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Silymarin content in *Silybum marianum* populations growing in Egypt

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ABSTRACT

Chemical variation of *Silybum marianum* growing in the north, middle, and south of Egypt was investigated. Variation was assessed according to the content of the individual silymarin components in the fruits of the plant. The fruits were distinguished according to location, plant variety, and fruit color (maturity). Accelerated solvent extraction was used to standardize the silymarin extraction. Quantitative analysis of the content of silymarin components was carried out using HPLC with qNMR-controlled reference standards of taxifolin and seven major flavonolignans including silybin A, silybin B, isosilybin A, isosilybin B, silychristin, isosilychristin, and silydianin. The quantification method was validated in accordance with ICH guidelines. Principal component analysis and hierarchical clustering were carried out to create homogeneous clusters of samples based on the content of the silymarin components. Taxifolin had the lowest correlation relative to other silymarin components, whereas silybin A was positively correlated with silybin B. The samples clustered into three classes: silydianin-rich samples, samples with an average silymarin content of <18.8 mg/g, and one class enriched in silymarin (>18.8 mg/g). *S. marianum* growing in the Nile delta showed the highest silymarin content. No correlation was found between fruit color and silymarin content, indicating that the fruit maturity stage has no significance.

1. Introduction

Milk thistle [*Silybum marianum* (L.) Gaertn., Asteraceae] is an annual or biennial herb, native to the Mediterranean and North African regions (Morazzoni and Bombardelli, 1995). The plant has an erect ridged stem that reaches to heights 150 cm and deeply lobed leaves characterized by white patches along its veins. The fruits of *S. marianum* are of achene type having shiny dark brown–black color. Two varieties of this species occur in Egypt; variety *purple* with purple corollas and variety *albiflorum* with white corollas (Boulos, 2002). The two varieties are growing wild along canal banks, roadsides, and waste ground. The plant is classified according to its ecological amplitude as a frequent species in the canal bank habitats of the Nile in Egypt (Mashaly et al., 2010).

Silymarin, in the form of a standardized extract prepared from milk thistle fruits, is widely used as hepatoprotective agent. Silymarin exerts its hepatoprotective activity through antiviral, anti-inflammatory, antioxidant, and immuno-modulatory actions in the liver and immune cells (Polyak et al., 2007; Ahmed-Belkacem et al., 2010; Morishima et al., 2010; Wagoner et al., 2010). Silymarin has also shown in vitro efficacy as a cancer chemopreventive agent by arresting human prostate carcinoma proliferation in cancer cell cultures (Tyagi et al., 2002) and in human cancer models (Singh et al., 2002). The name, silymarin, was originally introduced by Wagner et al. (1965) when describing the flavonolignans mixture as containing silybin, isosilybin, silydianin, and silychristin. Subsequently, this mixture has been further resolved into so far seven flavonolignans and one flavonoid (Fig. 1) that altogether comprise 65–80% of the milk thistle extract. Both silybin and isosilybin were subsequently resolved into pairs of diastereoisomers, silybin A & B and isosilybin A & B, respectively. The isosilybins A and B are regioisomers of silybin A and B, respectively. The absolute configurations of these four isomers studied using 2D NMR and CD spectroscopy (Kim et al., 2003).

In 2014, the plant was placed at the sixth position among the twenty top-selling herbal dietary supplements in the natural and health food market and position among the forty top-selling herbal supplements in the mainstream multi-outlet channel market in U.S., at about $9.2 million and $16,4 million, respectively (Smith et al., 2015). According to the European Pharmacopoeia and the United States National Formulary, mature fruits of *S. marianum* yield not less than 1.5–2% of silymarin.

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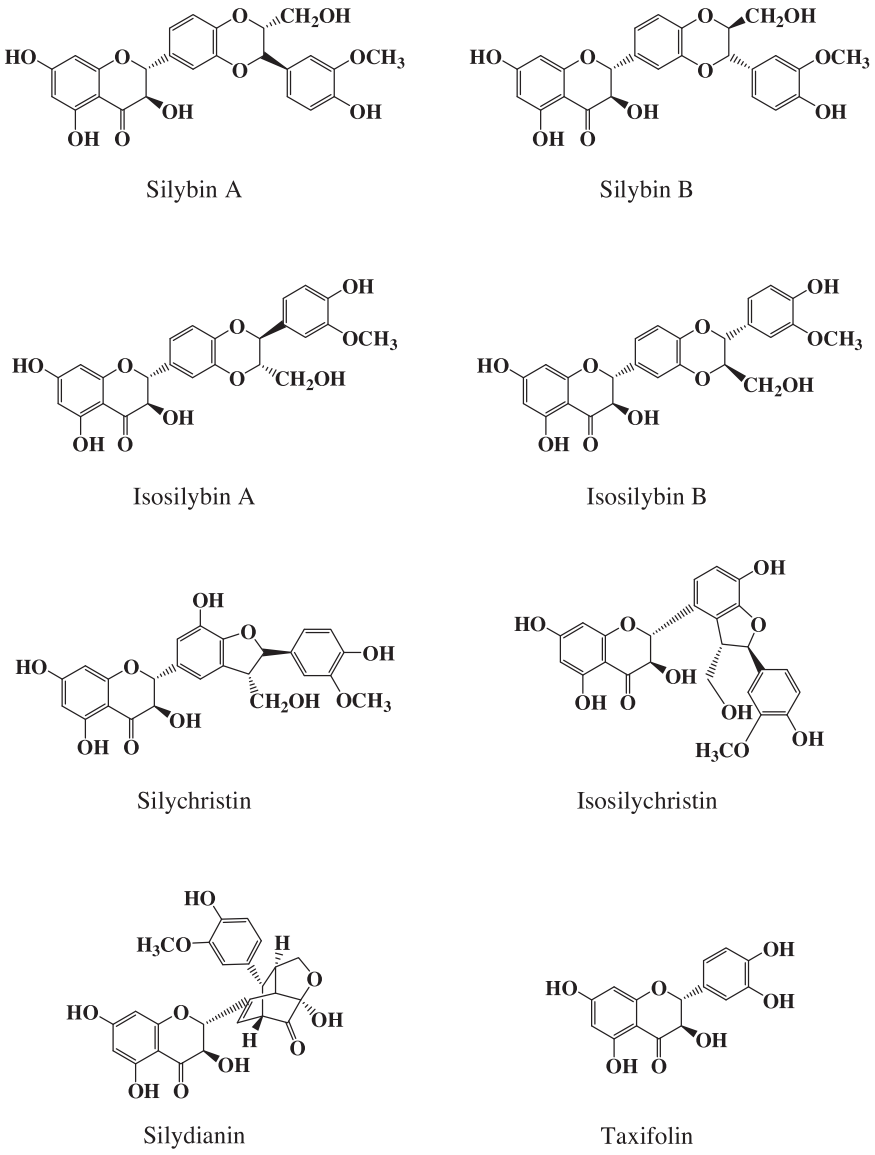

Fig. 1. Chemical structures of silymarin components.

It is well known that medicinal plants collected from different locations may considerably differ in their active constituents, resulting in potentially different therapeutic efficacy. These differences are caused by various environmental conditions and habitats in which the medicinal plants are grown. The plants develop locally adopted subpopulations, known as ecotypes (Shokrpour et al., 2007). Variation in the ecotypes can be characterized by chemical or genetic means (Mahmood et al., 2010; AbouZid, 2014). Analysis of chemical variation by chromatographic or spectroscopic provides a means of measuring and standardizing the metabolites responsible for the therapeutic effects of milk thistle.

The present study was aimed at differentiating wild populations of *S. marianum* growing in Egypt based on their silymarin content as a potential source for silymarin products. The study was also designed to determine whether links exist between the chemical variation and the location, plant variety, or fruit color as an indicator of maturity of the fruits.

2. Materials and methods

2.1. Chemicals

Silymarin (S0292-10G, Sigma, China), silybin primary reference standard 92.53% (HWI ANALYTIK GMBH pharma solutions, Rülzheim, Germany), and taxifolin analytical standard 85% (Sigma–Aldrich, St. Louis, MO, USA) were used as reference compounds. Isosilychristin, silychristin, and silydianin were prepared by preparative column chromatography and identified by ^{1}H NMR (Abraham et al., 1970; Diep et al., 2007; Napolitano et al., 2013). The solvents used in this study, *n*-hexane and acetone (Pharmaco-AAper, Brookfield, CT, USA) were of reagent grade and re-distilled. For analytical HPLC, methanol (Fisher Scientific, Fair Lawn New Jersey, USA), water and formic acid (Sigma–Aldrich, St. Louis, MO, USA) were of HPLC grade. Silica gel 60A was used for column chromatography (Fisher Scientific, New Jersey, USA, 200–425 mesh). Sephadex LH-20 (Sigma, Sweden) was used for column chromatography. Pre-coated silica gel plates ALUGRAM® SIL G/UV$_{254}$ for TLC (E-Merck, 10 × 20 cm) were used for analy-