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A RAPID TLC METHOD FOR ANALYSIS OF EXTERNAL FLAVONOID AGLYCONES IN PLANT EXUDATES

*M. Nikolova**, *S. Berkov*, and *S. Ivancheva*

Department of Applied Botany, Institute of Botany, Bulgarian Academy of Sciences, Acad. G. Bonchev str. 23, 1113 Sofia, Bulgaria

SUMMARY

A method for densitometric analysis of external flavonoid aglycones in plant exudates has been developed and applied to the quantification of apigenin in exudates from *Veronica chamaedrys* L. and quercetin in *Artemisia vulgaris* L.

INTRODUCTION

Lipophilic flavonoid aglycones can accumulate on plant surfaces [1]. External deposits of aglycones are related to their diverse functions, for example as antioxidants [2], for screening of UV-B radiation [3,4], as antifungal compounds [5] and as signalling agents both above and below ground between plants and other organisms [6]. It is necessary to find a rapid and sensitive method for study of the distribution and dynamics of surface flavonoids and the effect of different factors on their synthesis in plants. TLC–densitometry enables determination of flavonoids by exploiting their fluorescence under UV irradiation. TLC–densitometry is the method of choice for screening because it is simpler, more rapid, and less expensive than HPLC and has satisfactory sensitivity and reliability [7,8].

EXPERIMENTAL

Plant Material

Samples of *Veronica chamaedrys* L. were collected from the aerial parts of blossoming plants from two different populations 1500 and 2350 m above sea level on Rila mountain, Bulgaria. Samples of *Artemisia vulgaris* L. were collected at the blossoming stage from populations subjected to

industrial (Petrochemical plant, Bourgas) and background (Black sea health resort) pollution. Voucher specimens from the plant samples were deposited at the herbarium in the Institute of Botany (SOM) (Co659, Co655, Co519, and Co608).

Sample Preparation

Air dried (but not ground) plants (2 g) were rinsed with 20 mL acetone for 2 min. After evaporation of the acetone the dry extracts were dissolved in 250 μL methanol.

TLC Quantification

Apigenin (0.75, 1.5, and 3 μg $spot^{-1}$ from 0.3 μg μL^{-1} stock solution) and quercetin (0.75, 1, and 1.5 μg $spot^{-1}$ from 0.5 μg μL^{-1} stock solution) were applied, with 60 μL *V. chamaedrys* exudates or 40 μL *A. vulgaris* exudates of unknown concentration, to 10 cm × 20 cm aluminium-backed TLC plates coated with 0.2 mm layers of silica gel 60 F_{254} (Merck). Plant samples were spotted in triplicate. The mobile phase was toluene–dioxane –acetic acid, 95:25:4, for *Veronica* exudates and toluene–acetic acid, 40:20, for *Artemisia* exudates. The migration distance was 90 mm. Compounds were visualized by spraying with 'Naturstoffreagenz A' reagent. The fluorescence emission of apigenin ($R_F = 0.34$) and quercetin ($R_F = 0.37$) was recorded under UV radiation at 336 nm, by means of a digital camera, and the images were analysed by use of QuantiScan 2.1 Biosoft software. The amounts of the compounds in the samples were calculated by comparing densitogram peak areas from the samples with those from the three standards on the same plate. For determination of calibration equations 0.07, 0.09, 0.18, 0.37, 0.75, 1.5, 3, 4.5, and 6 μg $spot^{-1}$ for apigenin and 0.03, 0.06, 0.12, 0.25, 0.5, 0.75, 1, 2.5, 5, 10, and 20 μg $spot^{-1}$ for quercetin were chromatographed in triplicate.

RESULTS AND DISCUSSION

In this paper we describe a method for densitometric determination of two flavonoid aglycones, apigenin in *Veronica chamaedrys* L. exudates and quercetin in *Artemisia vulgaris* L. exudates. The method is suitable for rapid quantification of flavonoid aglycones in plant exudates. It requires little time for sample preparation and quantification. Our preliminary results showed that 1 min is sufficient time to extract almost all the externally deposited flavonoid aglycones; this is in agreement with the

results of Wollenweber [9]. As far as we are aware software for image analysis has not previously been used for quantification of external aglycones. Typical densitograms obtained from *A. vulgaris* and *V. chamaedrys* exudates are shown in Figs 1 and 2.

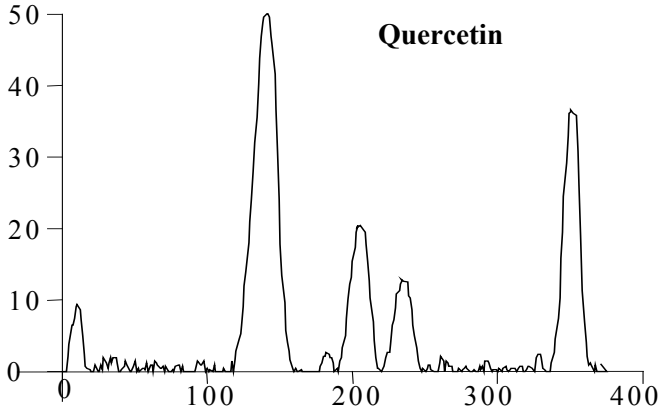

Fig. 1

Densitometric scan of exudate from *Artemisia vulgaris* after TLC separation

Calibration graphs for TLC quantification of apigenin and quercetin were linear between 0.35 and 4.5 μg spot^{-1} for apigenin and between 0.25 and 5 μg spot^{-1} for quercetin; the linear regression equations were $y = 30282x + 21815$ and $y = 3846.2x - 1552$, respectively, and the correlation coefficients 0.994 and 0.996, respectively. The limits of detection were 0.07 μg spot^{-1} for apigenin and 0.06 μg spot^{-1} for quercetin. Standard deviations from three replicate analyses of one sample, calculated as absolute amounts, were approximately ±0.07 μg for quercetin and ±0.04 μg for apigenin. It is necessary to chromatograph flavonoid standards on the same plates as the samples to overcome slight variations in the quantities on different plates.

Quantification of Apigenin and Quercetin in Plant Samples

Quantification of apigenin in acetone exudates of *V. chamaedrys* shows that the sample from the alpine region contains more apigenin than the sample from the lower altitude (2.20 and 0.68 μg g^{-1} dry weight, respectively). This result agrees with previous observations that plants from high altitudes accumulate more flavonoids than plants from low altitudes

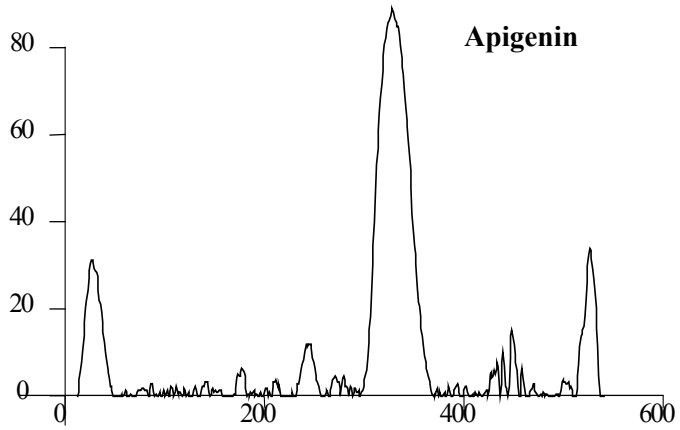

Fig. 2

Densitometric scan of exudate from *Veronica chamaedrys* after TLC separation

[10,11]. The higher quercetin content (2.8 μg g^{-1} dry weight) in the sample from the polluted site could be associated with the defensive role of flavonoids under conditions of environmental stress [12]. In comparison, the sample from the controlled site contained 1.5 μg g^{-1} dry weight quercetin. Other results suggest that atmospheric stress factors in highly polluted areas seem to affect accumulation of some flavonoids [13].

This technique for densitometric determination of apigenin and quercetin is suitable for routine assay of these compounds in a large number of samples. It could be used to study the effects of different abiotic and biotic ecological factors on the accumulation of these compounds in the plants.

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