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Comparison of classic and modern methods of furocoumarins extraction from Angelica officinalis Hoffm. and Pastinaca sativa L. fruits

Porównanie klasycznych i nowoczesnych metod ekstrakcji furanokumaryn z owoców *Angelica officinalis* Hoffm. i *Pastinaca sativa* L.

Extraction process of natural secondary metabolites from plant material is in many cases less expensive than chemical synthesis and in some cases it is the only way to obtain natural compounds from plant material. In this work optimisation of natural coumarins extraction is undertaken in selected species of *Apiacece* family. In *Archangelica officinalis* and *Pastinaca sativa* fruits the concentration of furocoumarins is high [14]. Natural coumarins and furocoumarins are described as phytoalexins in plants' defense system against fungi infection and UV radiation [1, 14]. *A. officinalis* and *P. sativa* frutis contain imperatorin, xanthotoxin, bergapten and isoimperatorin [3, 8]. Furocoumarins (especially xanthotoxin and bergapten) have application in dermatology as drugs in the PUVA therapy of vitiligo and psoriaris [6, 7, 11, 12]. Since imperatorin affects brain receptors, it poseses anticonvulsant activity [2, 5], and isoimperatorin shows a potent hepatoprotective effect [9]. Furocoumarins as apolar compounds are usually extracted with non-polar solvents as petrolatum ether or dichloromethan [3]. Classical methods of furocoumarins extraction are maceration and extraction on cooling water bath. More modern technics are maceration with shaking, sonication-assisted extraction and sonication-assisted extraction after homogenisation. In this work all these methods were tested and concentration of furocoumarins in particular extracts was determined by densitometry detection [15].

EXPERIMENTAL DESIGN

Solvents for extraction were purchased from POCh SA (Gliwice, Poland) or J.T. Baker, (USA). Standards of high purity were purchased from Sigma–Aldrich Chemie GmbH (Munich, Germany), Merck (Darmstadt, Germany) and from other suppliers. Fruits of *Archangelica officinalis* and *Pastinaca sativa* were collected in the Botanical Garden of Medical Univercity of Lublin in September 2007. The plant material was dried at room temperature, powdered and extracted with different modes.

Classic maceration (MAC). Pulverized fruits (5g) of *A. officinalis* and *P. sativa* were subjected to 24h maceration at room temperature with petroleum ether (50ml). The obtained extracts (A1 and P1) were filtered and evaporated to dryness in a vacuum evaporator under reduced pressure.

Maceration with shaking (MWS). Pulverized fruits (5g) of *A. officinalis* and *P. sativa* were subjected to 12h maceration with constant shaking at room temperature with petroleum ether (50ml). The obtained extracts (A2 and P2) were filtered and evaporated to dryness in a vacuum evaporator under reduced pressure.

Simple extraction method (SEM). Pulverized fruits (5g) of *A. officinalis* and *P. sativa* were subjected to 12h maceration and then to the extraction on water bath in the boiling temperature of the solvent (50ml of petroleum ether), under reflux for 40 min. The obtained extracts were filtered and the remaininig fruits were subjected twice to 40 min. extraction with 50ml of petroleum ether. All filtrates were combined and evaporated to dryness in a vacuum evaporator under reduced pressure. As a result of this procedure, extracts A3 and P3 were obtained.

Ultrasound-assisted extraction 80% (UAE-80) and 100% (UAE-100). Pulverized fruits (5g) of *A. officinalis* and *P. sativa* were subjected to 12h maceration at room temperature with petroleum ether (50ml). The macerats were extracted for 40 min. on the wather bath at 50ºC under reflux with 80% and 100% sonification. Then the obtained extracts were filtered and the remaininig fruits were subjected twice to 40min. extraction with 50ml of petroleum ether. All filtrates were combined and evaporated to dryness in a vacuum evaporator under reduced pressure. As a result of this procedure, extracts A4 and P4 (UAE-80) and A5 and P5 (UAE-100) were obtained.

Modified ultrasound-assisted extraction 100% (M-UAE). Pulverized fruits (20g) of *A. officinalis* and *P. sativa* were put in the mechanical homogenizer with 200ml of petroleum ether and mixed on low rotation. The homogenized mixtures were extracted for 40 min. on the wather bath at 50ºC under reflux with 100% sonification. Then the obtained extracts were filtered and the remaininig fruits were subjected twice to 40 min. extraction with 50ml of petroleum ether. All filtrates were combined and evaporated to dryness in a vacuum evaporator under reduced pressure. As a result of this procedure, extracts A6 and P6 were obtained.

In all methods used, dry residues were weighed (their content was calculated – Table 1), dissolved in methanol, transferred into 25ml volume flasks and filled up to their volume with methanol. In extracts: A3, A4, A5 and A6 after some time the residue precipitated. A3 and A6 were then quantitatively diluted to 500ml, A4 and A5 to 250ml, which was taken into consideration in the further calculations.

Table 1.Quantity of dry residue after extraction with petroleum ether

Extraction methods	Dry residue [mg/g] after extraction of *A. officinalis*	Dry residue [mg/g] after extraction of *P. sativa*
MAC	45.2	219.0
SHG	43.6	199.2
SEM	74.0	252.0
UAE-80	72.0	242.0
UAE-100	85.2	248.0
M-UAE	80.7	201.7

MAC–classic maceration, SHG–maceration with shaking, SEM–simple extraction method, UAE–80-ultrasound-assisted extraction 80%, UAE–100-ultrasound-assisted extraction 100%, M–UAE-modified ultrasound-assisted extraction 100%

TLC and densitometric analysis. TLC analysis was performed on HPTLC precoated plates Si 60 F254, 10x20cm (Merck, Darmstadt, Germany). Samples of 5 µl volume of extracts and standards were spotted on the chromatographic plates using Camag Automatic TLC Sampler III.(Camag, Muttenz, Switzerland). Plates with applied spots were developed in a horizontal DS-L chamber (Chromdes, Lublin, Poland) at the distance of 8 cm with the n-heksan – dichloromethan – ethyl acetate (5:5:1v/v/v) as a mobile phase. TLC chromatograms with separated coumarin extracts and standards were scanned (λ=306 nm for xanthotoxin, λ=316 nm for bergapten) with densitometer Camag TLC scanner3 with computer program CATS 4 (Camag). Visualisation of spots was performed under UV light (λ=254 and 366 nm) using Camag TLC Reprostar 3 with a computer program Videostore. Identification of coumarin compounds was carried out by means of comparing their UV spectra and their Rf with spectra and Rf values of adequate standards. Quantitative analysis of identified compounds was performed with the external standard, bergapten, imperatorin, isoimperatorin, xanthotoxin, by the calibration curve method. For the calibration curve bergapten and xanthotoxin were dissolved at methanol at the concentration of 1mg/ml, and were then diluted to 0.4, 0.33, 0.25 and 0.2 mg/ml each. Regression coefficients of bergapten and xanthotoxin calibration curves were $r = 0.9945$ and $r = 0.9731$, respectively.

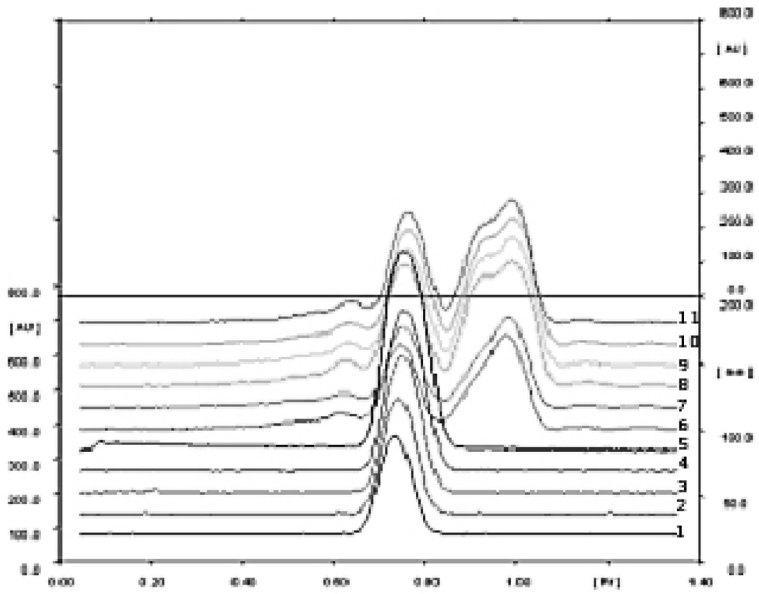

Fig. 1. Densitogram of TLC Si60 F_{254} glass plate with *P.sativa* samples developed with n-heksan – dichloromethan – ethyl acetate (5:5:1v/v/v). Lines 1-5 xanthotoxin standard (1, 1.25, 1.65, 2 and 5 µg/5µl), lines 6-11 extract prepared with method 1, 2, 3, 4, 5, 6. Detection in 306 nm (maximum absorption for xanthotoxin)

RESULTS AND DISCUSSION

As a results of our investigation, different content of furocoumarins in extracts obtained using traditional (classic) and modern extraction technics was compared. Shaking *P.sativa* seeds with

petroleum ether for 12h at room temperature gave 0.7071 mg/g of xanthotoxin and 1.1013 mg/g of sum of bergapten and imperatorin which was at the lowest estimate. Slightly better results were for maceration (24h) without shaking, which suggests that during 12 hours of time the amount of coumarins extracted did not cause saturation of the extract. Maceration with shaking was not better than maceration without any kind of mixing of the plant material. 12 hours was not enough to achieve equilibrium between the compounds extracted and as solvent that would stop passing coumarins to liquid medium. Extraction on the water bath at the temperature of the boiling solvent resulted in duplication of xanthotoxin and the sum of bergapten and imperatorin extracted comparing to the first and second methods (Table 2). Sonification 80% and 100% differ slightly in efficiency of coumarins extracted. The best yield of the extracted compounds was obtained by means of homogenization followed by ultrasonic-assisted extraction 100%: 1.8094 mg/g for xanthotoxin and 0.9670 mg/g for bergapten, respectively (Fig. 3). Only in case of imperatorin, homogenization before ultrasonic-assisted extraction was not the most effective method. The best results for imperatorin was obtained using sonication-assisted extraction 100% (0.7375mg/g).

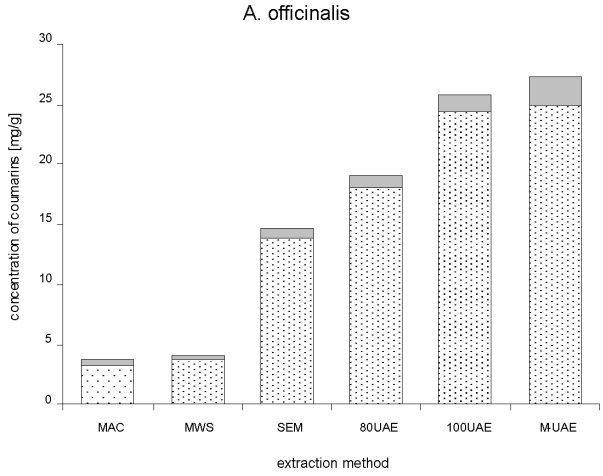

Fig. 2. Yield of furocoumarins extracted from *A. officinalis* with diferrent extraction methods. Lower column: bergapten+ imperatorin[mg/g], Upper column: xanthotoxin [mg/g], MAC–classic maceration, SHG–maceration with shaking, SEM–Simple extraction method, UAE–80-ultrasound-assisted extraction 80%, UAE–100-ultrasound-assisted extraction 100%, M–UAE-modified ultrasound-assisted extraction 100%

Investigation of *Archangelica officinalis* seeds revealed that the lowest amount of coumarins extracted gave 12 hours maceration with shaking at room temperature (Table 2). 24 hours' maceration at room temperature gave only slightly better results for xanthotoxin and the sum of bergapten and imperatorin. The methods of extraction on the water bath at the temperature of the boiling solvent under reflux, ultrasonic-assisted extraction 80% and 100% resulted in the quantities given in Table 2. Sonication as an additional energy input in extraction improves efficiency better than only high temperature used. In the strong ultrasound field in liquids extraction accelerates [10, 13]. The highest amount of coumarins was obtained by means of homogenosation of plant material followed by ultrasonic-assisted extraction 100% (Fig. 2). Homogenisaton enables breaking plant cells and eases

extrahent access. Preaparing plant material for extraction by blending it with the solvent provides even better efficiency than accelerated solvent extraction [15].

Table 2. Amount of coumarins[mg/g] *A. officinalis* and *P. sativa* extracted by different methods. Sum of bergapten and imperatorin was calculated on bergapten

Extraction methods	*Pastinaca sativa*		*Archangelica officinalis*	
	xanthotoxin	bergapten+ imperatorin		
	xanthotoxin	bergapten+ imperatorin	xanthotoxin	bergapten+ imperatorin
MAC	0.8258	1.3789	0.4633	3.8311
SHG	0.7071	1.1013	0.2232	3.2247
SEM	1.2737	2.3261	0.8336	13.9145
UAE-80	1.4604	2.5353	1.0298	18.0608
UAE-100	1.5222	2.9754	1.4199	24.4173
M-UAE	**1.8094**	**3.3109**	**2.2511**	**25.0069**

MAC–classic maceration, SHG–maceration with shaking, SEM–simple extraction method, UAE–80-ultrasound-assisted extraction 80%, UAE–100-ultrasound-assisted extraction 100%, M–UAE-modified ultrasound-assisted extraction 100%

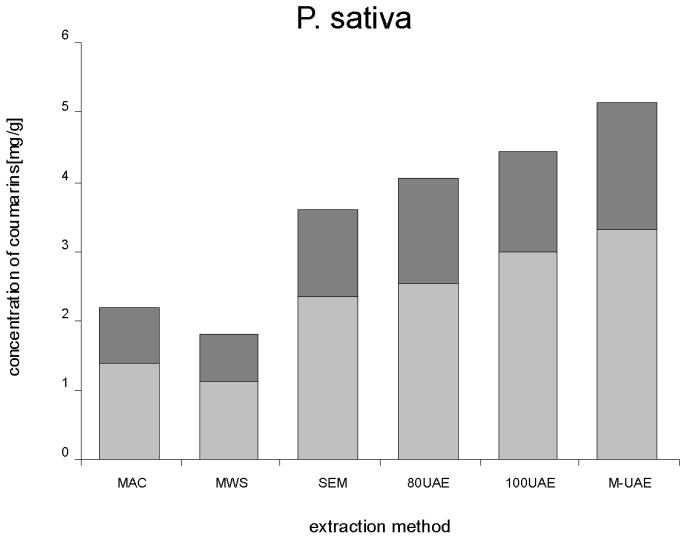

Fig. 3. Yield of furocoumarins extracted from *P. sativa* with diferrent extraction methods. Lower column: bergapten+ imperatorin [mg/g], Upper column: xanthotoxin [mg/g], MAC-classic maceration, SHG–maceration with shaking, SEM-simple extraction method, UAE-80-ultrasound-assisted extraction 80%, UAE-100-ultrasound-assisted extraction 100%, M-UAE-modified ultrasound-assisted extraction 100%

CONCLUSIONS

Petroleum ether was used as extrahent in all methods since non-polar solvent is the most suitable for extraction of furocoumarin compounds [3]. All extracts obtained using the aforementioned methods can be subjected to TLC and densitometric analysis without any additional purification. Among the investigated modified periodic extraction methods, used for *A.officinalis* and *P.sativa* fruits, the highest yield of furocoumarin compounds was achieved by means of homogenization followed by 100% sonification under reflux at the temperature of the boiling solvent. The following extraction methods using increasing efficiency can be enumerated: classic maceration, maceration with shaking, simple extraction method, ultrasound-assisted extraction 80%, ultrasound-assisted extraction 100%, modified ultrasound-assisted extraction 100%.

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SUMMARY

Simple extraction methods as macetarion, shaking, extraction, sonication-assistrd extraction(80%, 100%), homogenisation followed by sonication-assisted extraction were used to obtain coumarins from *A. officinalis* and *P. sativa.* The yield of coumarin extracted was compared by means of TLC and densitometry. The best results were obtained by means of modified ultrasound-assisted extraction: 1.8094 mg/g of xanthotoxin and 3.3109 mg/g sum of bergapten and imperatorin in *Pastinaca sativa* fruits and 2.2511 mg/g of xanthotoxin and 25.0069 mg/g sum of bergapten and imperatorin in *Archangelica officinalis* fruits.

STRESZCZENIE

W celu pozyskania kumaryn z *A. officinalis* i *P. sativa* wykorzystano klasyczne metody ekstrakcji, takie jak maceracja, wytrząsanie, ekstrakcja pod chłodnicą zwrotną, ekstrakcja wspomagana ultradźwiękami (80% i 100% mocy generatora) oraz ekstrakcja wspomagana ultradźwiękami poprzedzona homogenizacją matrycy roślinnej. Ilość uzyskanych związków kumarynowych została porównana za pomocą TLC i densytometrii. W toku analizy najlepszą z porównywalnych metod okazała się ekstrakcja wspomagana ultradźwiękami po uprzedniej homogenizacji badanych surowców roślinnych. W wyniku zastosowania tej metody uzyskano 1,8094 mg/g ksantotoksyny i 3,3109 mg/g sumy imperatoryny i bergaptenu w owocach *Pastinaca sativa* oraz 2,2511 mg/g ksantotoksyny i 25,0069 mg/g sumy imperatoryny i bergaptenu w owocach *Archangelica officinalis.*