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Short communication

Determination of khellin and visnagin in *Ammi visnaga* fruits by capillary electrophoresis

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Abstract

A new, simple and rapid capillary electrophoresis method was developed for the identification and quantitative determination of two medically active constituents—khellin and visnagin—in the extracts of *Ammi visnaga* fruits. Micellar electrochromatographic separation of khellin and visnagin was carried out using 10 mmol/l borate, 50 mmol/l sodium dodecylsulfate, 25% (v/v) acetonitrile at pH 9 as running buffer. *Ammi visnaga* fruits were extracted with methanol and the extracts were directly injected without any purification and pre-separation processes. Coumarin was used as internal standard for quantitation and the limits of detection for khellin and visnagin were 2.36 and 1.97 μg/ml, respectively using UV detection at 245 nm. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: Ammi visnaga; Plant materials; Pharmaceutical analysis; Khellin; Visnagin; Coumarin; Furanochromones

1. Introduction

Ammi visnaga is a commercial medicinal plant mainly grown in Mediterranean areas in open fields. The extract of fruits has been widely employed as herbal medicine in the treatment of coronary diseases and bronchial asthma; it has also been used as an important raw material in the pharmaceutical industry [1,2]. The major active components in the plant are khellin and visnagin which are both derivatives of furanochromones. The level of both compounds in the dry fruits varies widely depending on genetic factors and environmental conditions. It is therefore important to develop a simple and reliable analytical method for the active components to determine the quality of the wild plant.

A wide variety of analytical methods like spectrophotometry [3], polography [4], fluorometry [5], and in recent years HPLC [6–11] have been reported for the determination of khellin and visnagin in *Ammi visnaga* fruits. However, all the methods including HPLC necessitate the time and material consuming liquid or solid-phase extraction steps or thin-layer chromatography to obtain active species from the sample matrix prior to analysis.

More recently, capillary electrophoresis (CE) has been employed for the analysis of medicinal plants [12,13]. The advantage of the capillary electrophoretic methods is the considerable diminution in the sample preparation and analysis times, as well as in the reagent consumption. CE is particularly suitable

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in the analysis of complex natural matrices, owing to its higher resolving power.

Recently, a micellar electrokinetic chromatography (MEKC) method has been reported for the analysis of coumarins [14]. However, there are no data in the literature for the quantitative analysis of the pharmacologically active khellin and visnagin in *Ammi visnaga* fruits using capillary electrophoretic methods.

In the present study, we describe the development of a simple and rapid CE method for the simultaneous determination of khellin and visnagin in *Ammi visnaga* fruits collected from Hatay, in the southeast of Turkey.

2. Experimental

2.1. Apparatus

Separations were carried out with a commercial CE injection system (Prince Technologies, Emmen, Netherlands) in combination with an on-column variable-wavelength UV–visible detector (Lambda 1000, Bishoff, Leonberg, Germany). The wavelength was set at 245 nm. The fused-silica capillaries used for separation experiments were 50 μm I.D. and were obtained from Polymicro Technologies (Phoenix, AZ, USA). The total length of the capillary was 63 cm and the length to the detector was 49 cm. Data processing was carried out using commercial CE software (Prince Technologies).

2.2. Reagents

Fruits of *Ammi visnaga* plant were collected from the natural fields of Hatay, southeast of Anatolia. Khellin, visnagin (structures shown in Fig. 1), and coumarin were from Aldrich (Milwaukee, WI, USA). Methanol, sodium dodecylsulfate (SDS), and sodium tetraborate were obtained from Merck (Darmstadt, Germany). Acetonitrile (MeCN) was from Riedel (Seelze, Germany). All solutions were prepared with distilled water purified in an Elgacan C114 filtration system.

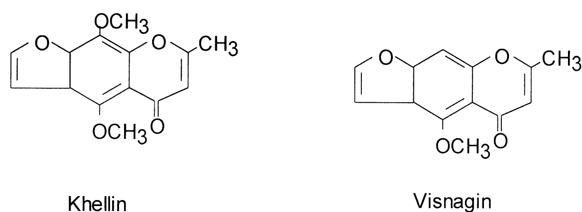

Fig. 1. The structures of khellin and visnagin.

2.3. Samples and standards

The fruits of *Ammi visnaga* were dried and powdered; 3 g of the powdered plant were refluxed with 150 ml of methanol for 2 h. The supernatant was evaporated to dryness under reduced pressure by a rotary evaporator. The residue was dissolved in 10 ml of running buffer solution (10 mmol/l borate, 50 mmol/l SDS, 25% MeCN at pH 9). The injected solutions were prepared from this stock extract by the addition of internal standard and dilution by buffer solution.

Stock standard solutions of khellin, visnagin and coumarin were prepared in acetonitrile.

2.4. Analytical method

The structures of khellin and visnagin shown in Fig. 1 suggest that they could not be separated by capillary zone electrophoresis (CZE) mode. In order to separate nonionic compounds in CE, an interaction with a charged carrier in the buffer should be provided. The most common technique to separate nonionic compounds is MEKC introduced by Terabe and coworkers [15,16]. By including a surfactant in the mobile phase, neutral molecules can be separated on the basis of their interaction with the micelles.

To separate khellin and visnagin in the plant extracts, SDS is used as surfactant. The addition of organic solvent to the buffer increased the selectivity and solubility of the furanochromones. Methanol and acetonitrile were used as organic modifier. Since separation time is much less in acetonitrile compared to methanol, buffer solutions were prepared from SDS and acetonitrile; 10 mmol/l borate, 50 mmol/l SDS, 25% MeCN at pH 9 were selected as optimal for the separation of standard samples.

Direct injections of extracts were performed after being filtered (0.45-μm cellulose acetate filter disc). A known amount of coumarin solution was added as internal standard to all injection samples.

At the beginning of each working day, the capillary was washed with 0.1 mol/l NaOH and with the separation buffer, for 5 min each. Washing for 2 min with 0.1 mol/l NaOH and 2 min with the buffer was applied between runs.

3. Results and discussion

The extracts of *Ammi visnaga* fruits were prepared as described earlier and after being filtered through a 0.45-μm cellulose acetate filter disc, injected directly to the optimized buffer solution. Fig. 2 shows the electropherogram of the extract of the fruit powder of *Ammi visnaga*. Peaks were identified by the addition of standard substances of khellin and visnagin. The electropherogram displays a good separation for khellin, visnagin, and the internal standard coumarin.

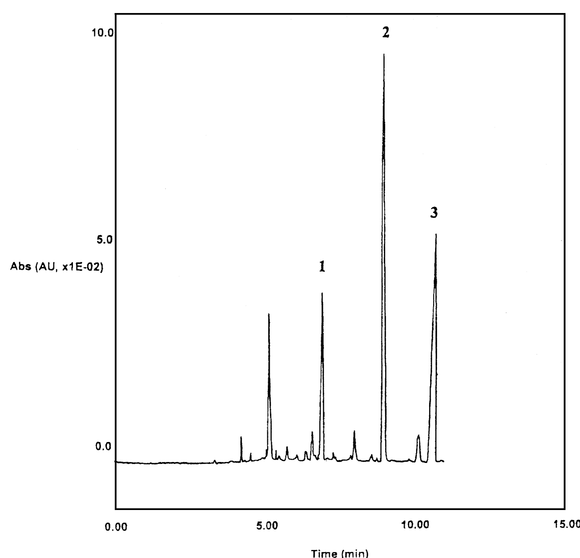

Fig. 2. Separation electropherogram of *Ammi visnaga* extract. Buffer: 10 mmol/l borate, 50 mmol/l SDS, 25% MeCN at pH 9. Voltage: 28 kV. Injection: 4×10^{-3} MPa for 6 s. 1, Coumarin (internal standard); 2, khellin; 3, visnagin. UV detection at 245 nm.

Table 1
Khellin and visnagin contents of *Ammi visnaga* fruits (mg/100 mg dry fruit)

Extract no.	Khellin	Visnagin
1	0.59	0.18
2	0.55	0.15
3	0.57	0.16
4	0.62	0.17
5	0.56	0.15
Mean	0.58	0.16
SD	0.03	0.01

Calibration curves between the concentrations of standard samples and the ratios of corrected peak areas of samples (s) and internal standard (i) $[(A_s/t_s)/(A_i/t_i)]$ were used for quantitative determinations. A linear correlation from 20 to 150 μg/ml was found for khellin ($y=1.41x+1.97\times10^{-4}$, $r^2=0.995$) and visnagin ($y=2.38x+3.11\times10^{-4}$, $r^2=0.989$), where y and x are the relative peak areas and the concentrations of analytes (mmol/l), respectively. The detection limits of the CE method corresponding to a signal/noise ratio of three are 2.36 and 1.97 μg/ml for khellin and visnagin, respectively. The run-to-run reproducibilities ($n=6$) of peak areas (A/t) of the analytes in the plant extract are 3.0% for khellin and 4.6% for visnagin.

Table 1 reports the amounts of khellin and visnagin in *Ammi visnaga* fruit obtained from five different extractions.

4. Conclusion

CE is a useful, simple, and rapid technique for the identification and determination of khellin and visnagin in *Ammi visnaga* fruits. The method does not require a pre-separation process. The main advantages of fused-silica capillaries compared to packed columns are that plant extracts are directly injected without any purification step, easily washed between runs and free of irreversible contamination of the matrix. The method promises to be applicable to the quality control of the plant and, since analysis time and cost are lower than with HPLC [6–11], is a good alternative to that method for plant analysis.

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