# CELL CULTURES OF THREE MEDICINAL PLANT SPECIES AS A SOURCE OF THERAPEUTICALLY IMPORTANT PHENOLIC ACIDS


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## INTRODUCTION

Phenolic acids possess valuable properties for therapy, e.g. antioxidant, anticancer and immunostimulating activities. Interesting large amounts may be produced in plant cell cultures.

## AIM

The studies are focused on phenolic acids produced by tissue cultures of *Aronia melanocarpa* (Michx.) Elliott, *Anethum graveolens* L. and *Schisandra chinensis* (Turcz.) Baill.

## MATERIAL AND METHODS

**I. *In vitro* cultures** (Fig. 1-4)

- medium: acc. to Murashige and Skoog (MS) and acc. to Linsmaier and Skoog (LS)
- plant growth regulators: BAP (6-benzyladenine) from 0.1 to 3.0 mg/l and NAA (α-naphthaleneacetic acid) from 0.1 to 2.0 mg/l
- temperature: 25 ± 2$^0$ C
- growth cycles: 4 – weeks, 3 series
- type of cultures: agar cultures
- light conditions: constant artificial light (4 W/m$^2$, LF-40W lamp, daylight, Piła)

**II. Plant material**

- fruits and/or leaves of native plants growing in the Rogów Arboretum, Warsaw University of Life Sciences, Forest Experimental Station in Rogów (Poland)

**III. Hydrolysis and extraction**

- methanolic extracts of biomass from *in vitro* cultures collected after 4 weeks growth cycles and from plant material were analyzed after acid hydrolysis (2 M HCl, 2h)

**IV. HPLC conditions**

- HPLC apparatus: Merck - Hitachi
- column: Kinetex™ 18C (150 x 4.6 mm, 2.6 µm)
- solvent system: 0.1 % trifluoroacetic acid (A) and acetonitrile (B) (gradient program) [1]
- injection volume: 5µl
- flow rate: 1ml/min
- detector UV: λ = 254 nm
- standards: caffeic, cinnamic, chlorogenic, protocatechuic, rosmarinic, salicylic, sinapic, syringic acids (Sigma); p-coumaric, ferulic, p-hydroxybenzoic, vanillic acids (Fluka)

## RESULTS

1. The maximum total contents of phenolic acids in cell cultures on LS and MS medium were as follows:
   - *A. melanocarpa* shoot cultures - 163.34 and 230.25 mg/100 g d.w., respectively [2] (Tab. 1)
   - *A. melanocarpa* callus cultures - 102.31 and 96.55 mg/100 g d.w. , respectively [3] (Tab. 2)
   - *A. graveolens* callus cultures - 49.38 and 120.40 mg/100 g d.w., respectively [4,5] (Tab. 3)
   - *S. chinensis* shoot-differentiating callus cultures - 115.64 mg/100 g d.w. (Tab. 4)
   - *S. chinensis* callus cultures - 165.40 mg/100 g d.w. (Tab. 4).
2. The cultures mainly produced p-hydroxybenzoic, salicylic, p-coumaric and chlorogenic acids (Tab. 1-4).

## CONCLUSIONS

The basic components and plant growth regulators (BAP and NAA) concentrations in culture media as well as the degree of differentiation in tissue cultures are of great influence on the accumulation of phenolic acids. Medicinal plant tissue cultures can become good source of some therapeutically important phenolic acids.

Fig. 1. *Aronia melanocarpa* shoot culture cultivated on the MS medium containing 2 mg/l BAP and 2 mg/l NAA

Tab. 1. Contents (mg/100 g d.w.) of phenolic acids in biomass extracts from shoot cultures of *Aronia melanocarpa* cultivated on different MS and LS medium variants and their contents in fruits of native plant

| Phenolic acids | MS medium variants BAP:NAA (mg/l) | | | | | | | LS medium variants BAP:NAA (mg/l) | | | | | Fruits |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *0.1:2* | *0.5:2* | *1:0.5* | *2:0.5* | *2:1* | *2:2* | *3:1* | *0.1:0.1* | *1:1* | *1:0.5* | *2:2* | *3:1* | |
| Caffeic acid | 2.74 | 6.45 | 5.91 | 3.40 | 12.35 | **13.74** | 10.62 | 5.73 | 7.62 | 11.14 | 10.00 | 1.57 | 5.26 |
| p-Coumaric acid | 22.21 | **65.24** | 54.91 | 46.31 | 42.14 | 39.33 | 47.64 | 2.95 | 6.44 | 14.40 | 18.46 | 2.17 | 3.24 |
| p-Hydroxybenzoic acid | 5.03 | 8.89 | 51.90 | 29.49 | 17.07 | 43.93 | 28.56 | 47.95 | **56.65** | 40.22 | 31.70 | 18.42 | 51.55 |
| Salicylic acid | 41.28 | 81.05 | 95.94 | 59.38 | 53.95 | **88.91** | 70.51 | 80.01 | 74.11 | 54.54 | 51.96 | 84.82 | 22.44 |
| Syringic acid | **31.82** | 34.31 | 11.35 | 15.26 | 18.22 | 28.40 | 16.31 | 19.09 | 5.24 | 4.25 | 5.03 | 9.81 | 4.46 |
| Vanillic acid | 1.34 | 17.42 | 10.24 | 13.37 | **15.87** | 14.39 | 13.82 | 7.61 | 6.52 | 18.63 | 14.39 | 7.12 | 3.65 |
| Total content | 104.43 | 213.35 | **230.25** | 167.22 | 159.60 | 228.69 | 187.46 | **163.34** | 156.58 | 143.18 | 131.54 | 123.92 | **90.60** |

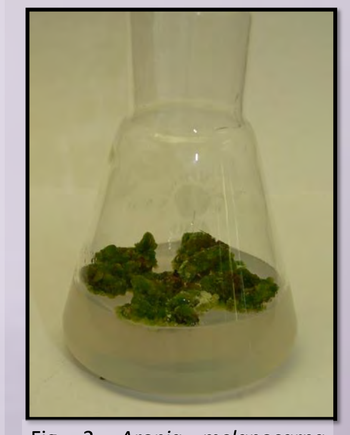

Fig. 2. *Aronia melanocarpa* callus culture cultivated on the MS medium containing 2 mg/l BAP and 1 mg/l NAA

Tab. 2. Contents (mg/100 g d.w.) of phenolic acids in biomass extracts from callus cultures of *Aronia melanocarpa* cultivated on different MS and LS medium variants and their contents in fruits of native plant (*nd – not detected)

| Phenolic acids | MS medium variants BAP:NAA (mg/l) | | | | | | | LS medium variants BAP:NAA (mg/l) | | | | | Fruits |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *0.1:2* | *0.5:2* | *1:0.5* | *2:0.5* | *2:1* | *2:2* | *3:1* | *0.1:0.1* | *1:1* | *1:0.5* | *2:2* | *3:1* | |
| Caffeic acid | nd* | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | 5.26 |
| p-Coumaric acid | 8.41 | 9.79 | 3.42 | 6.78 | 11.01 | 10.25 | 6.85 | 9.46 | **14.01** | 2.60 | 1.85 | 4.26 | 3.24 |
| p-Hydroxybenzoic acid | 19.82 | 23.86 | 24.29 | 23.35 | 24.70 | **35.33** | 18.23 | 27.71 | 23.43 | 23.86 | 23.51 | 27.23 | 51.55 |
| Salicylic acid | 20.97 | 13.89 | 19.00 | 10.50 | 16.98 | 27.53 | **31.80** | 10.50 | 9.31 | 9.22 | 11.81 | 20.52 | 22.44 |
| Syringic acid | 26.58 | 32.35 | 40.93 | 28.68 | 29.64 | 14.26 | 31.89 | 24.23 | 26.95 | **46.97** | 22.28 | 43.97 | 4.46 |
| Vanillic acid | 5.77 | 6.18 | 7.57 | **11.05** | 8.78 | 9.18 | 6.86 | 9.07 | 5.19 | 7.50 | 4.28 | 6.34 | 3.65 |
| Total content | 81.55 | 86.06 | 95.20 | 80.36 | 91.11 | **96.55** | 95.63 | 80.97 | 78.89 | 90.16 | 63.73 | **102.31** | **90.60** |

Fig. 3. *Anethum graveolens* callus culture cultivated on the MS medium containing 0.5 mg/l BAP and 2 mg/l NAA

Tab. 3. Contents (mg/100 g d.w.) of phenolic acids in biomass extracts from callus cultures of *Anethum graveolens* cultivated on different MS and LS medium variants and their contents in fruits of native plant

| Phenolic acids | MS medium variants BAP:NAA (mg/l) | | | | | | | LS medium variants BAP:NAA (mg/l) | | | Fruits |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | *0.1:2* | *0.5:2* | *1:0.5* | *2:0.5* | *2:1* | *2:2* | *3:1* | *0.1:0.1* | *1:1* | *2:2* | |
| Caffeic acid | 0.60 | 1.23 | 0.66 | 1.79 | 1.18 | 0.97 | 0.60 | 0.73 | 0.81 | **1.10** | 8.67 |
| Ferulic acid | 0.28 | 10.23 | 5.64 | **11.76** | 3.93 | 7.50 | 4.76 | 3.49 | 6.16 | 4.10 | 3.21 |
| p-Hydroxybenzoic acid | 22.33 | **42.55** | 26.38 | 26.50 | 31.00 | 36.74 | 28.98 | 22.93 | 23.34 | 25.80 | 11.75 |
| Salicylic acid | **61.46** | 58.98 | 47.78 | 43.89 | 58.77 | 58.41 | 33.92 | 11.47 | 11.04 | 11.57 | 71.76 |
| Syringic acid | 2.52 | 1.74 | 2.58 | 2.03 | **3.81** | 2.09 | 1.55 | 1.68 | 1.18 | 1.24 | 46.76 |
| Vanillic acid | 5.24 | 5.66 | 6.07 | 6.73 | **9.16** | 4.87 | 8.00 | 5.23 | 6.67 | 5.56 | 11.99 |
| Total content | 92.42 | **120.40** | 89.12 | 92.70 | 107.86 | 110.59 | 77.83 | 45.51 | 49.20 | **49.38** | **154.14** |

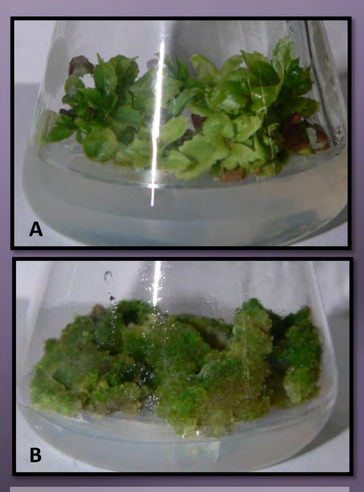


Fig. 4. *Schisandra chinensis* shoot-differentiating callus culture (A) and callus culture (B) cultivated on the MS medium containing 3 mg/l BAP and 1 mg/l NAA

Tab. 4. Contents (mg/100 g d.w.) of phenolic acids in biomass extracts from shoot-differentiating callus cultures and callus cultures of *Schisandra chinensis* cultivated on different MS medium variants and their contents in fruits and leaves of native plant

| Phenolic acids | Shoot-differentiating callus cultures | | | | | | Callus cultures | | Fruits | Leaves |
|---|---|---|---|---|---|---|---|---|---|---|
| | MS medium variants BAP:NAA (mg/l) | | | | | | | | | |
| | *0.1:2* | *0.5:2* | *2:0.5* | *2:1* | *2:2* | *3:1* | *2:2* | *3:1* | | |
| Caffeic acid | 1.04 | 3.63 | 3.33 | 1.38 | 0.74 | 1.87 | 1.60 | **3.62** | 7.37 | 0.86 |
| Chlorogenic acid | 18.92 | 39.14 | 17.00 | 22.41 | 29.16 | 43.03 | 70.54 | **82.10** | 44.35 | 2.02 |
| p-Coumaric acid | 3.59 | 15.75 | 9.23 | 4.11 | 6.57 | 12.82 | **13.19** | 11.59 | 2.87 | 1.83 |
| p-Hydroxybenzoic acid | 6.90 | 7.13 | 1.92 | 3.85 | 2.05 | 3.07 | 8.34 | **9.66** | 2.99 | 0.21 |
| Protocatechuic acid | 23.34 | 12.73 | 3.70 | 15.72 | 8.39 | 15.88 | **54.65** | 33.31 | 7.22 | 4.22 |
| Salicylic acid | 6.41 | **22.66** | 19.83 | 9.32 | 2.84 | 16.49 | 0.76 | 5.11 | 8.62 | 0.95 |
| Syringic acid | 3.14 | 6.89 | 6.55 | 1.65 | 5.29 | 9.19 | 9.41 | **9.58** | 15.42 | 0.47 |
| Vanillic acid | 1.26 | 7.72 | 5.31 | 1.55 | 0.83 | 3.12 | **6.92** | 6.15 | 6.09 | 3.60 |
| Total content | 64.61 | **115.64** | 66.87 | 59.99 | 55.86 | 105.45 | **165.40** | 161.13 | **94.91** | 14.16 |

## REFERENCES


1. Tian S. et al.: J. Chromatogr. A. 2005;1063:121-128.
2. Szopa A. Ekiert H.: Scientific Congress of the Polish Pharmaceutical Society (Naukowy Zjazd Polskiego Towarzystwa Farmaceutycznego), Białystok, Poland 2013; Abstracts p. 87.
3. Szopa A. Ekiert H.: 7$^{th}$ Polish-German Symposium on Pharmacyeutical Sciences, Gdańsk, Poland 2013; Abstracts p. 133-133; p.134-134.
4. Szopa A. Ekiert H.: Planta Med. 2013;79:1254-1254.
5. Szopa A. Ekiert H.: Planta Med. 2013;79:1253-1254.



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