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TLC screening in searching for active components in *Rhodiola rosea* L. roots

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Thin layer chromatography (TLC) has been used for screening analysis of extracts from *Rhodiola rosea* roots. TLC is a fast, simple and inexpensive screening technique, often used in qualitative analysis of plant substances. The results of TLC analysis confirmed to the possible presence of sugars, terpenes, terpenoids, saponines and propylpropanoids in *Rhodiola* roots. The extracts of *Rhodiola rosea* revealed strong antioxidant activity.

1. INTRODUCTION

Rhodiola rosea L. has been used for many years in traditional medicine to treat diarrhea, migraine, hysteria and cognitive dysfunction. The infusion of the root and rhizome of the *Rhodiola* reduces back pain and pain of kidney caused by stones, cures stomatitis and swelling. It also stimulates hair growth and alleviates the symptoms of various skin diseases. *Rhodiol*a has also been known as a plant that helps adapt to external conditions. *Rhodiola*, like other adaptogens, increases the ability of organism to adapt to environmental stressors and generates non-specific resistance [1, 2].

Summarizing, *Rhodiola* (mainly its roots and rhizome), is an adaptogen with anti-stress, antidepressant and antioxidant effects. It also has anticancer and immunostimulatory properties. Thanks to the content of numerous biologically active compounds, it has also a positive effect on memory, thinking and cognitive processes. These properties can be very important in the treatment of diseases associated with central nervous system disorders such as Alzheimer's disease.

Initially, it was believed, that for the adaptogenic properties of *Rhodiola* only two constituents were responsible, that is p-tyrosol and salidroside. Later, the important group of compounds was discovered, the so-called rosavins, which includes: rosin, rosavin and rosarin. In fact, both groups are responsible for the adaptogenic properties of *Rhodiola rosea*. The ratio of rosavins to salidroside in the raw material is 3:1 [2].

Rhodiola rosea biological properties have been investigated using spectrophotometric methods for bulk samples, e.g. DPPH assay [3], acetylcholinesterase (AChE) assay [4] revealing its antioxidant and AChE inhibiting properties, respectively. However, the composition of the plant has been analyzed mostly using chromatographic methods especially gradient HPLC [5-7].

Recently, chromatographic methods have been usually hyphenated with spectroscopic technics as HPLC-LC-MS [8] and GC-MS (essential oils) [9]. Thin layer chromatography (TLC) has been used as an alternative to HPLC, also in testing quality and authenticity of *Rhodiola* samples of different origin [10, 11]. However, there are no papers on separation and detection of bioactive compounds in parallel.

The method of choice is TLC, which is a simple and rapid method for analysis and screening for plant material. The great predominance of TLC over column chromatography is possibility to compare many samples in the one chromatographic run and to perform biological assays directly on TLC plate [12-14]. The aim of the study was TLC screening analysis for biologically active substances in the *Rhodiola rosea*, using chemical and biological-chemical detection.

2. EXPERIMENTAL

2.1. Chemicals

Methanol (99,8%), ethanol (96%), ethyl acetate (99,8%), toluene, chloroform, formic acid (85%), acetic acid (99,5-99,9%) and sulfuric acid (96-98%), all of the analytical grade, were from POCH (Gliwice, Poland), while p-anisaldehyde, thymol, DPPH (2,2-diphenyl-1-picrylhydrazyl) were from Sigma Aldrich (Poznań, Poland).

2.2. Sample preparation

The samples were prepared by maceration of grounded to a powder *R. rosea* dry root (manufactured by NatVita, Poland) with water/alcohol mixtures (Table 1). The plant (g) to solvent (mL) ratio was 1:10 (w/v). All extracts were stored in a refrigerator in dark glass vials.

Table 1. The extracts of *Rhodiola* used for further investigation.

Sample	Solvent	Maceration time	Extraction conditions
S1	Methanol	24h	Dark place, room temperature
S2	Methanol	72h	Dark place, room temperature
S3	Methanol	24h	Shaking
S4	Methanol	72h	Shaking
S5	Ethanol	72h	Dark place, room temperature
S6	70% methanol	72h	Dark place, room temperature
S7	70% ethanol	72h	Dark place, room temperature
S6*	70% methanol	72h	Dark place, room temperature *extract stored in a refrigerator for one year

2.3. Chromatography

Chromatography was performed on 20 cm × 10 m silica gel 60 F_{254} TLC plates and on 10 cm × 10 cm Diol F_{254} HPTLC plates (Merck, Germany). The plates were not activated. The samples (3 mm^3 and

5 mm^3) were applied using the Linomat 5 automatic applicator (Camag, Switzerland) as 5 mm and 10 mm bands, respectively, with application velocity at 0.15 mm^3/s. TLC plates were developed with mobile phases listed in the Table 2 [15, 16] to a distance of 8 cm using an unsaturated DS horizontal chamber (Chromdes, Poland). The air-dried developed plates were documented using TLC Visualiser (Camag, Switzerland) at UV and VIS light.

Table 2. The mobile phases used in experiments.

Number	Components	Proportions (v/v)
I	ethyl acetate: methanol: water [16]	70/30/10
II	ethyl acetate: methanol: water [15]	77/13/10
III	toluene: ethyl acetate: formic acid [16]	7/3/0,5
IV	chloroform: methanol: water [15]	26/14/3
V	ethyl acetate: chloroform [15]	75/25
VI	ethyl acetate: chloroform: methanol [16]	70/24/6

2.4. Chemical derivatization

2.4.1. AS

AS (p-anisaldehyde) is a good general reagent, used mostly for detection of terpenes, terpenoids, saponines and propylpropanoids. Plates were sprayed with solution of 0.5 cm^3 p-anisaldehyde in 85 cm^3 methanol, 10 cm^3 glacial acetic acid and 5 cm^3 concentrated sulfuric acid. Then plates were heated to 105°C for maximum visualization of spots. Chromatograms were documented in VIS light and UV light (366 nm) [17].

2.4.2. Thymol

Thymol reagent is used for detection of sugars. Plates were sprayed with a solution of 2-isopropyl-5-methylphenol (0.5 g) in 95 cm^3 ethanol and 5 cm^3 concentrated sulfuric acid. For visualization of sugars plates were heated 15-20 min at 120°C. Chromatograms were documented in VIS light [17].

2.4.3. DPPH

DPPH reagent is used for detection of antioxidants. Plates were sprayed with 0,2% 2,2-diphenyl-1-picrylhydrazyl solution in methanol. Results were observed after 30 min in VIS light. Radical scavengers appeared as yellow spots on the purple background [17].

3. RESULTS AND DISCUSSION

3.1. Chromatographic conditions

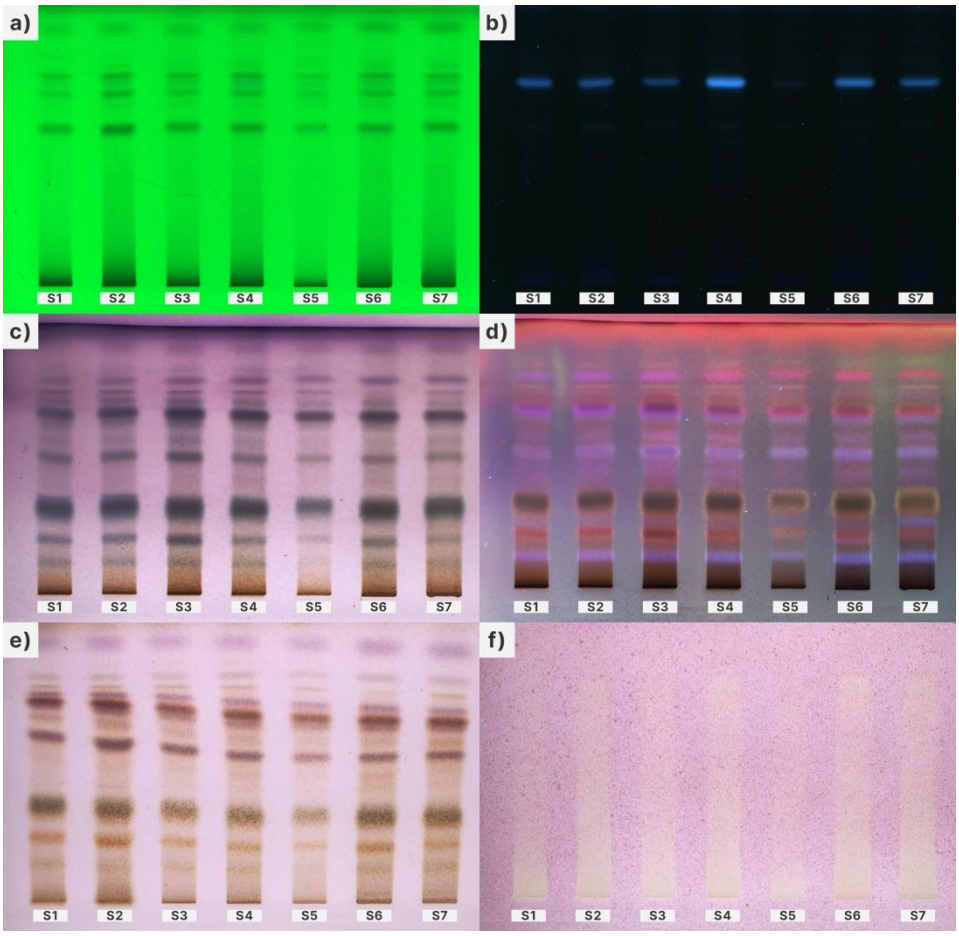

Fig. 1. Set of chromatograms documented using TLC Visualiser at UV and VIS light for *Rhodiola* extracts. Silica gel, mobile phase I. a) 254nm, b) 366nm, c) AS VIS, d) AS 366nm, e) Thymol VIS, f) DPPH VIS.

Preliminary TLC experiments were performed for various extracts (Table 1) to compare their composition and activity. The plant constituents were detected without any derivatization in UV light as well as after spraying with AS, Thymol and DPPH reagents. The mobile phases I and II (Table 2) were used according to the literature [15, 16].

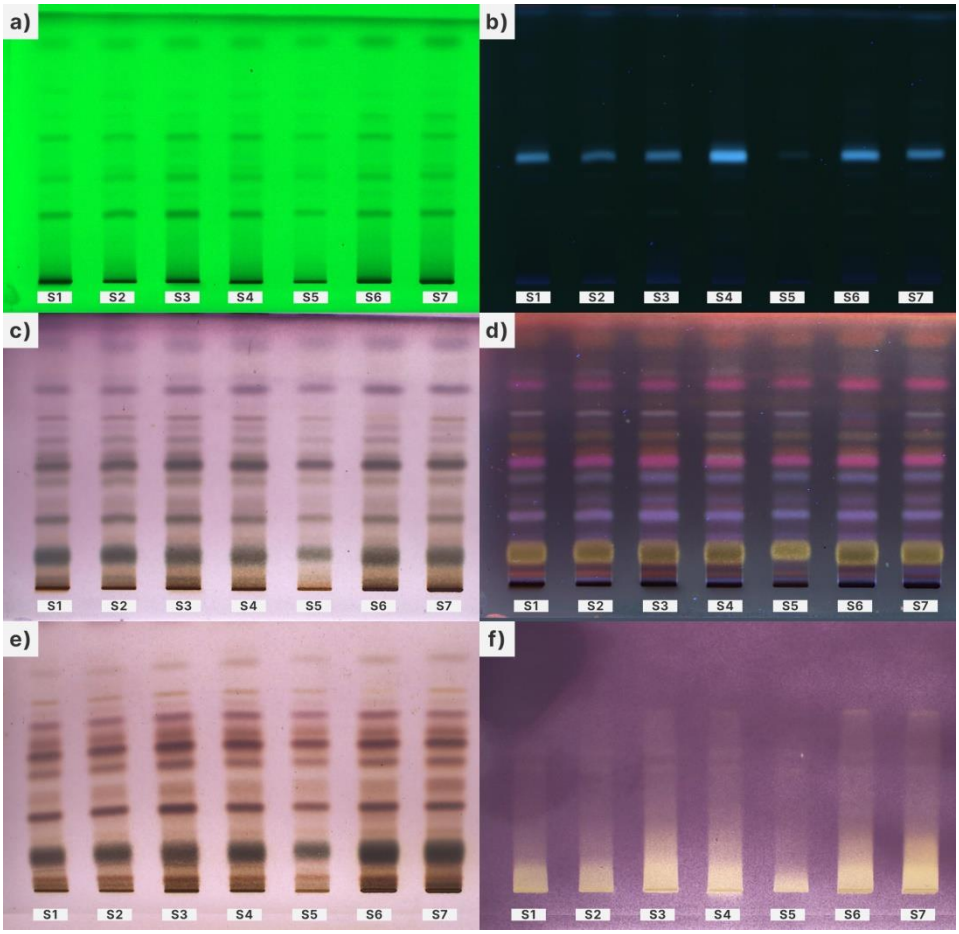

Figure 2. Set of chromatograms documented using TLC Visualiser at UV and VIS light for *Rhodiola* extracts. Silica gel, mobile phase II. a) 254nm, b) 366nm, c) AS VIS, d) AS 366nm, e) Thymol VIS, f) DPPH VIS

Figure 1 and Figure 2 present sets of chromatograms performed for *Rhodiola* roots extracts (Figure 1 – mobile phase I and Figure 2 – mobile phase II). Under these two conditions, the good separation of compounds was achieved. The investigated extracts revealed positive

reactions with AS and Thymol reagents pointing to the possible presence of terpenes, saponines, propylpropanoids and sugars. The DPPH test (Figure 1f and Figure 2f) confirmed antioxidant activity in all *Rhodiola* extracts. The S6 extract is the most rich in components and revealed the strongest antioxidant properties. Basing on these results (Figure 1 and Figure 2), the extract S6 were used for further analysis.

The samples (S6 and S6*) were analyzed using different mobile and stationary phases to find the optimal conditions for analytes. Besides the sample S6, i.e. fresh 70% methanol extract of *Rhodiola* roots, also the S6* (one year old 70% methanol extract of *Rhodiola* roots) was analyzed to compare the stability of the *Rhodiola* extract.

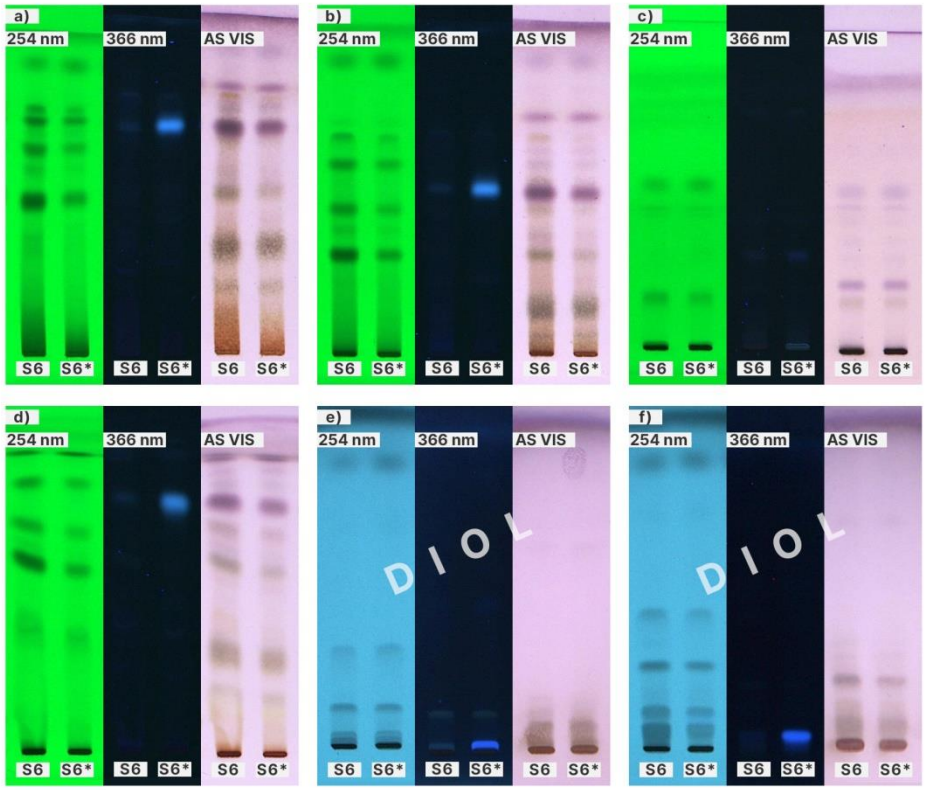

Figure 3. Set of chromatograms documented using TLC Visualiser at UV and VIS light for *Rhodiola* extracts. Silica gel: a-d; diol: e,f; the mobile phases: a) I, b) II, c) III, d) IV, e) V, f) VI.

Figure 3 showed that optimal chromatographic conditions for *Rhodiola* roots samples were obtained with the mobile phases I and II and silica gel 60 as the stationary phase. It is also evident, that the 70% methanol extract of *Rhodiola* roots slightly decomposed throughout a year. The S6 is richer in components compared to the S6*. Additionally, the fluorescence of the compound at R_f 0,6 is much stronger for the S6* (Figure 3a).

4. CONCLUSIONS

Rhodiola rosea has become very popular plant in the recent years. The investigated samples revealed strong antioxidant activity. The optimal chromatographic conditions were achieved with ethyl acetate: methanol: water 70/30/10 (v/v) I and ethyl acetate: methanol: water 77/13/10 (v/v) II mobile phases on silica gel 60 stationary phase.

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