THE THIN LAYER CHROMATOGRAPHY ANALYSIS OF SAPONINS BELONGING TO *SOLIDAGO* SPECIES

Dobjanschi Luciana*, Zdrinca Mihaela*, Muresan Mariana*, Vicas Simona, Antonescu Angela***

* University of Oradea, Faculty of Medicine and Pharmacy, 10 1Decembrie St., Oradea, Romania
** University of Oradea, Faculty of Environmental Protection, 26 Gen. Magheru St., 410048, Oradea, Romania

Abstract

Nowadays, saponins can be used to treat respiratory diseases, influenza, bronchitis and other respiratory diseases in order to ease expectoration; also, they can cure varicose ulcer, stomach and duodenal cancer, wounds which heal with difficulty, burns, frostbites, cutaneous staphylococcal infections, eczema; moreover, they are used during the convalescence period after infectious diseases, for erderly people`s general weakness, paralysis, cancer, metabolic diseases (Stănescu et al., 2002). The qualitative analysis using Thin layer chromatography plays an important role in the study of saponins.

Key words: saponins, Solidago.

INTRODUCTION

The isolation and determination of the studied species of saponins

The isolation of saponins is a rather difficult operation due to their high polarity and high molecular weight. Another inconvenient that appears when isolating pure saponins is the presence of complex mixtures of structurally related compounds which are slightly different due to aglycone or to carbohydrate fractions (the type, number and the biding site of monosaccharides).

MATERIAL AND METHODS

The extraction and isolation of crude saponins was conducted according to the method proposed by Cucu and Grecu (1971). The analyses were performed on dry plant material (***Solidago virgaurea, Solidago gigantea, Solidago canadensis***) (Tămaş, Roşca, 1988).

The dry plant material (previously degreased with chloroform) was extracted during ebb tide two consecutive times, using 10 parts of methanol. The combined methanol solutions were concentrated by circular evaporation until a syrupy consistency was obtained; then, they were poured into a thin stream under continuous stirring in 500ml acetone until the crude saponin was precipitated. The crude saponin was separated by vacuum filtration,

dried in desiccators over $CaCl_2$ and then weighted. The purification of the crude saponin was carried out by dissolving in methanol at a high temperature, followed by another precipitation in acetone and gravimetric determination.

After determining the amount of isolated saponin the following were found (Table 1):

Table 1

The concentration of saponin belonging to Solidago sp.

Species	The concentration of saponin g%
Solidago virgaurea	8,64%
Solidago gigantea	9,4%
Solidago canadensis	8,0%

As a result of the quantitative determination, we have observed that ***S. gigantea*** presents the largest amount of saponin (9,4%) compared to ***S. virgaurea and S. Canadensis*** (8,64% and 8%) in saponins.

The qualitative analysis using Thin layer chromatography

The qualitative analysis using Thin layer chromatography plays an important role in the study of saponins. Chromatographic plates used for Thin layer chromatography (usually silica gel) have the advantages that they can be used both for the analysis of pure saponins and for the crude extracts, they have a low cost, are easy to use and do not require a specific equipment.

Due to the fact that plants saponins are accompanied by strongly polar compounds such as sugars, colored phenolic compounds) they cannot be easily crystallized and often are hygroscopic. The characterization of pure saponins is quite challenging due to the absence of the crystalline form. Consequently, determining the purity and the identity of the substances by their physical constants (melting point, rotatory power) is difficult to achieve (Miyase et al., 1994; Saukel et al., 1986).

The purity of a compound can be established using methods such as Thin layer chromatography or HPLC.

The quantitative analysis of the saponins has been done by chromatography on a thin layer.

Material and method

Test solution: methanol solution of isolated saponins belonging to the 3 species of Solidago.

The stationary phase: silica gel GF 254 (Merck), standardized plates of 10x10 cm and 0,25 mm thick.

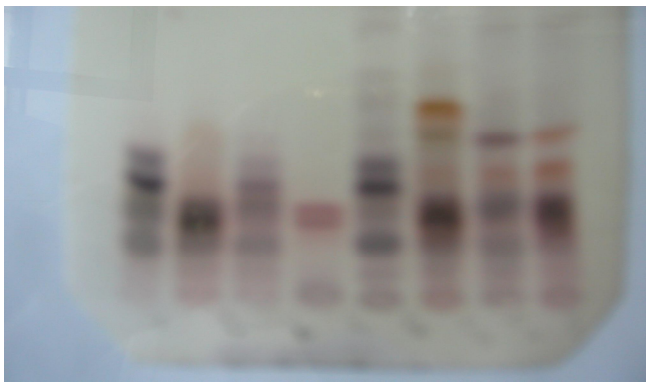
The mobile phase: chloroform: methanol: water (70: 44: 10)

The standard substance: methanol solution of Merck saponin 1%

The applied amount: 20 µl of sample and 10 µl of standard substance

Identification: Liebermann-Burchard reagent and then heated in oven at about 100^0C for about 5 minutes.

RESULTS AND DISCUSSION



1 2 3 4 5 6 7 8

Fig. 1. Thin layer chromatography- saponins: , 2- S. gigantean saponin, 3 - S. virgaurea saponin, 4 – Merck saponin, 6 - S. gigantea extract, 7 - S. virgaurea extract, 8 - S. canadensis extract

Based on the analysis of the chromatogram (Fig. 1), fractions of saponosides of each type can be emphasized. All saponosides give coloured reactions turning into brown or purple-pink as Merck saponin. In table no. 1, the Rf values and the colour of the isolated saponosides spots are presented. The extracts highlight more spots than isolated saponins and have greater Rf values than those of saponosides which indicate the presence of other substances which react with this reagent from the extracted solutions obtained from the four types (Dobjanschi, 2006).

Based on the analysis of the chromatogram. it can be stated that there are several differences between the three Solidago species taken into our study. The number of spots and the value of Rf varies. There are a couple of differences regarding the main fractions of ***S. virgaurea*** on one hand ***and S. gigantea and S. Canadensis*** on the other hand: the latter have also saponosides fractions. Thus, the chromatographic analysis on thin layer can differentiate the three species of Solidago.

Table 2

The Thin layer chromatography analysis of isolated saponin and extracts from Solidago and Anagalis arvensis species

Species	Rf	Colour
Solidago gigantea saponin	0.13	Grey
	0.2	Brown
	0.22	Grey
Solidago virgaurea saponin	0.13	Grey
	0.25	Brown
	0.3	Light purple
Merck saponin	0.2	Purple
	0.23	Purple
Solidago gigantea extract	0.15	Purple
	0.21	Brown
	0.27	Grey
	0.42	Brown
Solidago virgaurea extract	0.15	Grey
	0.25	Brown
	0.35	Light purple
	0.42	Purple
Solidago canadensis extract	0.15	Grey
	0.21	Brown - purple
	0.33	Purple
	0.42	Purple

The spots that have appeared for saponins isolated from Solidago extracts are identical to those from Solidago extracts. It can be observed that all studied saponins give the same colour reaction as Merck saponin.

CONCLUSIONS

The analysis of the saponins from the studied species found the following:

- The quantitative determination showed that ***S. gigantea*** contains the largest amount of saponin (9. 4%) compared to ***S. virgaurea and S. canadensis*** (8. 64% and 8%) in saponins.
- The chromatographic analysis on thin layer can differentiate

between the 3 species of ***Solidago.***

- ***S. gigantea and S. canadensis*** also contain saponosides fractions compared to ***S. virgaurea.***
- All the studied saponins give the same colour reaction as Merck saponin.

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