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Silymarin from *Silybum marianum* by Naviglio's extractor: a new and very efficient approach

Anna De Marco[a], Giovanni Luongo[b], Cinzia Di Marino[b], Gaetano De Tommaso[b], Giovanni Di Fabio[b] and Armando Zarrelli[b]

[a]Department of Biology, University of Naples "Federico II", Naples, Italy; [b]Department of Chemical Sciences, University of Napoli "Federico II", Naples, Italy

ABSTRACT

The aim of this work is to compare new and traditional extraction methods to obtain silymarin from *Silybum marianum*, a biennial herbaceous plant of the Asteraceae family, present throughout the Mediterranean basin and used to treat several diseases. Silymarin primarily contains flavonolignans and flavonoids and is used in some pharmaceutical preparations to improve of liver function and as a protective against some hepatotoxins. In six extracts obtained by new and traditional extraction methods, the total contents of silymarin and its main flavonolignans, total phenols and condensed tannins were evaluated in addition to their respective antioxidant capacities. By the Naviglio extractor, that is a rapid solid-liquid dynamic extraction method, it is possible to obtain a fraction quantitatively more abundant than other methods, and with a lower content of tannins and phenolic compounds but with a higher content of flavonolignans, rare and expensive, and therefore easier to separate and purify.

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CONTACT Armando Zarrelli zarrelli@unina.it

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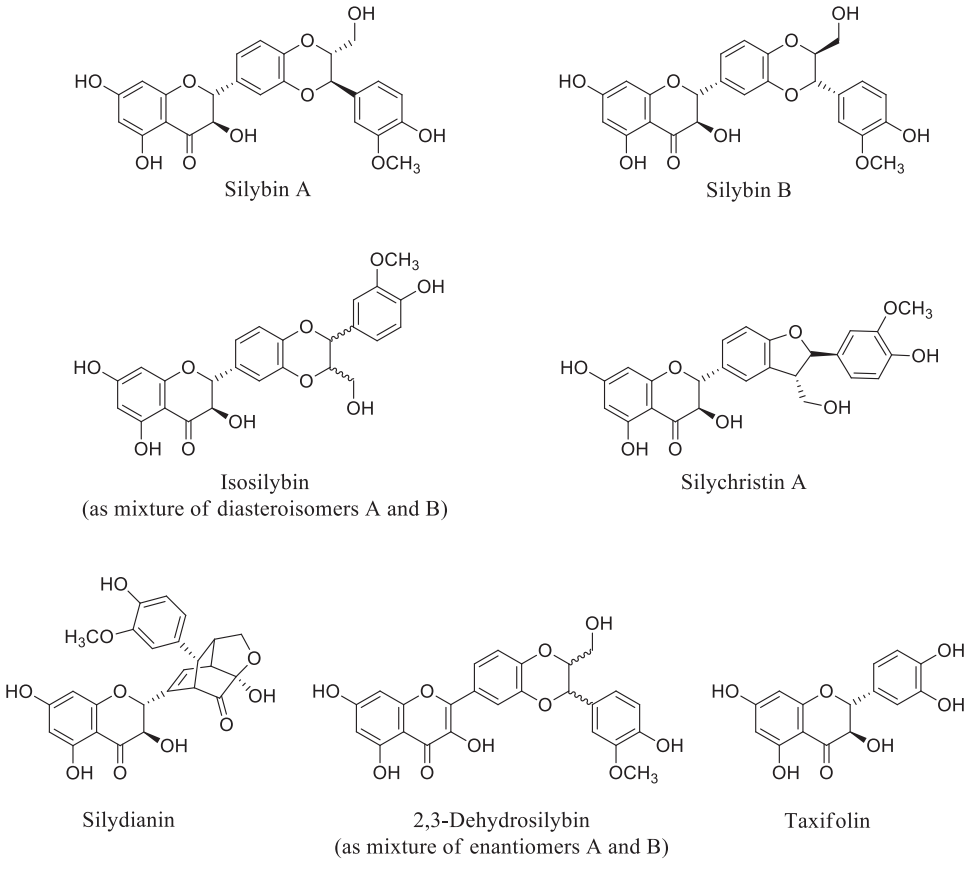

Figure 1. Main extracted compounds from *S. marianum* [L. Gaertn. (Asteraceae)].

1. Introduction

Milk thistle (*Silybum marianum*) is a biennial herbaceous plant of the Asteraceae family which is present throughout the Mediterranean basin (Bijak 2017). Native to Mediterranean countries, Southern Russia and North Africa, this plant is naturalized in California and in the Eastern United States, where it spontaneously thrives in dry and hot desert areas and can also be grown in gardens. Milk thistle seeds have been used for more than 2,000 years to treat hepatitis, cirrhosis, and jaundice and to protect the liver against poisoning from environmental chemicals and natural toxins, including snake and insect bites, fungal poisoning and alcohol. This plant's seeds contain 1.5–3% of a mixture of flavonolignans collectively referred to as Silymarin (Gaertner and Mariusz 2015). A standardized extract may be obtained from the seeds of *S. marianum,* containing 40–80% of flavonolignans and approximately 60–20% of a chemically indefinite fraction, formed in large part by oxidized polymeric compounds and polyphenols. The major component of the silymarin complex is silibinin, a mixture of two diastereomers, silybin A and silybin B, in a respective 45:55 ratio (Di Fabio et al. 2013). The additional most abundant flavonolignans present are the isosilybins A and B, silychristin A and silydianin, some flavonoids, primarily taxifolin (Figure 1) and trace of 2,3-dehydrosilybin (Di Fabio et al. 2013).

Silibinin is an active component in some pharmaceutical preparations (Silymarin - Forte™, Legalon™) which is widely used in therapies for the improvement of liver function and as a hepatoprotective agent against some hepatotoxins (Federico et al. 2017). In the last ten years, silibinin has received particular attention thanks to its alternative beneficial activities, which are not directly bound to its hepatoprotective and/or antioxidant effects (Federico et al. 2015; Zhu et al. 2016). In fact, recent studies have shown that silibinin is able to reduce the proliferation of tumour cells of various kinds (prostate, ovary, breast, lung, skin and bladder) by inhibiting a series of proteins involved in such processes as gene expression, mitosis, differentiation, proliferation and cell survival.

Unfortunately, the bioavailability and the therapeutic efficacy of silibinin are rather limited by its scarce solubility in water (ca 400 μg/L), which is also the limiting factor of studies aimed at understanding the mechanisms of action (Romanucci et al. 2017). For this reason, various derivatives have been synthesized, which are considerably more soluble and equally active (Zhang et al. 2008; Wang et al. 2009; Theodosiou et al. 2011; Zarrelli et al. 2011, 2013, 2014). In most of the published manuscripts, aspects such as the optical purity of silibinin and of other flavonolignans of the silymarin complex are neglected. However, when silibinin is used for different applications, not just as a mere antioxidant in an isotropic environment, stereochemistry plays an extremely important role and the respective biological activities would be evaluated with respect to the optically pure metabolites (Biedermann et al. 2016). In fact, relatively few data exist regarding the pharmacological activity of its two components, the diastereomeric silybins A and B, but it has recently been shown that silybin B interacts with the oestrogenic receptor while its diastereomer silybin A is correspondingly inactive (Sciacca et al. 2017). Continuing our studies dedicated to the isolation of secondary metabolites from plants of the Mediterranean area (Cutillo et al. 2004; Fiorentino et al. 2007), and to the evaluation of their properties in the function of possible applications (Della Greca et al. 2003, 2007), the aim of the current study was to evaluate the total content of silymarin and its main flavonolignans, phenols and condensed tannins in three ethanolic extracts and three aqueous extracts obtained from *S. marianum* with a new generation method based on rapid solid-liquid dynamic extraction (RSLE), using a *Naviglio* extractor (Naviglio 2003; Biagi et al. 2014; Caprioli et al. 2017; Gigliarelli et al. 2017; Daliu et al. 2019), and three traditional methods, such as maceration (ME), decoction (DE) and Soxhlet (SE) extractions. The literature shows numerous publications concerning isolation of silymarin with novel extraction techniques, both for laboratory and industrial applications, inter alia: microwave assisted extraction (Zheng et al. 2009), enzyme assisted extraction (Qiao et al. 2011), pressurized hot water (Engelberth et al. 2008), pressurized liquid extraction (Wianoswska and Wisniewski 2014), and supercritical CO_2 (Rahal et al. 2015).

However, the use of these techniques presupposes the use of expensive and not always available equipment as well as not rarely rather long extraction times. Therefore, it would be useful to develop a new extraction process to overcome these limitations, for applications on small or large scale.

The *Naviglio* extractor, small in size, compact, easy to use and affordable, allows the acquisition of abundant fractions, in short times and almost devoid of lipid components without the necessity of a preliminary defatting stage, which is necessary in the

case of traditional methods. With the *Naviglio* extractor, such solvents as water and ethanol are used instead of organic solvents, which should then be disposed of, thereby obtaining fractions that are compatible with subsequent nutraceutical and/or pharmaceutical applications.

2. Result and discussion

A preliminary analysis was performed to quantify the mass fraction and the silymarin obtained from the different extraction techniques, in addition to major constituents, such as silybins A and B, silychristin A, silydianin, isosilybin, taxifolin (Figure S2 and Table S1 in the Support Informations), which were identified by an LC/MS/MS technique applied to each fraction.

The masses extracted range between 2.6 and 5.5% of the dry mass of the plant. The lowest value is related to the aqueous decoction (H_2O/DE), while the highest value is associated with the ethanolic extraction derived by maceration (EtOH/ME). The amount of silymarin, on the other hand, ranges between 1,095 mg of the aqueous fraction obtained by means of the *Naviglio* extractor (H_2O/RSLE) and 1,452 mg of the ethanolic fraction obtained by maceration (EtOH/ME). In particular, then, the ethanolic fraction obtained by the *Naviglio* extractor (EtOH/RSLE) was the one containing the smallest amount of taxifolin (5.3%), almost the same as the aqueous fraction obtained with the same technique (H_2O/RSLE) (5.4%) and almost half of the content in the ethanolic fraction obtained by Soxhlet (EtOH/SE). In contrast, the ethanolic fraction obtained by the Naviglio extractor (EtOH/RSLE) was the one containing the highest quantity of silybins A and B (18.1 and 20.0%, respectively). The aqueous fractions obtained by the same technique (H_2O/RSLE) (8.3 and 7.8%, respectively) and the aqueous fractions obtained by maceration (H_2O/ME) (6.8 and 8.2%, respectively) are those that contained less silybins. Similarly, the content of silychristin A, silydianin and isosilybin was always higher in the ethanolic fraction obtained from the Naviglio extractor (EtOH/RSLE), with percentages of 14.5, 7.6 and 3.5, respectively, at least 50% more than all the others fractions.

Therefore, the results demonstrate how the rapid liquid-solid extraction (RSLE) can provide a fraction quantitatively comparable in mass to that obtained with ethanol by maceration (EtOH/ME), the most abundant, and much more abundant than aqueous fractions obtained by decoction and maceration, on the other hand in very short times (approximately 40 min vs. hours). In particular, the fraction EtOH/RSLE contains the same quantity of silymarin of the other fractions, but richer in precious flavonolignans (consider that the diastereoisomeric mixture of the two silybin isomers is commercially available at a cost of nearly 350 euros/25 mg), and poorer with respect to flavonoids such as taxifolin and condensed tannins, which make the process of purification of the themselves flavonolignans difficult, long and expensive.

3. Conclusions

In conclusion in this work four extraction methods (decoction, Soxhlet extractor, maceration and the *Naviglio* extractor) were compared to obtain silymarin complex

from *S. marianum*. The last two methods offer undoubted advantages in terms of mass of the extracted fractions and relative quantities of some their precious components. In particular, the *Naviglio* extractor allows extraction times very short, just over half an hour instead of at least 72 h and without the use of solvents, that facilitates the use of raw fraction without further manipulation and also reducing toxicity and risks for both operators and environment. In addition, the fraction obtained by the *Naviglio* extractor presents the same amount of silymarin contained in the infusion by maceration and much more than the infusions obtained by Soxhlet and decoction. The low content of tannins and taxifolin, less than 67% of that present in silymarin obtained by maceration, favors the purification of the precious flavonolignans, for example silybins A and B, not only commercially available or if they are available at prohibitive prices.

Disclosure statement

No conflict of interest was reported by the authors.

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ORCID

Giovanni Luongo http://orcid.org/0000-0002-8308-9682
Giovanni Di Fabio http://orcid.org/0000-0003-2912-4827

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