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Short communication

Phytochemical and pharmacological studies on *Orthosiphon stamineus* Benth. (Lamiaceae) hydroalcoholic extracts

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Abstract

The main components of *Orthosiphon stamineus* Benth. leaves and extracts are the pharmacologically active polyphenols: the polymethoxylated flavonoids and the caffeic acid derivatives. Two tinctures, having different alcohol concentration, were studied from phytochemical and pharmacological point of view. The main polyphenols were identified and quantitatively determined by HPLC. Comparison of the retention parameters and UV–Vis spectra of standards and those of the separated compounds performed the identification of caffeic-, cichoric- and rosmarinic acids, respectively, of sinesetine and eupatorine. The quantitative determination was performed by external standard method. The diuretic, saluretic and uricosuric actions of the studied tinctures were compared by experiments on rats.

Keywords: *Orthosiphon stamineus* Benth.; Hydroalcoholic extracts; Tinctures; Polyphenols; Caffeic acid derivatives; Polymethoxylated flavonoids; HPLC; Qualitative and quantitative determination; Diuretic action; Saluretic action; Uricosuric action

1. Introduction

The most important components of *Orthosiphon stamineus* Benth. leaves are the polyphenols: the polymethoxylated flavonoids: sinesetine (**IV**), eupatorine (**V**), etc. and the caffeic acid derivatives: rosmarinic acid (**III**), cichoric acid (**II**), caffeic acid (**I**), etc.

The polyphenols from *O. stamineus* Benth. leaves were studied by different chromatographic and spectral methods: TLC [1], HPLC–UV–Vis spectrometry [1–4], HPLC-NMR [5], UV–Vis spectrometry [4].

This paper presents a HPLC method for identification and quantitative determination of main polyphenols (**I**–**V**) from two different *O. stamineus* Benth. tinctures and a comparison of the pharmacological action of these tinctures.

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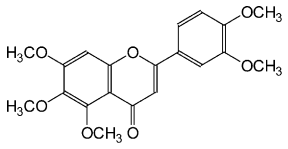
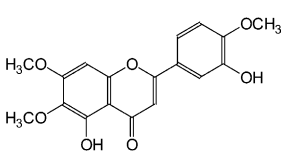


IV



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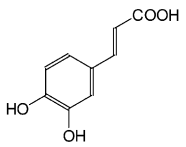
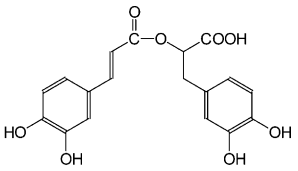


I



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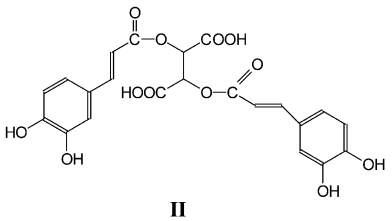

II

2. Experimental

2.1. Materials

The dry leaves of *O. stamineus* Benth. were obtained from Caesar & Loretz, Germany and the 96% vol. ethanol from Agronad, Romania.

The HPLC determinations were performed on RP-18 LiChrosphere 5 μm, 4 × 4 mm guard column and an RP-18 LiChrosphere 5 μm, 125 × 4 mm column from Merck, Germany. The solvents were of HPLC purity from Merck. The standards were: caffeic acid and rosmarinic acid from Roth, Germany, cichoric acid from Dalton, USA and sinesetine and eupatorine from Faculty of Pharmacy, Cluj, Romania.

The pharmacological experiments were performed on white Wistar Bratislava male rats. The quantitative determinations of sodium, potassium and uric acid from urine were performed by using a Vitros 250 Chemistry System (Johnson & Johnson Clinical Diagnostics).

2.2. Sample preparation

Each extract was prepared from 100 g of plant and 500 g solvent. 50% v/v Ethanol was used to obtain the tincture A and 70% v/v ethanol for tincture B. The extraction was performed by cold extraction (maceration) in 5 days with daily mixing. The filtrated extracts were used as sample [6].

2.3. Experimental conditions—HPLC method

The identification and quantitative determination of polyphenols from the studied tinctures were performed by HPLC. A Shimadzu Class-VP chromatograph was used coupled with a diode array UV–Vis detector. An RP-18 LiChrosphere 5 μm, 4 × 4 mm guard column and an RP-18 LiChrosphere 5 μm, 125 × 4 mm column was used as stationary phase. The mobile phase was a gradient made by changing the content of solvent A (acetonitrile–phosphoric acid 99.9:0.1, v/v) from 15 to 100% in 32 min, the solvent B being water–phosphoric acid (98:2, v/v). The flow rate was 0.5 ml/min and the column temperature 40 °C. The detection was performed at 340 nm. Each extract was injected without dilution. The standards used were: caffeic acid 0.01025 mg/ml, cichoric acid 0.01 mg/ml, rosmarinic acid 0.0108 mg/ml, sinesetine 0.6 mg/ml and eupatorine 0.4 mg/ml. 20 μl was injected from each standard and sample.

2.4. Experimental conditions for pharmacological studies

The used experimental conditions for determination of diuretical action were presented by Tamas et al. [7,8].

The experimental animals were white Wistar Bratislava male rats (ca. 200 g each). All animals were maintained in standard condition and they were selected by checking the diuresis with water. The rats having a minimal 40% diuresis in 2 h were

selected. In last 24 h before experiments the rats do not eat or drink anything.

Four series of five rats were used and the samples were administrated orally.

It was administrated:

- series I (the blank series): 1 ml water/rat;
- series II (the comparison series): 30 mg furosemid/kg rat;
- series III: 700 mg tincture A/kg rat;
- series IV: 700 mg tincture B/kg rat.

The urine of each rat was collected after 24 h and then the urine was analyzed for determination of sodium, potassium and uric acid content.

3. Results and discussions

3.1. Identification

The identification of the main polyphenols was performed by comparison of retention times and the wavelength with maximum absorbency from UV–Vis spectra of standards and the separated compounds in same chromatographic conditions.

The retention times and the wavelength with maximum absorbency of the standards are presented in Table 1. Fig. 1(A and B), respectively, Fig. 2(A–E) show the obtained chromatograms and UV–Vis spectra.

The chromatograms show a good separation of the main components—the method is specific. The order of elution can be explained by the separated compounds' polarity. The most polar compounds (as caffeic acid derivatives) are eluting before the less polar compounds (as polymethoxylated flavonoids) because the stationary phase is non-polar.

Table 1
The retention times and the wavelength with maximum absorbency for the standards

Standard	Retention time (min)	Wavelength with maximum absorbency (nm)
Caffeic acid	6.71	321
Cichoric acid	12.20	329
Rosmarinic acid	13.55	328
Sinesetine	26.51	273 and 336
Eupatorine	30.27	266 and 321

On basis of these results it was identified in both tinctures: caffeic-, cichoric- and rosmarinic acids from caffeic acid derivatives and sinesetine and eupatorine from flavonoids.

3.2. Quantitative determinations

The quantitative determinations were performed in the same HPLC conditions using the external standard method. The cichoric acid was determined using a correction factor (equal to 1.21) relative to caffeic acid. The results are the mean of six determinations. The RSD for repeatability (precision) and the RSD for accuracy were calculated.

Table 2 shows the results of quantitative determination and the calculated RSD for repeatability and the RSD for accuracy.

The results show that the main caffeic acid derivatives from tinctures is the rosmarinic acid. The cichoric acid is an important component too. The most important component from the flavonoid class is the sinesetine. Qualitatively the flavonoids are better represented as the caffeic acid derivatives in both tinctures.

Comparing the two studied tinctures a significant quantitative difference can be noticed in case of caffeic acid and rosmarinic acid. The quantity of the determined compounds is higher in tincture A, an exception being the rosmarinic acid which is more in tincture B. The most polar caffeic acid is extracted more in tincture A with most water and the less polar rosmarinic acid is extracted more in tincture B with most ethanol.

Considering as validation criteria for repeatability a RSD not higher than 2% and for accuracy a RSD not higher than 1%, on basis of results presented in Table 2, the used method is precise and accurate.

3.3. The pharmacological studies

The diuresis was calculated as ml/24 h per kg rat, the saluresis as mEq/24 h per kg rat and the uricosuresis as mg/24 h per kg rat. These were

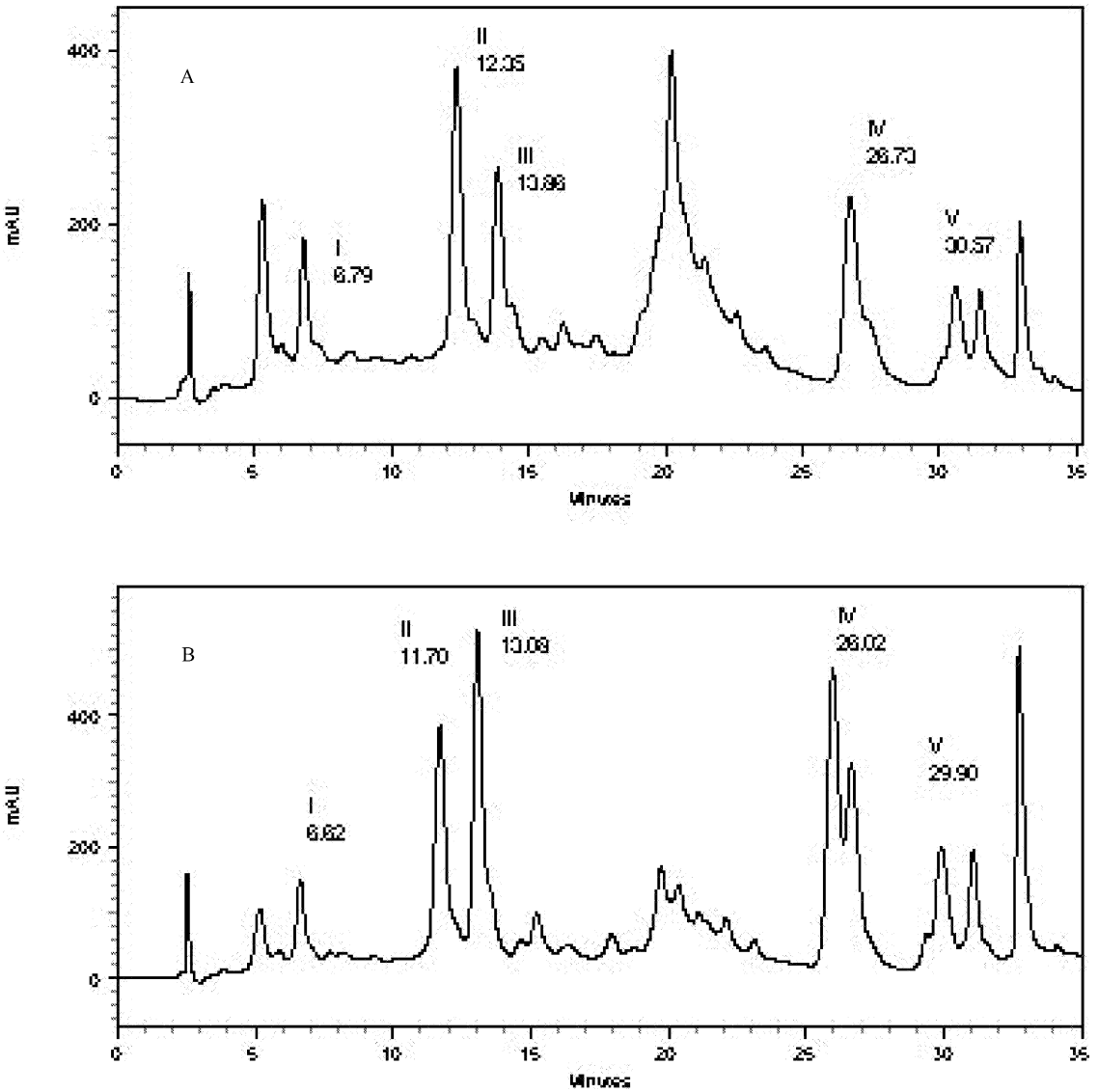

Fig. 1. Chromatograms of (A) tincture A, (B) tincture B.

calculated as ratio between the samples and blank the diuretic index, the sodium–saluretic index, the potassium–saluretic index and the elimination of uric acid in percent.

Table 3 presents the results of pharmacological studies.

The results show that the tincture A has a better diuretical action than the tincture B. The tincture A has eliminated better the sodium as the tincture B or the furosemid, usefully diuretic, but it has preserved the potassium for body better than the furosemid or the tincture B. The experiments on same tincture A indicated a very good elimination of uric acid.

The tincture A has a higher content of main compounds as the tincture B and it has more water. By correlation of phytochemical and pharmacological results can be affirmed that the phytocomplex formed from more polar compounds like the caffeic acid derivatives and the

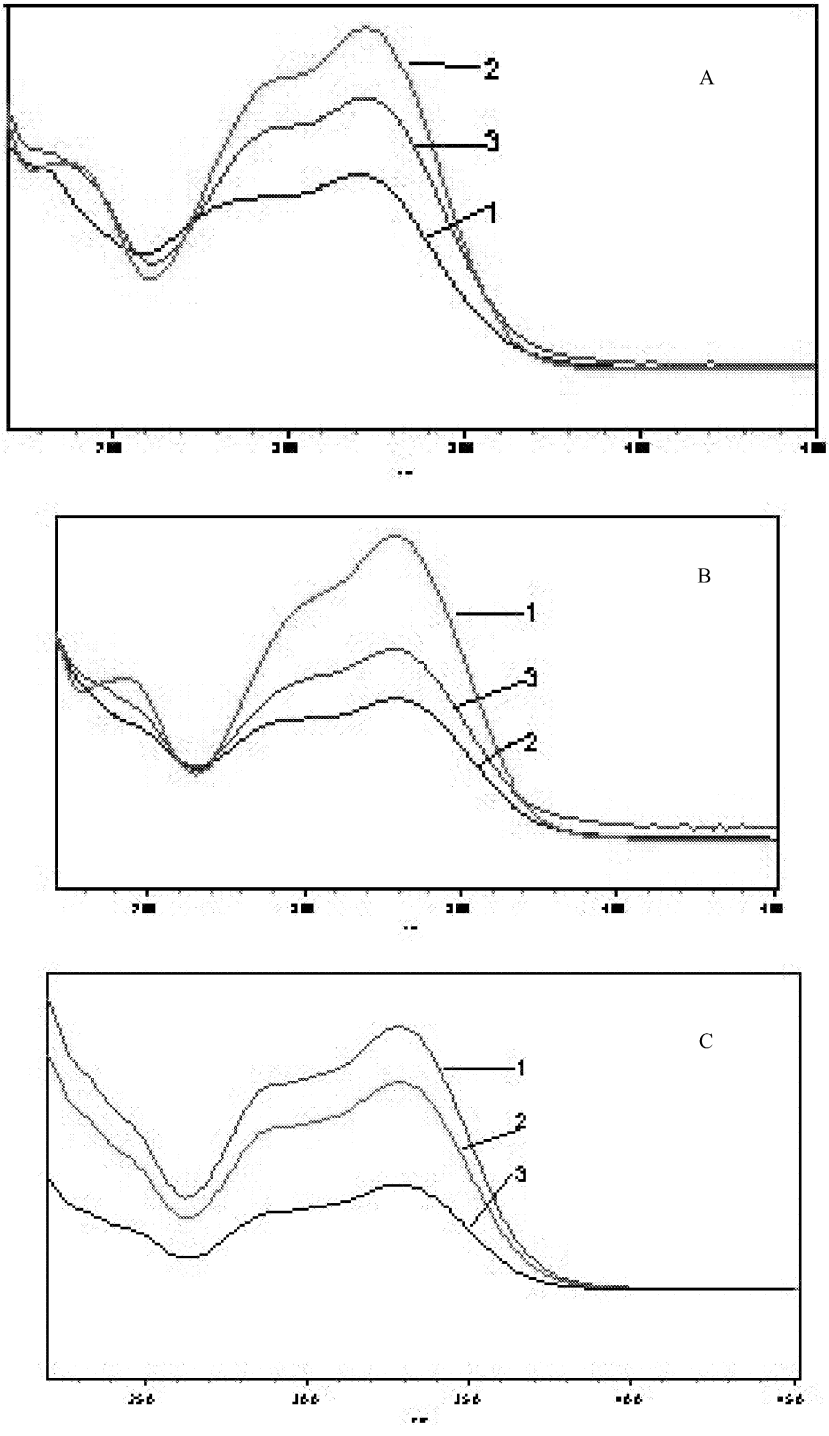

Fig. 2.

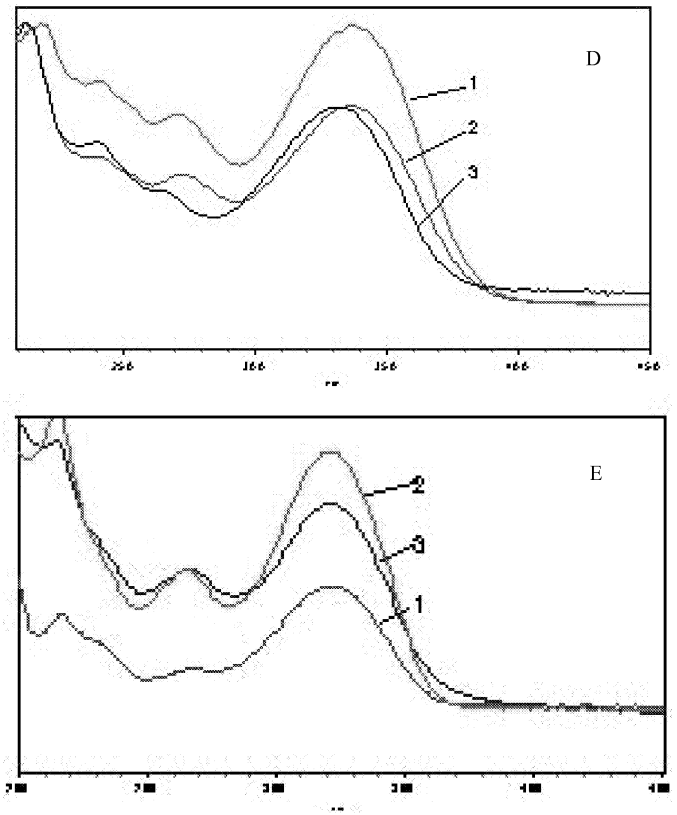

Fig. 2. UV–Vis spectra of (A) standard caffeic acid (1), caffeic acid separated from tincture A (2), caffeic acid separated from tincture B (3); (B) standard cichoric acid (1), cichoric acid separated from tincture A (2) cichoric acid separated from tincture B (3); (C) standard rosmarinic acid (1), rosmarinic acid separated from tincture A (2), rosmarinic acid separated from tincture B (3); (D) standard sinesetine (1), sinesetine separated from tincture A (2), sinesetine separated from tincture B (3); (E) standard eupatorine (1), eupatorine separated from tincture A (2), eupatorine separated from tincture B (3).

Table 2
The results of quantitative determination and the RSD value for repeatability (RSD_r) and accuracy (RSD_a)

Compounds	Samples					
	Tincture A			Tincture B		
	Concentration (mg/ml)	RSD_r	RSD_a	Concentration (mg/ml)	RSD_r	RSD_a
Caffeic acid	0.025 ± 0.0004	1.58%	0.64%	0.017 ± 0.0003	1.70%	0.70%
Cichoric acid	0.071 ± 0.0004	0.51%	0.21%	0.073 ± 0.0005	0.65%	0.27%
Rosmarinic acid	0.091 ± 0.0016	1.69%	0.69%	0.117 ± 0.0018	1.45%	0.59%
Sinesetine	3.000 ± 0.0415	1.32%	0.54%	2.850 ± 0.0378	1.26%	0.52%
Eupatorine	1.530 ± 0.0196	1.22%	0.50%	1.430 ± 0.0179	1.19%	0.49%

Table 3
The results for pharmacological tests

Series	Samples	Diuresis (ml/24 h per kg rat)	Diuretic index		
1	Blank	32.41 ± 3.44	–		
2	Furosemid	80.61 ± 6.12	2.49		
3	Tincture A	41.19 ± 4.37	1.27		
4	Tincture B	28.87 ± 3.89	0.89		
Series	Samples	Saluresis (mEq/24 h per kg rat)		Saluretic index	
		Sodium	Potassium	Sodium	Potassium
1	Blank	3.56 ± 0.41	0.55 ± 0.08	–	–
2	Furosemid	4.45 ± 0.35	2.51 ± 0.20	1.25	4.56
3	Tincture A	5.72 ± 0.60	1.14 ± 0.12	1.61	2.07
4	Tincture B	4.59 ± 0.61	0.85 ± 0.11	1.29	2.34
Series	Samples	Quantity of uric acid (mg/24 h per kg rat)	Uric acid elimination (%)		
1	Blank	3.72 ± 0.58	–		
3	Tincture A	6.96 ± 0.73	187.10		
4	Tincture B	4.96 ± 0.66	133.33		

less polar compounds like the polymethoxylated flavonoids is responsible for the diuretic and uricosuric action of tincture A.

On basis of these results the tincture A can be indicated as diuretic and in gout.

4. Conclusions

The main polyphenols: sinesetine, eupatorine, rosmarinic-, cichoric- and caffeic-acids were identified and quantitatively determined in two different *O. stamineus* Benth. tinctures. The used HPLC method was evaluated. The two studied tinctures were compared as pharmacological action. The results were discussed. The *O. stamineus* Benth. tincture obtained with 50% vol. ethanol has a higher content of the determined compounds and a better pharmacological action: diuretic and uricosuric.

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