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The silymarin composition… and why does it matter???

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ABSTRACT

The extract from milk thistle (*Silybum marianum* (L.) Gaertn. (Asteraceae)), known as silymarin, contains a variety of flavonolignans and displays antioxidant, anti-inflammatory, immunomodulatory and hepatoprotective properties. As silybin is the main component of silymarin, the literature mainly focuses on this compound, ignoring all other components. This leads to problems in reproducibility of scientific results, as the exact composition of silymarin is often unknown and can vary to a certain degree depending on the processing, chemovariety of the plant used and climatic conditions during the plant growth. There are studies dealing with the analytical separation and quantification of silymarin components as well as studies focused on silymarin content in clinically used drugs, in various plant parts, seasons, geographic locations *etc*. However, no comparison of detail flavonolignan profiles in various silymarin preparations is available to date. Also, as a result of the focus on the flavonolignans; the oil fraction, which contains linoleic, oleic and palmitic acids, sterols, tocopherol (vitamin E) and phospholipids, has been neglected. Due to all these factors, the whole plant is used *e.g.* as animal feed, the leaves can be eaten in salads and seed oil, besides culinary uses, can be also utilized for biodiesel or polymer production. Various HPLC separation techniques for the determination of the content of the flavonolignans have been vastly summarized in the present review.

Chemical compounds mentioned in this article

Silybin (PubChem CID: 31553)
Silymarin (PubChem CID: 7073228)
Silydianin (PubChem CID: 11982272)
Silychristin (PubChem CID: 441764)
Taxifolin (PubChem CID: 439533)
Isosilybin (PubChem CID: 3085830)
Oleic acid (PubChem CID: 445639)
Linoleic acid (PubChem CID: 5280450)
Palmitic acid (PubChem CID: 985)

1. Introduction

Silybum marianum (L.) Gaertn. (Asteraceae) is an annual or a biennial herb native to the Mediterranean and North African regions (Khan, Blackshaw, & Marwat, 2009). The plant is called by different names such as Marian thistle, Mary thistle, blessed milk thistle, or milk thistle (AbouZid & Ahmed, 2013) and many others in different languages (Table 1).

The origin of the name comes from a legend that when the Virgin Mary was sheltering under a bower of thorny leaves from the milk thistle, a drop of the Virgin Mary's milk fell whilst she was nursing the infant Jesus, causing the distinctive white veins on the leaves.

Milk thistle was already used by ancient Greek and Roman physicians, each called the plant by different names. Dioscorides' named it ‘silybon’, Theophrastus ‘pternix’ and Pliny the Elder called it ‘silybum’. For Dioscorides the preparation of tea was good “for those that be bitten of serpents”, whilst Pliny the Elder wrote that the juice of the plant mixed with honey was good for “stimulating the flow of bile”.

There exist two varieties of *S. marianum*; a purple flowering variety, which contains flavonolignans (Fig. 1) having a hydroxyl group in position C-3, so called taxifolin-derived flavonolignans (Biedermann, Vavříková, Cvak, & Křen, 2014) and the white flowering variety of milk thistle containing non-taxifolin derived flavonolignans (Chambers, Valentová, & Křen, 2015). The white variety has been overlooked and

Abbreviations: ADME, absorption, distribution, metabolism, and excretion; ASTM, American Society for Testing and Materials; NHDF, normal human dermal fibroblasts; NHEK, normal human epidermal keratinocytes; PE, petroleum ether; LC-MS, liquid chromatography–mass spectrometry; HPLC-UV, high-performance liquid chromatography-with ultraviolet detector; HSCCC, high-speed counter-current chromatography; UHPLC, ultra-performance liquid chromatography; SCH, silychristin; SD, silydianin; SB-A, silybin A; SB-B, silybin B; isoSB-A, isosilybin A; isoSB-B, isosilybin B; TX, taxifolin

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Table 1
Names of *Silybum marianum* in different languages.

Language	Name	Language	Name
Arabic	سيليبوم ماريانوم [sialibum marianum]	Hindi	दुग्ध रोम [dugdh rom]
Armenian	կաթ ուղտափուշ [kat' ughtap'ush]	Hungarian	máriatövis
Bulgarian	бял трън [byal trŭn]	Italian/Spanish /Portuguese	cardo mariano
Catalan	Card marià	Japanese	オオアザミ [ooazami]
Chinese	薊 [Nǎi jì]	Latin	Lac Carduus
Croatian	mlijeko čička	Polish	ostropest plamisty
Czech	ostropestřec mariánský	Romanian	armurariul
Danish	mælk tidsel	Russian	Молочный чертополох [molochnyy chertopolokh]
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English	milk thistle	Serbian	Расторóпша пятни́стая [rastorópsha pyatnístaya]
Finish	maarianhohdake	Slovak	mlieko bodliak
French	Chardon-Marie	Swahili	maziwa mbigili
German	Mariendistel	Swedish	mariatistel
Greek	γαϊδουράγκαθο γάλα [gaïdouránkatho gála]	Turkish	Süt dikeni
Hebrew	גדילן מצוי [dilan mazui]	Vietnamese	cây kế sữa

much research has centered on the purple variety. Besides this, silybin B (110 mg/1500 g) was also isolated from dried whole plants of *Gentiana apiata* N.E. Br. (Gentianaceae) from Qinling Mountain, Shaanxi Province, China (Zhou et al., 2009).

Silymarin flavonolignans from the purple variety of *S. marianum* are derived from taxifolin and coniferyl alcohol by one of two plausible mechanisms: a) the traditional Freudenburg's hypothesis and b) Althagafy proposal (Fig. 2). Freudenburg's hypothesis (a) suggests a radical oxidative coupling of a 3′,4′-dihydroxy group of the dihydroflavonol B-ring of taxifolin with the radical of coniferyl alcohol. A one-electron free radical of taxifolin is formed which then reacts with a free radical of coniferyl alcohol to provide the intermediate, which cyclizes through attack of the phenol nucleophile on the quinone methide system to provide the two *trans* diastereomeric flavonolignans, called silybins A and B (Dewick, 2009; Merlini, Zanarotti, Pelter, Rochefort, & Hansel, 1980; Schrall & Becker, 1977). However, Althagafy (b) suggests that a single radical from coniferyl alcohol is formed. This radical reacts with the 3′,4′-dihydroxy group of the dihydroflavonol B-ring of taxifolin and after the further oxidization the silymarin components are formed (Althagafy, Meza-Aviña, Oberlies, & Croatt, 2013). Since both proposed mechanisms are radical they are typically non-regio and non-stereoselective, which results in the immense variety of regio- and stereomers formed. Yet, the conformations at C10-C11 positions are characteristically *trans,* which suggests this to be thermodynamically preferred configuration.

The seeds (botanically correct: fruits – achenes, cypselae – contain only single seed; for simplicity, this work will use the term "seed") (Biedermann et al., 2014) from the purple milk thistle contain a mixture of compounds, especially flavonolignans and polymeric polyphenols commonly known as silymarin (Graf, Cech, Polyak, & Oberlies, 2016; Kuki et al., 2012; Rainone, 2005; Wallace, Carrier, Beitle, Clausen, & Griffis, 2003) and lipids (Khan, Khattak, Ullah, & Bangash, 2007). Silymarin flavonolignans have numerous biological activities such as antioxidant, anti-inflammatory and immunomodulatory effects that are connected with its hepatoprotective properties (Polyak et al., 2010). The extract from milk thistle is effective in the treatment of liver diseases such as acute and chronic viral, drug- or toxin-induced and alcoholic hepatitis (Flora, Hahn, Rosen, & Benner, 1998). Silymarin flavonolignans act through multiple molecular mechanisms that could block all stages of carcinogenesis (initiation, promotion and progression) resulting in their anticancer effect. The combination of chemotherapy with silymarin treatment reduced the toxicity associated with chemotherapy both *in vitro* and in clinical trials (Ramasamy & Agarwal, 2008) and usually does not compromise chemotherapeutical activity.

The main component of silymarin is silybin (which is in fact a quasi-equimolar mixture of diastereoisomers A and B), and due to this fact biological activity of the whole complex extract is often assigned to this compound. Another reason why silybin is so popular is because of its very easy method of isolation from silymarin: ethanol precipitation from acetone silymarin extract (Biedermann et al., 2014). However, the other flavonolignans in silymarin could contribute to or even be responsible for distinct beneficial effects of silymarin. For example, only silybin B and taxifolin were found to stimulate an estrogen responsive reporter plasmid construct in T47D breast cancer cells *in vitro* (Plíšková et al., 2005). Isosilybin B was consistently the most potent suppressor of cell growth of prostate carcinoma cells in culture (Davis-Searles et al., 2005). The inhibitory potency of pure silybin A and silybin B toward cytochrome P450 2C9 was superior to that of isosilybin A and isosilybin B (Brantley, Oberlies, Kroll, & Paine, 2010). On the other hand, isosilybin B was the most potent inhibitor of cytochrome P450 3A4 in human liver microsomes and human intestinal microsomes, followed by silychristin (Biedermann et al., 2016), silybin B, and silybin A; isosilybin A was almost inactive (Brantley, Graf, Oberlies, & Paine, 2013). *In vivo*, entirely different metabolic profiles of optically pure silybin A and silybin B (administered *p.o.*) in rat plasma were observed (Marhol et al., 2015) and 2,3-dehydrosilybin inhibited basal cell carcinoma allograft tumor growth more than silybin in mice (Tilley et al., 2016). A recent seminal study with optically pure 2,3-dehydrosilybin A and B demonstrated clearly superiority of 2,3-dehydrosilybin A over its isomer B in promotion of lifespan extension of the worm *Caenorhabditis elegans* (Filippopoulou et al., 2017). Studies with pure diastereoisomers