

HPLC ANALYSIS OF SALICYLIC DERIVATIVES FROM NATURAL PRODUCTS

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Abstract

Salicylate-containing herbs have been used as analgesic and anti-inflammatory remedies from ancient times. In this work we performed a comparative HPLC study of salicylic derivatives in three natural products: *Salicis cortex*, *Populi gemmae* and *Ulmariae folium*. We have identified and measured by a new, easy and fast HPLC method both salicylic acid and salicin, before and after acid hydrolysis, in ethanolic extracts of vegetal products. *Ulmariae folium* is the richest source of salicylic acid (0.295-0.487%), while *Populi gemmae* and *Salicis cortex* contain higher quantities of salicylic alcohol derivatives (0.351-0.366% and respectively 0.199-0.315% salicin).

Rezumat

Plantele conținând derivați salicilici au fost utilizate în medicina populară pentru proprietățile analgezice și antiinflamatoare încă din timpuri străvechi. În această lucrare am realizat un studiu HPLC comparativ al derivaților salicilici din trei produse naturale: *Salicis cortex*, *Populi gemmae* și *Ulmariae folium*. Printr-o metodă nouă, simplă și rapidă de cromatografie de lichide de înaltă performanță am identificat și am determinat concentrația acidului salicilic și salicinei, atât înainte, cât și după hidroliza acidă a extractelor etanolice preparate din produsele vegetale. *Ulmariae folium* este cea mai bogată sursă de acid salicilic (0,295-0,487%), în timp ce *Populi gemmae* și *Salicis cortex* conțin cantități mai mari de derivați ai alcoolului salicilic (0,351-0,366% și respectiv 0,199-0,315% salicină).

Keywords: salicylic acid, salicin, HPLC

Introduction

Salicylates are non-steroidal anti-inflammatory agents derived from salicylic acid: esters of organic acids substituted at the hydroxyl group or esters or salts substituted at the carboxyl group. The main salicylic acid derivatives are acetylsalicylic acid, methylsalicylate, salicylaldehyde and salicyl alcohol. Natural products with salicylic derivatives have widespread uses in traditional therapy as antirheumatic and analgesic remedies. The anti-inflammatory properties are attributed to salicylic acid obtained by hydrolysis of its esters or by oxidation of the salicyl alcohol, formed upon intestinal hydrolysis of salicin [1,5,8].

The flowering tops and leaves of *Filipendula ulmaria* L. Maxim (meadowsweet, *Rosaceae*) contain flavonol glycosides, tannins (10-20%),

phenolic compounds (monotropitoside, salicylaldehyde), essential oil [1,3,4,7,8,9]. *Filipendula ulmaria* is a medicinal plant traditionally used for its anti-inflammatory, antirheumatic, analgesic, astringent, antiulcerogenic, antacid and diuretic properties [1,3,5]. Unlike acetylsalicylic acid, the combination of salicylates, tannins and other constituents in meadowsweet acts to protect the inner lining of the stomach and intestines, while providing the anti-inflammatory effect of salicylates [5].

The bark of *Salix alba* L. (willow, *Salicaceae*) is rich in tannins (3-12%), flavonoids, glycosides of phenols and phenolic acids, like: salicin 0.5-1% (salicyl alcohol glucoside) and its esters: salicortin, tremulacin, populin. [1,3,6,16].

The buds of *Populus nigra* (black poplar, *Salicaceae*) contain phenolic glycosides (salicin, populin), essential oil, tannins, and they are used in traditional medicine for their anti-inflammatory, antiseptic, astringent properties [3,10].

Considering the lack of quantitative studies on these compounds, the aim of this work was to perform a comparative HPLC analysis of salicylic acid and salicin from natural products: *Salicis cortex*, *Populi gemmae* and *Ulmariae folium*. We have analysed salicylic derivatives by an original, easy and fast HPLC method before and after acid hydrolysis in ethanolic extracts of vegetal products obtained from indigenous sources.

Materials and methods

We analysed the following natural products: *Salicis cortex*, *Populi gemmae* and *Ulmariae folium*. The leaves of *Filipendula ulmaria* were harvested from Cluj, Romania in May 2006, while the willow bark and the poplar buds were obtained from commercial samples. The analysed solutions were prepared as follows: 2g of powdered vegetal product were extracted with 70% ethanol for 30 min on a water bath at 80°C. After extraction, the mixtures were centrifuged at 4000 rpm. In order to study the presence of salicylic acid and salicin after hydrolysis, we mixed these solutions together with 2 M hydrochloric acid, and the solutions were heated at 80°C for 60 min on a water bath. The mixtures were centrifuged at 4000 rpm. The solutions were diluted with distilled water in a 10 mL volumetric flask and filtered through a 0.45 µm filter before injection [13,14].

HPLC determinations:

Apparatus and chromatographic conditions: We used an Agilent 1100 HPLC Series (Agilent, USA) equipped with a degasser G1322A, HP 1100 Series binary pump, a Zorbax SB-C18 reversed-phase analytical

column 100 mm x 3.0 mm i.d., 3.5 μ m particle (Agilent technologies, USA), and we operated at 45°C. The mobile phase was a binary gradient: distilled water with 0.1% (v/v) orthophosphoric acid 85% and acetonitrile. The linear gradient started at 5% acetonitrile for 2 minutes, followed by isocratic elution with 25% acetonitrile over the next 3 minutes. The flow rate was 1 mL/min and the injection volume was 10 μ L [2,11,12,14,15].

Detection: The fluorescence detector at 310 nm as excitation wavelength and 450 nm as emission wavelength. Salicylic acid and salicin were identified by the external standard method and by comparing of the retention times (R_T) with the ones of the standards, in the same chromatographic conditions and quantified by the external standard method.

The wavelengths for fluorescence detection were chosen in order to give an optimal sensitivity and selectivity to this method.

Standards of salicylic acid (Sigma, Germany) and salicin (Sigma, Germany) were used in order to perform quantitative determinations in distilled water solutions with concentrations between 68-21960 ng/mL, and respectively 64-20540 ng/mL (Table I). The HPLC chromatogram for standards is presented in Figure 1.

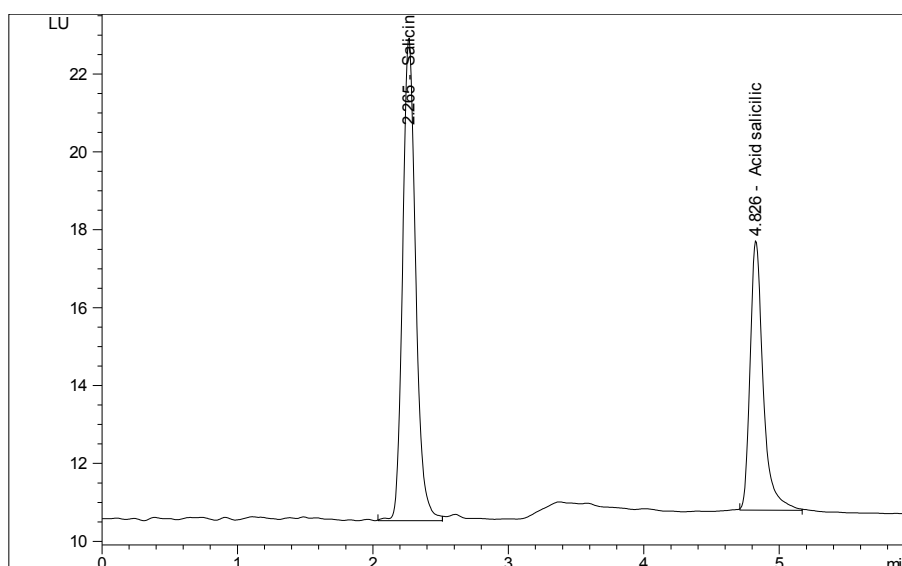


Figure 1
HPLC chromatogram for standards: salicin ($R_T=2.2$ min)
and salicylic acid ($R_T=4.8$ min)

Table I
The concentrations of salicylic acid (ng/mL) and salicin (ng/mL) for the calibration curves

Salicylic acid		Salicin	
Concentration (ng/mL)	Area (mAU•sec)	Concentration (ng/mL)	Area (mAU•sec)
68.625	6.48	64.187	10.46
137.25	12.07	128.375	21
274.5	22.8	256.75	40.16
549	46.21	513.5	81.1
1098	85.7	1027	146.1
2196	156.5	2054	278.2
5490	449.7	5135	721.7
10980	918.2	10270	1459.1
21960	2040.1	20540	3103.4

The calibration curve of salicylic acid standard was linear between 68.625-21960 ng/mL (Figure 2a), and the calibration curve of salicin standard was linear between 64.187-20540 ng/mL (Figure 2b).

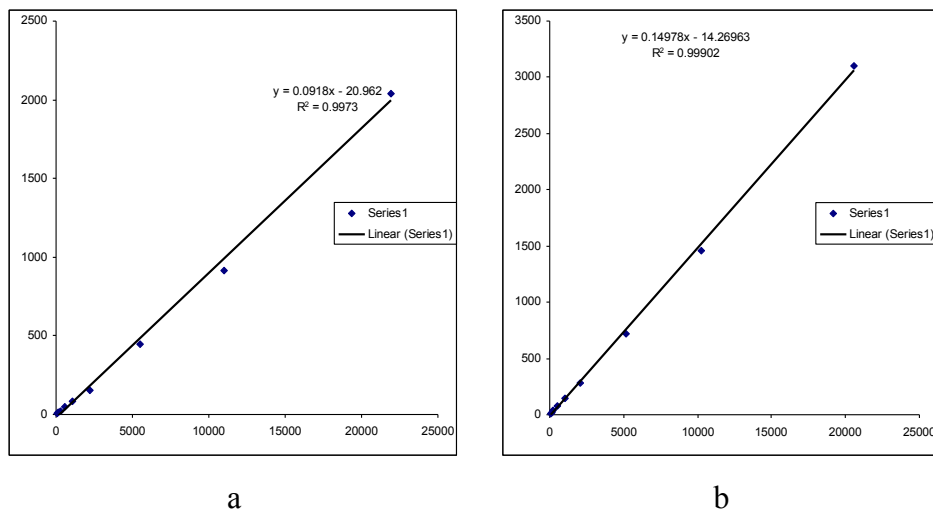


Figure 2
The calibration curves of salicylic acid (a) and salicin (b)

Results and discussion

The results of the quantitative determination of phenolic compounds from the three natural products: *Ulmariae folium*, *Populi gemmae* and *Salicis cortex* are shown in table II.

Table II
Results of the quantitative determinations of salicin and salicylic acid

Natural Product	Salicin (%)		Salicylic acid (%)	
	Before hydrolysis	After hydrolysis	Before hydrolysis	After hydrolysis
<i>Ulmariae folium</i>	0	0	0.295	0.487
<i>Populi gemmae</i>	0.351	0.366	0.120	0.133
<i>Salicis cortex</i>	0.199	0.315	0	0.20

The HPLC chromatograms of the hydrolysed extracts of *Populi gemmae* and *Salicis cortex* are presented in Figure 3.

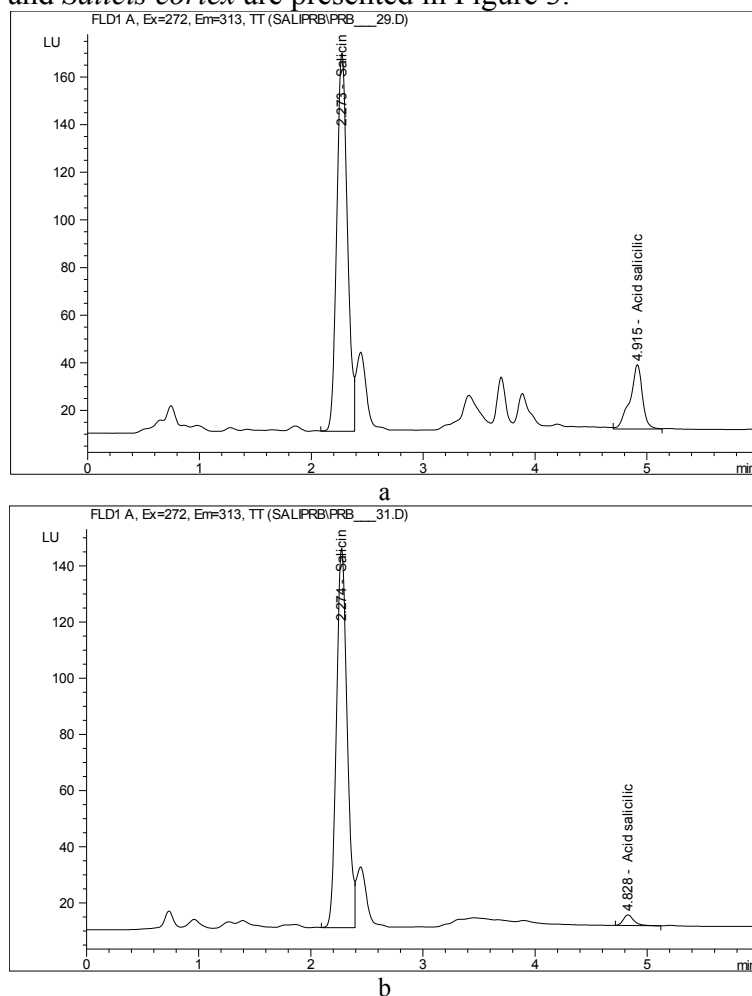


Figure 3
HPLC chromatograms of the hydrolysed extracts of *Populi gemmae* (a)
and *Salicis cortex* (b)

After the acid hydrolysis, the quantity of these compounds increased. We can consider that salicylic acid is present in natural products both free and as esters derivative. The higher concentration of salicin can be explained by the hydrolysis of some glycosidic esters (salicortin, tremulacin, populin) from the natural products and we can suppose that the glycosidic bound was not hydrolysed in the used conditions.

Ulmariae folium contains the highest quantity of salicylic acid and derivatives, while salicin and its derivatives are missing. *Populi gemmae* contains salicin, salicylic acid and a small quantity of their derivatives. *Salicis cortex* contains mainly salicin and its derivatives.

Because the identified compounds are very important for their therapeutic activities, the use of *Salicis cortex*, *Populi gemmae* and *Ulmariae folium* as raw material, in order to obtain anti-inflammatory drugs, is justified.

Conclusions

A fast and easy HPLC method was used to identify and quantify salicylic derivatives in three natural products: *Salicis cortex*, *Populi gemmae* and *Ulmariae folium* obtained from indigenous flora.

Ulmariae folium is the richest source of salicylic acid, while *Populi gemmae* and *Salicis cortex* contain higher quantities of salicylic alcohol derivatives, so they can be recommended as anti-inflammatory products.

References

1. Bruneton J., Pharmacognosy. Phytochemistry. Medicinal Plants, 2nd Edition, Ed. Lavoisier, Paris, 1999, 251-253.
2. Croci A.N., Cioroiu B., Lazar D., Corciova A., Ivanescu B., Lazar M.I., HPLC evaluation of phenolic and polyphenolic acids from propolis, *Farmacia*, 2009, 57(1), 52-57.
3. Istudor V., Farmacognozie, fitochimie, fitoterapie. Vol. 1. Editura Medicală, București, 1998, 107-110.
4. Krasnov E.A., Raldugin V.A., Shilova I.V., Avdeeva E.Y., Phenolic compounds from *Filipendula ulmaria*, *Chem Nat Comp*, 2006, 42(2), 148-151.
5. Mills S., Bone K., Principles and practice of phytotherapy. Modern herbal medicine, Churchill Livingstone, Edinburgh, 2000, 60-61, 148-149, 479-482.
6. Palade M., Dinu M., Codreanu M.V., Pavel M., Cercetări farmacobotanice în vederea valorificării frunzelor de *Salix babylonica* L., *Farmacia*, 53 (3), 2005, 32-40.
7. Papp I., Apati P., Andrasek V., Blazovicz A., Balasz A., Kursinzi L., et al., LC-MS analysis of antioxidant plant phenoloids, *Chromatographia*, 2004, 60, s93-s100.
8. Papp I., Simandi B., Blazics B., Alberti A., Hethelui E., Szöke E., Kery A., Monitoring volatile and non-volatile salicylates in *Filipendula ulmaria* by different chromatographic techniques, *Chromatographia*, 2008, 68, s125-s129.
9. Tămaș M., Roșca M., Suci-Iorga I., Târnoveanu D., Cercetări fitochimice comparative asupra florilor de *Filipendula ulmaria* (L.) Maxim și *F. hexapetala* (L.) Gilib, *Herba Romanica*, 1993, 12, 89-96.
10. Tămaș M., Botanică farmaceutică. Vol.III Sistematica-Cormobionta, Editura Medicală Universitară "Iuliu Hațieganu", Cluj-Napoca, 1999, 66, 144-145.

11. Tero-Vescan A., Imre S., Vari C.E., Oşan A., Dogaru M.T., Csödö C., Determination of some isoflavonoids and flavonoids from *Genista tinctoria* L. by HPLC-UV, *Farmacia*, 2009, 57(1), 120-127.
12. Toiu A., Muntean E., Oniga I., Tămaş M., Pharmacognostic research on *Viola declinata* Waldest. Et Kit. (*Violaceae*), *Farmacia*, 2009, 57(2), 218-222.
13. Toiu A., Vlase L., Oniga I., Tămaş M., HPLC analysis of salicylic acid derivatives from *Viola* species, *Chem Nat Comp*, 2008, 44(3), 357-358.
14. Toiu A., Vlase L., Oniga I., Tămaş M., Quantitative analysis on some phenolic compounds from *Viola* species tinctures, *Farmacia*, 2008, 56(4), 440-445.
15. Toiu A., Vlase L., Oniga I., Tămaş M., LC-MS analysis of flavonoids from *Viola tricolor* L. (*Violaceae*), *Farmacia*, 2007, 55(5), 509-515.
16. Wagner H., Bladt S., Plant Drug Analysis. Springer Verlag, Berlin, 1996, 249-250.

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