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Note

Determination of phenolic compounds in *Colladonia triquetra* L.

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Mangiferin is well-known for its cardiotonic, spasmolitic, diuretic, choliretic and antiphlogistic actions[1]. It was established as the major xanthone glycoside in the Bulgarian endemic medicinal plant *Colladonia triquetra* L. The development of suitable generation of *C. triquetra* is connected not only with agricultural problems, but also with an adequate analytical assay method. There are various different methods published in the literature[2–4], however, we found that they could not be applied successfully for the plant investigated.

The aim of the present report is to demonstrate the possibilities of two chromatographic techniques — thin-layer chromatography (TLC) and high-performance liquid chromatography (HPLC)— as assay methods for mangiferin in *C. triquetra* L. Two other terpinoid coumarins, colladin and colladonin, are isolated for the first time from this plant[5,6] and are determined separately.

EXPERIMENTAL

TLC conditions

TLC pre-coated plates of silica gel 60 F_{254} (0.25 mm thick) are from Merck (Darmstadt, F.R.G.); they are not activated before use.

Finely powdered seeds of *C. triquetra* L. (2 g) were extracted with methanol in a Soxhlet for 2 h. The extract was evaporated to dryness *in vacuo*. The residue was dissolved in a dioxan–water (1:1) mixture, filtered and then made up to 25.0 ml (sample solution I). A 5.0-ml volume of I was diluted with dioxan–methanol (1:1) to 50.0 ml (sample solution II).

Determination of mangiferin

A standard solution was made up by dissolving 0.0100 g of mangiferin in dioxan–methanol (1:1) to 100.0 ml (III); 5.0, 6.0, 7.0, 8.0 and 9.0 μl of this solution were applied. Sample solution II was applied in aliquots of 1.0, 2.0, 3.0 and 4.0 μl.

A mobile phase of ethyl acetate–formic acid–water (67:13:20) was used to saturate the plate, at development distance 10 cm. The plate was then left to dry. The spots were scanned by fluorescence, using a Helena Laboratories Cliniscan densitometer (U.S.A.). Scanning conditions were: low-pressure mercury lamp with neutral filter, plate speed 2.54 cm/s, chart speed 35 mm/s. Quantitation was by integral calculation of the peak areas.

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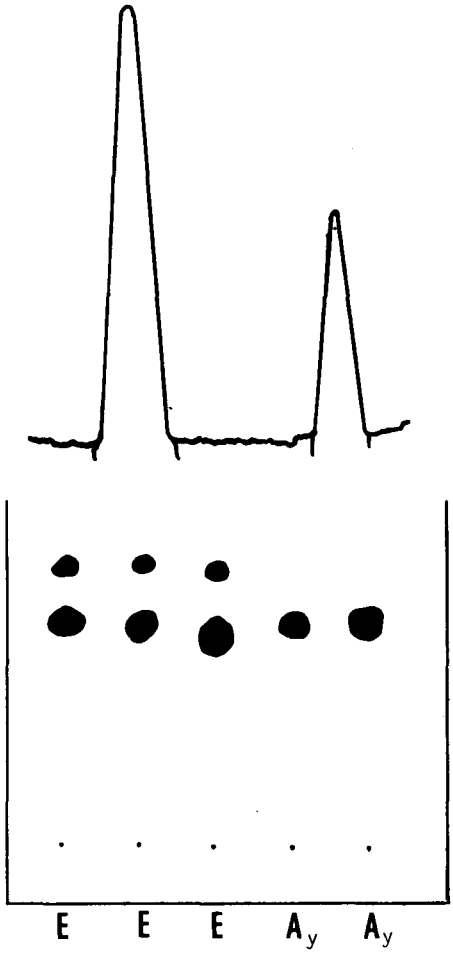

Fig. 1. Thin-layer chromatogram and densitometric scan of *C. triquetra* seed extract (E) and standard mangiferin (A) in ethyl acetate–formic acid–water (67:13:20). Migration distance: 8.4 cm. Visualization by fluorescence at 366 nm. y = Yellow fluorescence.

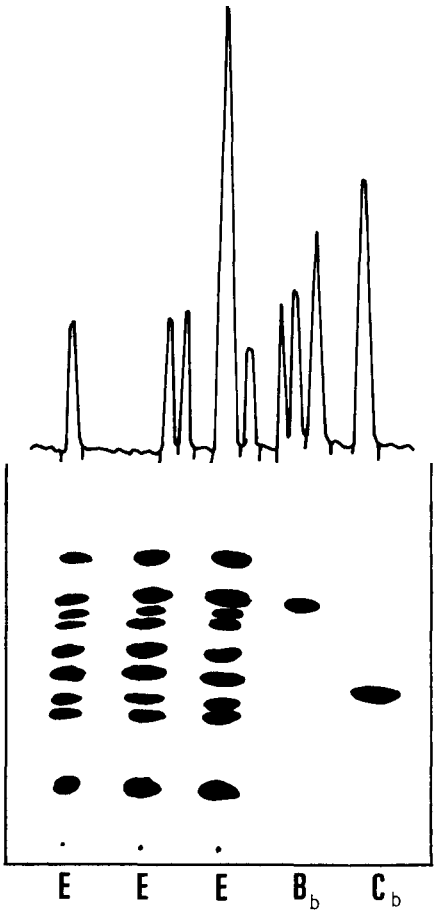

Fig. 2. Thin-layer chromatogram and densitometric scan of *C. triquetra* seed extract (E), standard colladin (B) and colladonin (C) in benzene–methyl ethyl ketone–formic acid (9:1:1). Migration distance: 7.5 cm. Visualization by fluorescence at 366 nm. b = Blue fluorescence.

Determination of colladin and colladonin

Standard solutions were made up by dissolving 0.0100 g of colladin and colladonin in dioxan–methanol (1:1) to 100.0 ml (standard solutions IV and V, respectively); 1.0, 2.0, 3.0, 4.0 and 5.0 μl of IV and V were applied. Sample solution II was applied in aliquots of 5.0, 7.0 and 10.0 μl.

A mobile phase of benzene–methyl ethyl ketone–formic acid (9:1:1) was used. The densitometric evaluation was carried out as mentioned above for A.

HPLC conditions

The HPLC system used consisted of a Series 2/2 pump, a UV detector Model LC-75 (Perkin Elmer, Norwalk, CT, U.S.A.), a Rheodyne injector Model 7125 and

a 10-μm LiChrosorb RP-8 column (250 × 4 mm I.D.; Merck, Darmstadt, F.R.G.). The detector was set to 320 nm, 0.16 a.u.f.s. sensitivity. The mobile phase was methanol–0.2% sulphuric acid–water, gradient from 25 to 95% methanol in 55.0 min. The flow-rate was 1.0 ml/min at ambient temperature. A 10.0-μl volume of II was injected.

TABLE I

COMPARISON OF MANGIFERIN (A), COLLADIN (B) AND COLLADONIN (C) CONTENT OF THE SAME SAMPLE BY TLC AND HPLC

Values are expressed as percentages ± relative standard deviation.

Compound	*TLC*	*HPLC*
A	2.96 ± 0.079	3.03 ± 0.131
B	0.105 ± 0.0084	0.106 ± 0.0045
C	0.595 ± 0.011	0.604 ± 0.0064

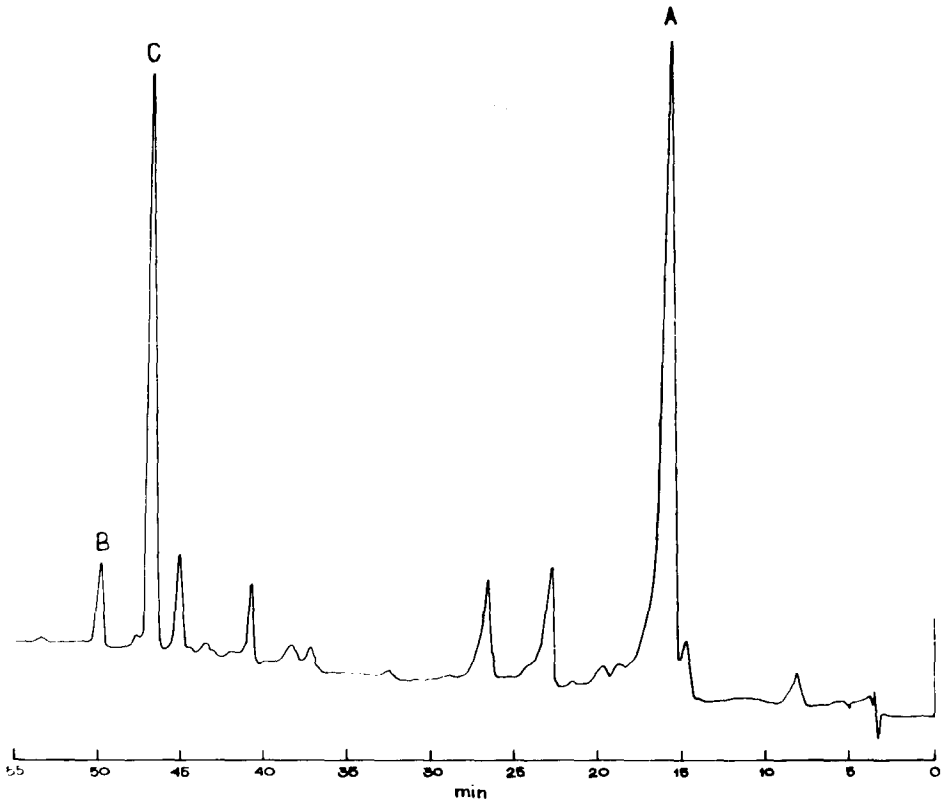

Fig. 3. Separation of mangiferin (A), colladin (B) and colladonin (C) from *C. triquetra* in methanol–0.2% sulphuric acid–water. Gradient from 25 to 95% methanol in 55 min; UV detector at 320 nm; 0.16 a.u.f.s.

RESULTS AND DISCUSSION

In spite of the presence of closely related xanthone glycosides with similar chromatographic behaviour and numerous coumarine in the seeds, the mobile phases afforded separation suitable for TLC densitometric evaluation, with an R_F value of 0.60 for mangiferin (see Fig. 1), and 0.60 for colladin and 0.40 for colladonin (see Fig. 2). The use of cellulose thin layers did not improve xanthone separation, and the use of a spray reagent did not improve the sensitivity. The limit of detection in fluorescent mode was 0.1 μg for mangiferin, 0.05 μg for colladin and 0.05 μg for colladonin.

The TLC method is simple and permits determination of mangiferin, colladin and colladonin with satisfactory precision (see Table I). To increase the accuracy of the method, however, it is suggested that a calibration curve should be derived for each plate.

The HPLC assay procedure on a reversed-phase column has been studied. A linear gradient elution with methanol–sulphuric acid–water achieved separation of mangiferin, colladin and colladonin in a reasonable time [15.8, 49.8 and 46.8 min, respectively (see Fig. 3)]. The linearity was investigated by standard curves from 100 to 400 μg/ml for mangiferin, from 5 to 18 μg/ml for colladin and from 20 to 80 μg/ml for colladonin.

The results obtained from both TLC and HPLC determinations are in good agreement (see Table I). Both methods could be applied successfully to quantitate the compounds in crude extracts from the plant.

The proposed methods also afford an opportunity to quantitatively determine other xanthone glycosides and coumarins present in *C. triquetra* seeds.

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