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Two-dimensional thin-layer chromatography of selected *Polygonum* sp. extracts on polar-bonded stationary phases

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ABSTRACT

Two-dimensional thin-layer chromatographic systems on cyano-bonded polar stationary phases were used for the separation of some phenolic compounds extracted from two species of *Polygonum*: *Polygonum hydropiper* L. and *Polygonum cuspidatum* L. Non-aqueous solvents were used in the first direction and aqueous solvents were used in the second direction on CN silica TLC plates. For the separation of phenolics' standards optimal chromatographic systems were chosen from the retention data collected in one-dimensional TLC experiments by plotting graphs of R_F vs. R_F dependencies. Using above described method the satisfactory results of separations were obtained.

1. Introduction

Plant extracts are usually rich in groups of substances of various physicochemical properties. In the group of phenolics, among others, flavonoid aglycones, flavonoid glycosides, phenolic acids are widely presented in various plant organs. Similar physicochemical properties cause some difficulties in their separation. Separation of these compounds, having similar chromatographic properties, is often impossible in one chromatographic run. For such difficult purposes multidimensional separations by using systems of various selectivities are often applied [1–5]. This needs, however, special equipment and complicated procedures in case of HPLC systems [6–10].

Two-dimensional separations can be much easily performed on one chromatographic plate with two eluents of different selectivities or on two various chromatographic plates (graft TLC).

Polar bonded stationary phases (cyanopropyl, aminopropyl and diol) are the special types of stationary phases which can be used in both normal-phase (NP-TLC) and reversed phase (RP-TLC) systems. In this case, two-dimensional thin layer chromatography can be performed without technical problems in the connection of various types of stationary phases (e.g. silica – RP phases). It makes possible the separation of multicomponent natural mixtures on one plate by the use of non-aqueous and perpendicularly aqueous eluents (various properties and selectivities) [11–17].

Polygonum hydropiper L. also known as smartweed has a long history of herbal use, both in Eastern and in Western herbalism. It is not very often used, and it is seen more as a domestic remedy being valued especially for its astringent properties which makes it useful in treating bleeding, skin problems, diarrhoea, etc. The leaves have anti-inflammatory, astringent, carminative, diaphoretic, diuretic, emmenagogue, stimulant, stomachic, and styptic properties. They contain rutin, which helps strengthen fragile capillaries and thus helps prevent bleeding. The seeds are carminative, diuretic and stimulant. The whole plant, either on its own or mixed with other herbs, is decocted and used in the treatment of a wide range of ailments including diarrhoea, dyspepsia, itching skin, excessive menstrual bleeding and haemorrhoids. A poultice of the plant is used in treating swollen and inflamed areas. In Chinese tests, the plant was ranked 20th in a survey of 250 potential antifertility drugs. A homeopathic remedy is made from the leaves. It is used in the treatment of piles, menstrual pains and other menstrual complaints [18].

Polygonum cuspidatum L. (also known as Japanese Knotweed, *Polygonum sieboldii*, *Reynoutria japonica*) has antiphlogistic, bechic, depurative, diuretic, emmenagogue, emollient, febrifuge, stomachic and vulnerary activity. It is also used in the treatment of women's complaints. A decoction is used in the treatment of burn injuries, boils and abscesses, poisonous snakebites, acute hepatitis, appendicitis, traumatic injuries and menstrual irregularities. The leaves can be crushed and applied externally as a poultice to abscesses, cuts, etc., whilst the dried roots can be ground into a powder and applied externally. Extracts of the plant have shown antitumour activity [18,19]. Thin layer chromatographic determination of resveratrol in extract from *P. cuspidatum* was performed by Zhao [20] and Babu et al. [21].

The aim of this paper was the investigation of retention behavior of phenolic compounds in some selected non-aqueous and

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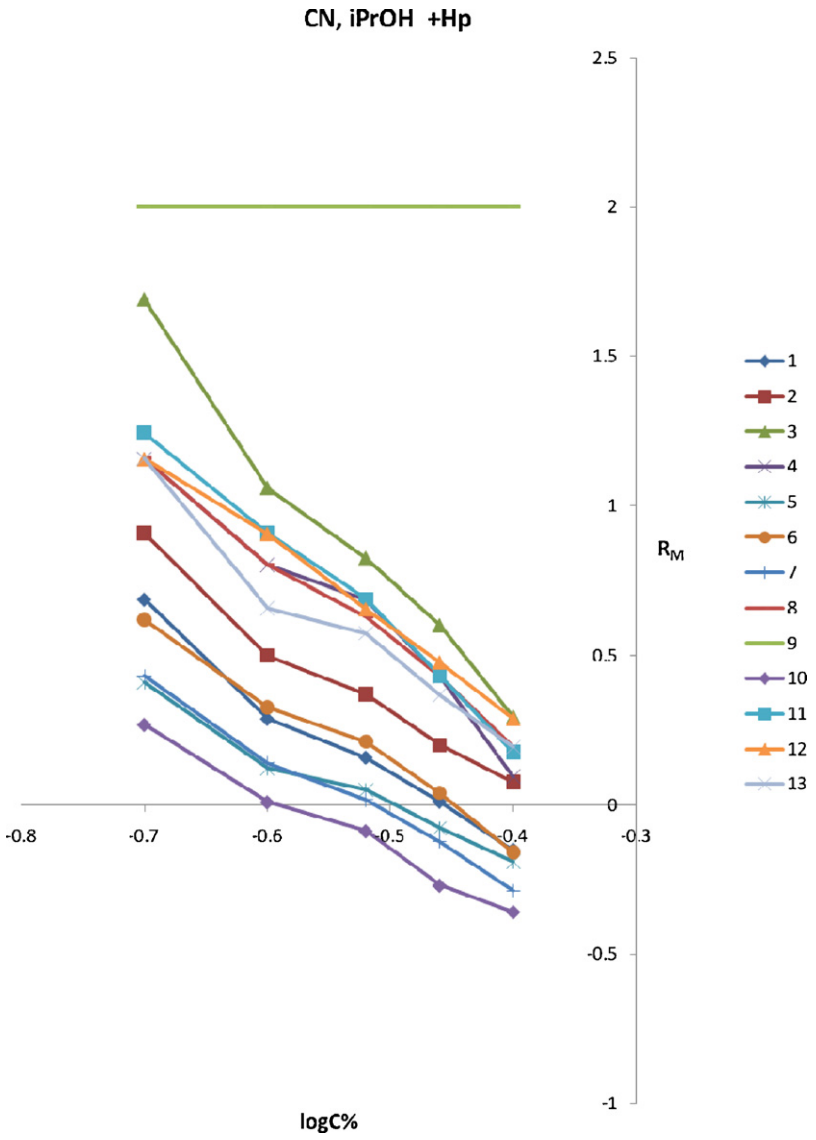

Fig. 1. R_M vs. log *c*% relationships for tested compounds in the system: cyano bonded stationary phase – propan-2-ol + n-heptane as mobile phase. Numbers in legend as in Table 1.

aqueous mobile phases to compare their separation selectivities and to use optimized most selective systems for the separation of some phenolic compounds occurred in two species of *Polygonum*: *P. hydropiper* and *P. cuspidatum*. This method can also be used as the instrument for comparison of the composition of plant extracts (fingerprints).

2. Experimental

HPTLC CN F254s 10 × 10 cm (Merck, Darmstadt, Germany) plates were used in all experiments. Solvents: propan-2-ol, ethyl acetate, n-heptane and methanol pro analysis grade and were purchased from Polish Reagents (POCh, Gliwice, Poland). Distilled water was mixed with methanol to obtain aqueous phases and n-heptane was mixed with propan-2-ol and ethyl acetate to obtain non-aqueous solvents for 2D-TLC.

All test substances (kaempferol, quercetin, rutin, hyperoside, ferulic acid, gallic acid, caffeic acid, chlorogenic acid, chinic acid, p-coumaric acid, catechin, epicatechin and resveratrol) were acquired from various manufacturers (Sigma, Aldrich, Fluka, Roth). 2,2′-Diphenyl-1-picrylhydrazyl (DPPH) was from Aldrich. 2-(diphenylboryoxy)-ethylamine and PEG4000 – Naturstoff reagent was produced by Merck (Merck, Darmstadt, Germany).

P. hydropiper and *P. cuspidatum* herbs were obtained from Herbapol (Lublin, Poland).

5 g of each herb was closed in the paper case and extracted in Soxhlet apparatus on water bath during 10 h using 350 mL of dichloromethane to get rid of ballast substances (fats, chlorophyll, etc.) and next after drying in air it was extracted by 350 mL of methanol during 12 h. After extraction methanol was evaporated on water bath under reduced pressure and the remnant was washed out with hot water and put in the refrigerator for 12 h. The mixture was filtered using paper filter and the solution was extracted 5 times by the use of 100 mL portions of ethyl acetate. Ethyl acetate extracts were coupled and the solvent was evaporated under low pressure on water bath and the dry residue was dissolved in methanol in 10 mL flask [22]. These extracts were examined in all experiments.

Some mobile phases consisted of propan-2-ol and n-heptane (concentrations: 20%, 25%, 30%, 35% and 50% (v/v)) and ethyl acetate and n-heptane (concentrations: 40%, 50%, 55%, 60% and 70% (v/v)) were prepared to optimize the separation of test substances in non-aqueous systems using CN-bonded chromatographic plates as