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A PRELIMINARY INVESTIGATION OF FLAVANOLIGNANS OF SILYBUM MARIANUM (L) GEARTN FRUITS GROWING IN EGYPT

L. Dranik, L. Dolganenko, A. Grysodub, M. Levin and I.M. Eshami*

- All-Union Research Inst. of Drug Chem. and Technol., Kharkov, USSR.
** Department of Pharmacognosy, Faculty of Pharmacy,*
Tanta University, Tanta, Egypt

ABSTRACT

The flavanolignans of the fruits were isolated by column chromatography and identified by TLC and HPLC in addition to m.p., ms, uv and optical rotation measurements. A comparative study by TLC and HPLC of the flavanolignans from the purple-flowered and the white-flowered plants of Silybum marianum was performed. The major flavanolignans of the white flowered plants were identified as silybin, silychristin, silydianin and taxipholin. The major flovanolignans of purple-flowered plants were found to be silybin, silichristin and taxipholin.

INTRODUCTION

The fruits of Silybum-marianum (L.) Geartn, exert remarkable anti-hepatotoxic activities[1]. Silymarin, the major constituent of the fruits, consists of the isomers silybin, silydisanin and silychristin[2,3]. Silychristin inhibits damage induced by Amanita phalloids, pyrrolizidine alkaloids and some rare metals (e.g. praseodyminum). These flavanolignans appear in several popular products currently marketed in Europe and U.S.A. A German drug, Legalon, contains just silymarin, while the American Health Food Supplement, Arista Herb's liver cleanser, has the entire Silybum marianum (L.) Geartn fruits, combined with powdered beets, dandelion, golden-seal roots and black-radish seeds[3].

The genus Silybum of the family Asteracea (Compositae) includes two species, *S. marianum* (L.) Geartn and *S. eburneum* (Coss). Two varieties of *S. marianum* were also known, purple-flowered and white-flowered, both may have a large population, depending on the region of growth. The white-flowered and purple-flowered plants growing in Hungary and other localities of Europe was found to contain flavanolignans which differ qualitatively[4,5]. On the other hand, the Egyptian authors do not agree with this statement[7]. The varieties growing in Egypt were studied, the macromorphology of the capitula of both plants were found to be without any significant differences other than colours[7]. The full grown ripe fruits of the less common white-flowered variety were subjected to a study of the flavonoid contents. A preliminary TLC examination of the purified ethyl acetate extract using polyamide and cellulose sheets revealed the presence of seven flavonoid spots. The purple-flowered plants, however, were found to contain flavonoids from which five could be characterised (m.p.; chem. reactions, cochromatography, UV, IR) as silybin, silydianin, silychristin, taxifolin and quercetin[7]. Accordingly, the white-flowered and purple-flowered plants were considered to be similar, concerning their flavonoidal contents[7].

The aim of this work is the confirmation of this statement and comparison of the two varieties of

Silybum marianum (L.), regarding the flavanolignan contents of the fruits using HPLC methods.

EXPERIMENTAL

Plant material:

The fully grown mature but unripe fruits of the white-flowered and purple-flowered *S. marianum* (L.) Geartn., were obtained from the wild growing plants in the Nile Delta at the date of flowering and early fruiting in June 1988. The plant was kindly identified by Dr. Abdel Fattah Bader, Prof. of Taxonomy, Dep. of Botany, Faculty of Science, Univ. of Tanta, Egypt, and a voucher specimen of the investigated plants was kept at the museum of the Dep. of Pharmacognosy, Faculty of Pharmacy, Tanta University.

Isolation of flavanolignans:

The powdered plant material (fruits), was extracted with 80% ethanol and the flavanolignan mixture was isolated by the method developed by two of the authors[8]. Silybin and silydianin were isolated on column chromatography on polyamide, using gradient elution with ethanol-water mixture, while silychristin and taxifolin were silated by column chromatography on silica gel using a mixture of chloroform-ethanol (9:1) as an eluent.

Thin layer chromatography (TLC)

Using TLC Silufol (Czechoslovakia), solvent system: chloroform-acetone-formic acid (9:2:1).

High performance liquid chromatography (HPLC)

Instrument: Millichrom (USSR), column, Separon $C_{18}R_5$ 64X2 mm., mobile phase: water-acetonitrile-acetic acid (70:20:100 v/v), flow rate: 0.1 mm/min., temp.: 220°C, wavelength: 280 nm., paper speed: 5mm/min.

RESULTS AND DISCUSSION

The pure mixture of flavanolignans of the purple-flowered and white-flowered plants were separated using the known patented procedure[8]. The yield of flavanolignans separated from the fruits for the purple-flowered plants was (3.5%) and for the white-flowered plants 94%), mixtures were composed of 70-75% of the flavanolignans. The separation of the pure crystalline flavanolignans from the mixtures was achieved by separation on column chromatography on polyamide and silica gel. The separated flavanolignans were identified as taxipholin, silybin, silydianin and silychristin on the basis of physicochemical properties in comparison with those given in the literature[4-6].

The composition of the flavanolignans of the fruits of the purple-flowered and white-flowered varieties was found to be qualitatively similar, but different in their quantitative composition. Thus, the major flavanolignans of the fruits of purple-flowered variety were silychristin followed by silybin; silydianin and taxipholin were minor components. It seems that silychristin and silybin are formed in that variety at the expense of the two minor flavanolignans. The fruits of the white variety, on the other hand, contained silychristin and taxipholin as the two major flavanolignans, while silybin and silydianin were still present in respectable amounts (Fig. 1 and Table 1). It is interesting that the unknown components present in the flavanolignans mixture appear to be the same from HPLC data. Further work needed to isolate and identify such compounds from the flavanolignan mixture of both varieties of *S. marianum* L. Geartn.

Spectroscopic analysis:

Silybin:

$C_{25}H_{22}O_{10}$ (482.45, M^+ 482), m.p. 166-168°C, (190 MeOH), UV-Spectrum: λ_{max}^{EtOH} 288, 330 nm; λ_{max}^{EtOH} + KOH 255, (320), 355 nm; $(\alpha)^{25}$ + 11°.

Silydianin:

$C_{25}H_{22}O_{10}$ (482.45, M^+ 482), m.p. 173-180°C, (1% MeOH), UV-Spectrum: λ_{max}^{EtOH} 292, 328, λ_{max}^{EtOH} + KOH 245, 330 nm, $(\alpha)^{25}$ + 180°.

Silychristin:

$C_{25}H_{22}O_{10}$ (482.45, M^+ 482), m.p. 174-176°C (corrected), (1% MeOH), UV-Spectrum: λ_{max}^{EtOH} 288, 325, λ_{max}^{EtOH} + KOH 250, (320), 330 nm, $(\alpha)^{25}$ + 81°.

Taxipholin:

$C_{15}H_{12}O_7$ (304.26, M^+ 304), m.p. 235-237°C (corrected), (1% MeOH), UV-Spectrum: λ_{max}^{EtOH} 288, 331, λ_{max}^{EtOH} + KOH 255, (288), 325 nm, $(\alpha)^{25}$ + 63°.

Table (1): Relative composition of the flavanolignans of S. marianum fruits*

Flavanolignans %**	Purple-flowered variety	White-flowered variety
Taxipholin	4.92	20.55
Silychristin	39.61	28.25
Silydianin	10.23	24.58
Unknown	24.47	13.34
Silybin	20.76	13.26

* Area was determined by triangulation method.

** These were identified according to retention times as compare with authentic compounds

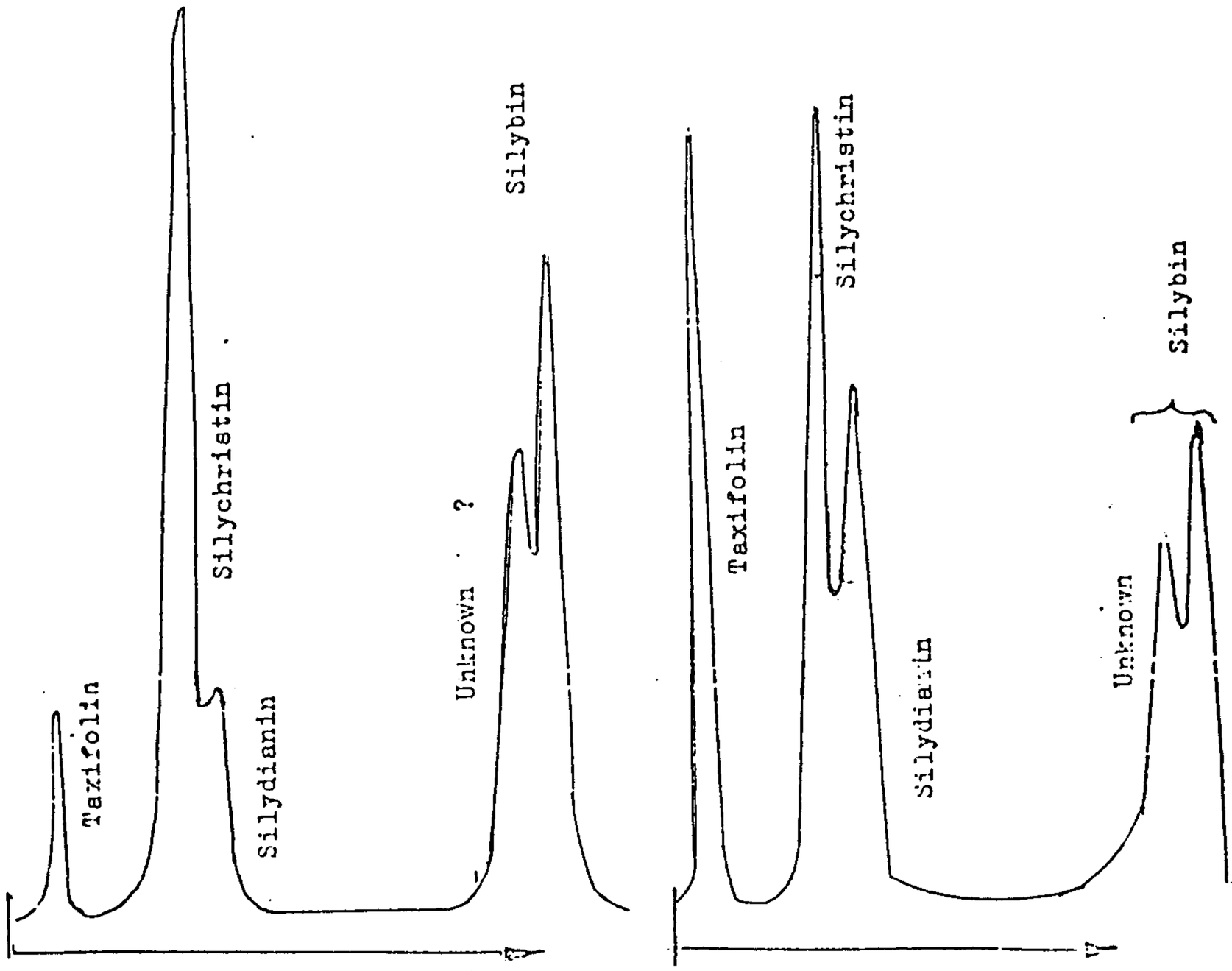

Fig. 1: HPLC chromatogram of flavanolignans of fruits of S. marianum purple-flowered variety (A) and fruits of S. marianum white-flowered variety (B).

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ل. درانيك ، ل. دلجاننكو ، أ. جريسوب ، م. لينين و أ م. الشامى*

معهد الاتحاد السوفيتى لابحاث كيمياء العقاقير وتكنولوجيا الادوية - خاركوف جمهورية أوكرانيا*

قسم العقاقير - كلية الصيدلة - جامعة طنطا - طنطا - مصر

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