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Note

Quantitative determination of biologically active constituents in crude extracts of medicinal plants by thin-layer chromatography–densitometry

II. *Eleutherococcus senticosus* Maxim., *Panax ginseng* Meyer and *Picrorrhiza kurroa* Royle

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In a continuation of our study related to the standardization of medicinal plants extracts[1], three new drugs were analysed by thin-layer chromatography (TLC)–densitometry: *Eleutherococcus senticosus* roots (eleutheroside E determination), *Panax ginseng* roots (simultaneous determination of ginsenosides Rb_1, Rb_2, Rc, Rd, Re and Rg_1) and *Picrorrhiza kurroa* roots (picrosides I and II determination). In addition, a high-performance liquid chromatography (HPLC) method was developed for eleutheroside E determination and used to control the TLC–densitometry results.

EXPERIMENTAL

Plant material

Spray-dried extracts Extrenorm were obtained from Expansion Aromatique Française (Ivry-sur-Seine, France).

Standards

Picrosides I and II and ginsenosides were obtained from Sarsynthex (Mérignac, France).

Eleutheroside E was isolated from an aqueous *E. senticosus* extract using reversed-phase column chromatography (Lobar RP-8, Art. 10629, E. Merck, Darmstadt, F.R.G.) eluted with ethanol–water mixtures, followed by fractionation on a silica gel column (Lobar Si 60, Art. 10402, E. Merck) using chloroform–methanol–water systems; eleutheroside E was crystallized from methanol and its identity confirmed by direct comparison with an authentic sample.

Equipment

For the TLC–densitometry measurements, a Shimadzu (Kyoto, Japan) high-speed TLC-scanner CS-920 was used with the following settings: beam sizes: 0.4 ×

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0.4 mm (*P. ginseng*), 1.2 × 1.2 mm (*E. senticosus* and *P. kurroa*); linearizer on position 1; AZS off; wavelengths are given in the figure captions.

The HPLC determinations were performed on an apparatus consisting of a Model 6000M pump (Waters Assoc., Milford, CT, U.S.A.), a Model U6K injector (Waters Assoc.) and a Model SPD-2AM variable-wavelength detector (Shimadzu) or a Model 1040A detection system (Hewlett-Packard, Palo Alto, CA, U.S.A.) and equipped with a Hibar RP-18 column (mean particle size, 5 μm; Art. 50333, E. Merck).

Sample preparation

Solutions of the spray-dried extracts in water were diluted to 10 ml with meth-

TABLE I

PREPARATION CONDITIONS OF THE EXTRACTS AND OF THE STANDARDS SOLUTIONS, R_F, VALUES OF DETERMINED CONSTITUENTS AND QUANTITATIVE DETERMINATIONS (CONDITIONS AND RESULTS)

Experimental conditions	*Plant material*		
	Eleutherococcus senticosus	*Panax ginseng*	*Picrorrhiza kurroa*
Extract nature	Aqueous ethanol spray-dried extract		
Extracted yield (extract weight dried plant material)	1/6	Not specified	
Determined constituents (R_F values)	Eleutheroside E (0.42)	Ginsenosides Rb_1 (0.33), Rb_2 (0.36), Rc (0.39), Re (0.45), Rd (0.49) and Rg_1 (0.55)	Picrosides I (0.67) and II (0.47)
Solvents for extracts and standards dissolution	Water–methanol (1:1)		
Extracts concentration	1 g in 10 ml		
Standards concentrations (lower and upper limits)	2.5–10 mg in 10 ml	10–30 mg in 10 ml	Picroside I: 5–25 mg in 10 ml Picroside II: 2.5–10 mg in 10 ml
Spotted volumes	3 × 1 μl (band)	0.5 μl	1 μl
Values (correlation coefficient of the calibration graph)	0.995	0.998–1.000	0.999
Concentration in the extract (%)	0.29	Ginsenosides Rb_1 (1.83); Rb_2 (0.67); Rc (0.96); Re (0.81); Rd (0.42); Rg_1 (1.16)	Picroside I (20.16); Picroside II (8.28)
Variation coefficient (%)	8.5	5.4	3.2
Recovery (%)	96	95–97	99

anol and filtered to give the concentrations listed in Table I; solubilization was improved using an ultrasonic bath for 15 min.

Thin-layer chromatography

TLC plates precoated with silica gel $60F_{254}$ (10 × 20 cm; normal, Art. 5729; for HPTLC, Art. 5642; E. Merck) (see figure captions) were used for TLC performed in unsaturated tanks.

The solutions (volumes given in Table I) were spotted 15 mm from the lower edge of the plates and developed with the solvent systems described in figure captions. For *E. senticosus*, three spots were associated to form a band (distance between spots, 3 mm).

Calculations

Each determination corresponded to the mean value calculated from the integration results of four chromatograms obtained on the same plate [one spot of the extract solution and three different standard concentrations repeated four times (*P. ginseng* and *P. kurrod*) or twice (*E. senticosus*)].

RESULTS AND DISCUSSION

The determinations were performed on spray-dried aqueous ethanol extracts of the drugs without preliminary fractionation and were related to the main biologically active constituent(s) selected according to current pharmacological knowledge. Chemical, pharmacological and chromatographic analyses of *P. ginseng* and *E. senticosus* have been recently reviewed and investigated[2–4]. The pharmacological activity of picrosides isolated from *P. kurroa*, mainly picroside II, has been published[5], but no study on picrosides determination has been carried out until now. For *E. senticosus*, the assay was restricted to eleutheroside E, which is considered to be the most active constituent among the glycosides[6].

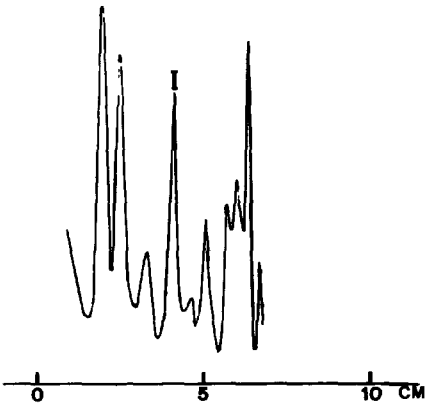

Fig. 1. Scanning profile of a spray-dried extract of *E. senticosus* Adsorbent, silica gel 60 (F_{254}) HPTLC plate; mobile phase, 1,2-dichloroethane–ethanol–methanol–water (65:22:22:7); detection, the chromatogram was sprayed with a mixture (1:1) of a 1% vanillin ethanolic solution and a 5% sulphuric acid ethanolic solution, heated at 120°C for 2.5 min and measured at 530 nm (absorption) after colour stabilization (5 min). Peak I = eleutheroside E.

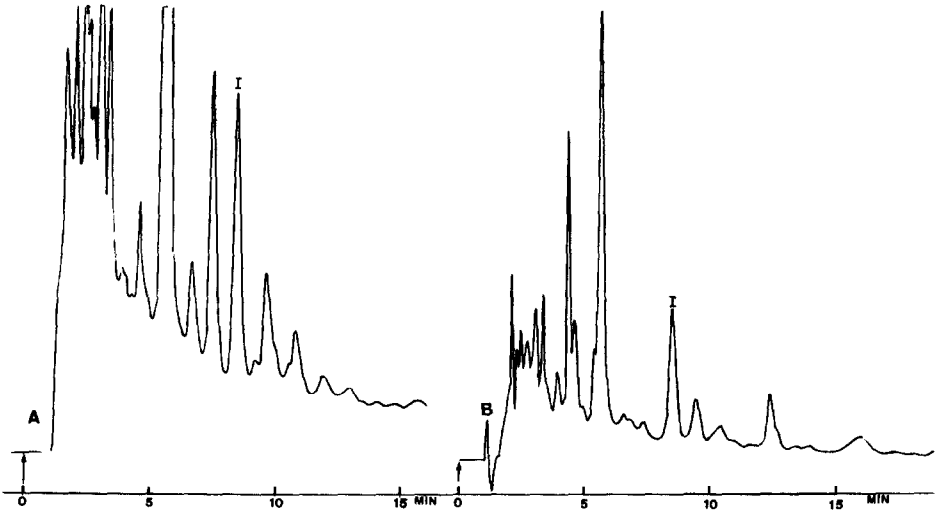

Fig. 2. HPLC chromatograms of *E. senticosus* extracts. (A) Spray-dried extract (unknown origin); (B) extract of Russian origin. Column, Hibar RP-18 (5 μm; Art. 50333, E. Merck); mobile phase, water–ethanol–acetic acid (84:16:1); flow-rate, 1.1 ml/min; UV detection at 235 nm. Peak I = eleutheroside E.

Eleutheroside E concentration was found to be very low in the crude extracts of *E. senticosus*; moreover, the vanillin sulphuric reagent must be considered only as a compromise between specificity and sensitivity for eleutheroside E detection (Fig. 1). Therefore, the multiple spotting technique was essential, and the results were controlled by HPLC. The HPLC conditions used by Wagner *et al.*[3] were modified in order to avoid solvent programming; however, under these new conditions, it was necessary to wash the column with 1 ml of methanol after each injection. The specificity of eleutheroside E detection was carefully investigated by calculations of the absorbance ratio at different wavelengths and by UV spectra recording; under the experimental conditions, no interference was observed when UV detection was performed at 235 nm (Fig. 2).

From the comparison between HPLC and TLC–densitometry (Table II), it

TABLE II

COMPARISON BETWEEN HPLC AND TLC–DENSITOMETRY METHODS FOR THE DETERMINATION OF ELEUTHEROSIDE E IN A CRUDE EXTRACT OF *ELEUTHEROCOCCUS SENTICOSUS*

Method	*Eleutheroside E concentration (%) in a spray-dried extract of E. senticosus*	*Eleutheroside E lower measurable concentration (mg/ml)*
HPLC	0.293 (ten determinations; C.V. = 4.95)	0.02
TLC–Densitometry	0.285 (six determinations: C.V.% = 8.52)	0.20

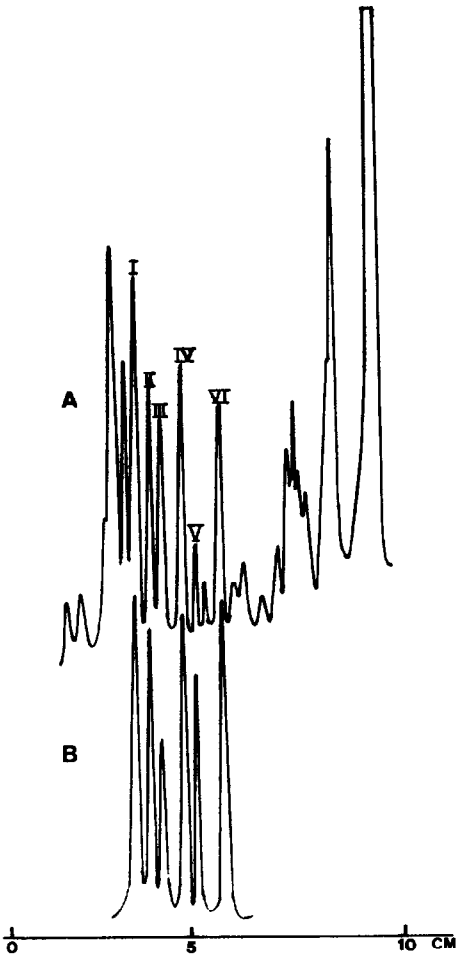

Fig. 3. Scanning profile of a spray-dried extract of *P. ginseng* (A) compared with that of a synthetic mixture of ginsenosides (B). Adsorbent, silica gel 60 (F_{254}) HPTLC plate; mobile phase, 1,2-dichlorethane–ethanol–methanol–water (65:22:22:7); detection, the chromatogram was sprayed with a mixture (1:1) of an 1% vanillin ethanolic solution and a 5% sulphuric acid ethanolic solution, heated at 120°C for 5 min and measured at 530 nm (absorption) after colour stabilization (5 min). Peaks: I = ginsenoside Rb_1; II = ginsenoside Rb_2; III = ginsenoside Rc; IV = ginsenoside Re; V = ginsenoside Rd; VI = ginsenoside Rg_1.

appears that the results obtained by the two methods were very similar, except that TLC–densitometry gave a C.V.% value twice as high and a sensitivity ten times lower.

Ginsenoside determination by TLC–densitometry has been recently studied by Furuya *et al.*[7] This method was improved by modifying the mobile phase composition in order to increase the resolution of ginsenosides and by using the vanillin sulphuric regent for detection (Fig. 3).

As the UV maxima of picrosides I and II occurred at 285 and 268 nm, re-

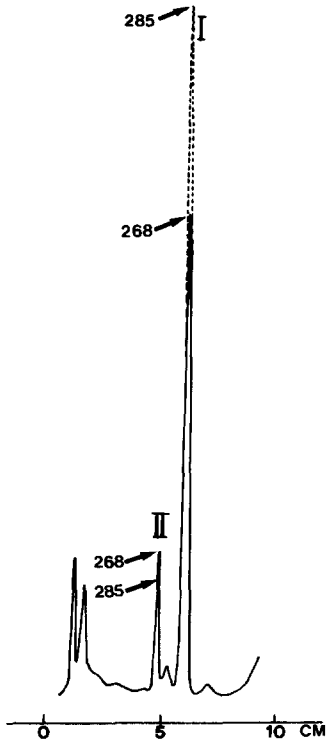

Fig. 4. Scanning profile of a spray-dried extract of *P. kurroa*. Adsorbent, silica gel 60 (F_{254}); mobile phase, dichloromethane–methanol–water (40:10:1); detection at 268 and 285 nm (absorption). Peaks: I = picroside I; II = picroside II.

spectively, specificity and selectivity could be increased by separated UV densitometry at these two wavelengths (Fig. 4).

Addition (1:1, v/v) to the crude extracts solution of standard solutions (upper limit concentration given in Table I) allowed for the drugs under investigation to control the recovery of the measured constituents (see Table I); a minimum of 95% recovery was found.

As noted in ref. 1, the problem of standardization of plant drugs would be usefully resolved using the TLC–densitometry method; it is convenient, accurate, precise and gives good recoveries (Table I).

ACKNOWLEDGEMENT

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