used term is misleading because flavonolignans are not composed of a flavonoid and a lignan unit. Lignans are a group of phenolic secondary plant metabolites that are formed by oxidative coupling of two phenylpropanoid units (C_6C_3). In classical lignans the C_6C_3 units are linked through the central carbons (β) of their side chains. Neolignans are also condensation products of phenylpropanoid units, but the actual bond varies and involves no more than one β carbon of the C_3 parts. Flavonolignans, termed as “non-conventional lignans” or “hybrid lignans”, are biogenetically related to lignans and neolignans as they have similar biosynthetic pathways. Formally, flavonolignans are derived from two phenylpropanoid units, but have an additional structural part that places them also under the flavonoids. Flavonolignans have a broad structural diversity in consequence of the C—C or C—O linkage of the C_6C_3 unit to the flavonoid nucleus in different positions, affording dioxane, furan, cyclohexane rings or simple ether side chains. In general, these compounds contain several chiral centres, hence they usually occur in the form of stereoisomers in nature. Flavonolignans are characteristic compounds of Asteraceae, Berberidaceae, Chenopodiaceae, Flacourtiaceae, Fabaceae, Poaceae, and Scrophulariaceae species. The first known source of flavonolignans was milk thistle (*S. marianum*). Flavonolignans isolated from its fruits are the most important, biologically active constituents of the plant; to date 23 compounds were identified from this species (Fig. 1) [11,12].

Numerous scientific papers have been published on the chemistry, pharmacology, clinical efficacy, safety, bioavailability, pharmacokinetics and chemical analysis of milk thistle extracts, and several review articles dealing with different aspects of *S. marianum* and its flavonolignans have also been published. The majority of recently published reviews assessed the pharmacology, chemistry and clinical studies of milk thistle (Table 1). No previous survey