ORIGINAL ARTICLE

PHYTOCHEMICAL AND PHYTOBIOLOGICAL RESEARCH UPON AERIAL PARTS AND SEEDS FROM *PEUCEDANUM OFFICINALE* L.

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Abstract

Several samples consisting of aerial parts and seeds from *Peucedanum officinale* L., harvested from ecologic crops belonging to S.C. Dacia Plant, Bod, Brasov, Romania were analysed. The aim of our research was the determination of the phytochemical profile (qualitative and quantitative tests), antioxidant activity (DPPH method) and cytotoxic properties (phytobiological test, Constantinescu method) of both herbal products. Aerial parts and seeds samples have various contents of active compounds, depending on the solvent used for extraction, i.e. 0.6517 - 0.7121 g % phenolcarboxylic acids (expressed as chlorogenic acid), 0.4890 - 0.6050 g % flavonoids (expressed as rutin) and 1.8515 - 2.0047 g % total polyphenols (expressed as tannic acid) – for aerial parts, 0.7003 - 0.7907 g % phenolcarboxylic acids, 0.8739 - 0.9872 g % flavonoids and 1.8659 - 2.1519 g % tannic acid – for seeds, respectively. Using HPLC, we have identified chlorogenic acid, caffeic acid, ferulic acid, rutin and quercetin in both raw materials. The aerial parts have 40.147 mg % rutin and 8.634 mg % quercetin, while seeds contain 29.4303 mg % rutin and 25.7465 mg % caffeic acid. The seeds have a higher antioxidant capacity than aerial parts. Bergapten and 8–methoxypsoralen were identified in both samples. These compounds might be responsible for the cytotoxic effect.

Rezumat

Au fost analizate părţile aeriene şi seminţele recoltate de la specia *Peucedanum officinale* L., introdusă în cultură ecologică la SC Dacia Plant, Bod, România. Cercetările au urmărit determinarea profilului fitochimic (analize chimice calitative şi cantitative), determinarea acţiunii antioxidante (metoda DPPH) şi comportamentul soluţiilor extractive asupra celulei vegetale (test fitobiologic, metoda Constantinescu). Dependent de solventul de extracţie probele din partea aeriană pot avea un conţinut de 0,6517 - 0,7121 g % acid clorogenic, 0,4890 - 0,6050 g % rutozidă, 1,8515 - 2,0047 g % acid tanic, iar cele din seminţe 0,7003 - 0,7907 g % acid clorogenic, 0,8739 - 0,9872 g % rutozidă, 1,8659 - 2,1519 g % acid tanic. Prin HPLC a fost decelată prezenţa acizilor clorogenic, cafeic şi ferulic, precum şi a rutozidei şi cvercetolului în cele două tipuri de produse vegetale analizate. Cantitativ, partea aeriană are un conţinut de 40,147 mg % rutozid şi 8,634 mg % cvercetol, iar cea de seminţe 29,4303 mg % rutozid şi 25,7465 mg % acid cafeic. Seminţele prezintă o capacitate antioxidantă crescută faţă de partea aeriană a produsului vegetal. În ambele probe s-au identificat bergaptenul şi 8-metoxipsoralenul, compuşi posibil responsabil de efectul uşor citotoxic.

Keywords: *Peucedanum officinale*, phytochemical analysis, antioxidant activity, cytotoxic effect

Introduction

There are twelve spontaneous species of the genus *Peucedanum* in Romanian flora. These are: *P. officinale* L., *P. longifolium* Waldst. Et Kit., *P. tauricum* M. Bieb., *P. ostruthium* (L.) W. D. J. Koch., *P. arenarium* Waldst. Et Kit., *P. carvifolia* Vill., *P. latifolium* (M. Bieb.) D. C., *P. alsaticum* L., *P. palustre* (L.) Moench., *P. cervaria* (L.) Lapery., *P. austriacum* (Jacq.) W. D. J. Koch and *P. oreoselium* (L.) Moench [3].

Previous researches conducted on different species of *Peucedanum* targeted: quantitative evaluation of polyphenolcarboxylic acids from fruits of *Peucedanum alsaticum* L. and *P. cervaria* (L.) Lap. [22]; antihyperlipidaemic effect of an extract obtained from aerial parts of *Peucedanum pastinacifolium* [17]; identification of coumarins, furanocoumarins and phyranocoumarins from *Peucedanum ostruthium*, *P. praereptorum*, *P. japonica*, *P. wulongense* [8, 10-12].

The present research concerns the aerial parts and seeds from *Peucedanum officinale* L., harvested from ecologic crops belonging to S.C. Dacia Plant, Bod, Brasov, Romania (geographic coordonates 45°45′48″N 25°38′23″E45°45′48″N 25°38′23″E) [9]. The aim of our study is a phytochemical and phytobiological characterization of these raw materials and the evaluation of their antioxidant properties.

Materials and Methods

Raw materials consisted of aerial parts (*Peucedani herba* - PH) and seeds (*Peucedani semen* - PS) harvested in 2013 from ecologic crops belonging to S.C. Dacia Plant, Bod, Brasov, Romania.

The following chemical tests were performed: qualitative chemical analysis, thin layer chromatography (TLC) and high performance liquid chromatography (HPLC) analysis, and spectrophotometric assays of phenolic compounds. Moreover, the antioxidant activity and the cytotoxic effect upon wheat caryopses were evaluated.

The qualitative chemical analysis had in view the identification of active compounds by using general and specific reactions.

Preparation of test solutions for qualitative chemical analysis: raw materials (PH and PS) were successively extracted with ether, ethanol and purified water, on a reflux condenser in a water-bath, for 30 minutes. Half of these solutions were hydrolysed with hydrochloric acid 100g/L, for 15-30 minutes [4].

Thin layer chromatography (TLC) for coumarins and furanocoumarins was performed using silica gel GF plates (Merck) and toluen:ether = 1:1 (saturated with acetic acid 100 g/L) as eluent system [21]. Hydroalcoholic solutions – PHi 70%, PHi 50%, PSi 70% and PSi 50% (prepared as described below) were used as test solutions. Bergapten, 7-hydroxi-coumarin, 8-methoxy-psoralen and scopoletin were used as references. All standards were purchased from Roth. Plates were examined in UV light (λ = 365 nm) using a Camag Reprostar Lamp with Epson Photo PC850.

Preparation of test solutions for TLC: 5.0 g of raw materials (PH and PS) were heated twice with 50.0 mL of solvent (70 per cent v/v solution of ethanol and 50 per cent v/v solution of ethanol, respectively), on a reflux condenser, for 30 and 15 minutes.

Spectrophotometric determination of total polyphenols (expressed as tannic acid equivalent) was performed with *Folin-Ciocâlteu* reagent according to Singleton & Rossi method (1965) [19] modified by Makkar *et* al. (1993) [15]. Flavonoid and phenolcarboxylic acids (PCA) contents (expressed as rutin and chlorogenic acid equivalents) were estimated based on the chelating reaction with aluminium chloride and formation of oximes with *Arnow* reagent, respectively [23]. For all determinations a Jasco V-530 (Jasco, Japan) spectrophotometer was used. Hydroalcoholic extractive solutions (previously described for TLC) were used as test solutions. Calibration curves of tannic acid (linearity range: 1.21-9.18 µg/mL, R^2 = 0.9994, n = 8), rutin (linearity range: 5-35 µg/mL, R^2 = 0.9997, n = 11) and chlorogenic acid (linearity range: 0.0113-0.0527 mg/mL, R^2 = 0.9998, n = 6) were used to calculate the active substances contents.

HPLC analysis was carried out using a Jasco HPLC series system, equipped with binary gradient pump, column thermostat and diode array detector. The separation was achieved on a reverse-phased analytical column (Nucleosil – C18, 25 x 0.4 mm, 5 µm). The mobile phase consisted of a mixture of water and phosphoric acid = 999:1 (v/v) (solvent A) and acetonitrile (solvent B). The gradient used was: 90% A / 10% B, 0 min.; 90% → 78% A / 10% → 22% B, 0-13 min; 78% → 60% A / 22% → 40% B, 13-14 min.; 60% A / 40% B, 14-20 min. The flow rate was 1.5 mL/min and the injection volume 20 µL. The monitoring wavelength was 310 nm. The analytical data was evaluated using a Jasco data processing system (Chrompass). Hydroalcoholic solutions (PHi 70%, PHi 50%, PSi 70% and PSi 50%, previously described for TLC) and the corresponding hydrolysed solutions (PHh 70%, PHh 50%, PSh 70% and PSh 50%) were used as test solutions. Methanolic solutions of chlorogenic acid, caffeic acid, ferulic acid, quercetin, kaempferol, apigenin-7-glucoside, isoquercitrin and rutin were used as standards. Calibration curves of chlorogenic acid, caffeic acid, rutin and quercetin in the 4.06 - 370 µg/mL range had a good linearity (R^2 > 0.99, n = 5). Phenolic compounds from samples were identified based on their chromatographic retention times and UV spectra and quantified by comparing integreated peak areas to calibration curves obtained for the mentioned standards.

The antioxidant activity of both products was evaluated based on their scavenger capacity upon DPPH free radical [1, 2, 6]. Hydroalcoholic extractive solutions (previously described for TLC) were used as test solutions. Briefly, 2 mL of 0.06 M DPPH methanolic solution was added to 2.0 mL of sample solutions (0.4 - 2.8 mg/mL). The mixture was kept protected from light. The absorbances of the previous solutions were measured at 516 nm after 30 min., using a blank solution of methanol. It was used a Jasco V-530 (Jasco, Japan) spectrophotometer was used. The inhibition (%) was calculated using the following formula:

$$I\ \% = (A_{control} - A_{sample}) / A_{control} \times 100,$$

where: $A_{control}$ = the absorbance of the DPPH without sample solution; A_{sample} = the absorbance of the DPPH with sample solution, after 30 min.

The concentration of extract that inhibited 50% of the DPPH free radical activity (EC_{50}, mg/mL) was determined graphically from the linear regression curve plotted between percent (%) of inhibition and test solutions concentration.

The phytobiologic assay (*Triticum* test, Constantinescu method) aimed to determine the highest dilutions of extracts that influence the

kariokynetic film, depending on exposure time [5, 20]. 5.0, 2.5 and 0.33 % aqueous extractive solutions (prepared as described below) were used as test solutions.

Preparation of test solutions for Triticum test: 5.0 g of raw materials (PH and PS) were heated with 100 mL of purified water, on a reflux condenser, for 30 minutes. The liquids were filtered through absorbent cotton, and diluted to 100 mL with water. Thus, 5 % aqueous solutions were obtained. Afterwards, 2.5 and 0.33 % aqueous extractive solutions were obtained, by diluting the previous solution.

Statistical analysis. The data analysis was performed using Microsoft Excel 2007 software. The spectrophotometric determinations represent the average ± standard deviation (SD) of three independent replicates.

Results and Discussion

The qualitative chemical analysis revealed the presence of lipophilic and hydrophilic compounds in both herbal products; the chemical reactions intensity was dependent on the sample type. Several compounds were identified: sterols, carotenoids, flavonoids and coumarins aglycons (in etheric solutions), flavonoids, coumarins, proanthocyanidins (in alcoholic solutions), polyholosides, tannins, saponins (in aqueous solutions).

TLC analysis of coumarins showed the presence of bergapten (R_f = 0.55, greenish fluorescence) and 8-methoxypsoralen (R_f = 0.60, greenish fluorescence) in all samples (Figure 1). These compounds were also identified by other scientists in roots of *P. oreoselinum* L. (Moench) (bergapten) and *P. praeruptorum* Dunn. (8-methoxypsoralen) [18]. Although Kuzmanov B. & al. [13] foresaw the presence of 7-hydroxicoumarin in aerial parts of P. *officinale* L., this compound was only identified by us in the seeds (R_f = 0.39, blue fluorescence).

According to our **spectrophotometric results** (Table I) *Peucedani semen* have a higher content of flavonoids compared to *Peucedani herba*. Regarding total polyphenols and PCA, the content is similar for both herbal products.

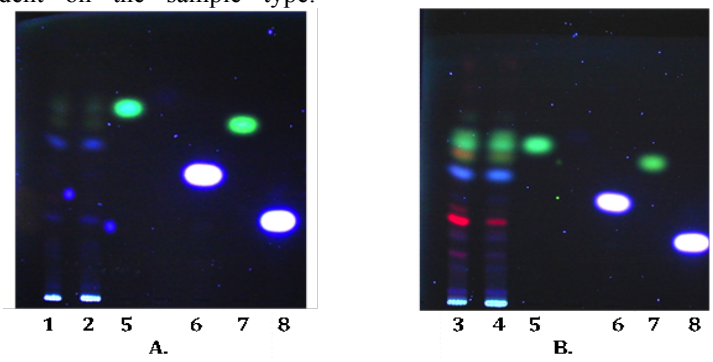


Figure 1.

TLC chromatograms of *Peucedanum officinale* samples: A. TLC chromatogram of *Peucedanii herba*; B. TLC chromatogram of *Peucedanii semen*; 1. PHi 70%; 2. PHi 50%; 3. PSi 70%; 4. PSi 50%; 5. bergapten; 6. 7-hydroxicoumarin; 7. 8-methoxypsoralen; 8. scopoletin.

Table I

Spectrophotometric determination of polyphenols

Sample	PCA g% chlorogenic acid	Flavonoids g% rutin	Total polyphenols g% tannic acid
PHi 70%	0.6517 ± 0.0261	0.6050 ± 0.0241	2.0047 ± 0.1391
PHi 50%	0.7120 ± 0.0213	0.4889 ± 0.0182	1.8515 ± 0.0335
PSi 70%	0.7906 ± 0.0234	0.9872 ± 0.0419	1.8658 ± 0.0526
PSi 50%	0.7002 ± 0.0467	0.8738 ± 0.0555	2.1519 ± 0.0537

The HPLC results are presented in Tables II, III and IV, in Figure 2 and 3.

Table II

Retention time for standards

Compound	Retention time (min.)
Chlorogenic acid	7.122
Caffeic acid	7.960
Ferulic acid	13.145
Rutin	15.198
Quercetin	18.872
Kaempferol	20.25
Apigenin-7 -glucoside	17.65
Isoquercitrin	15.68

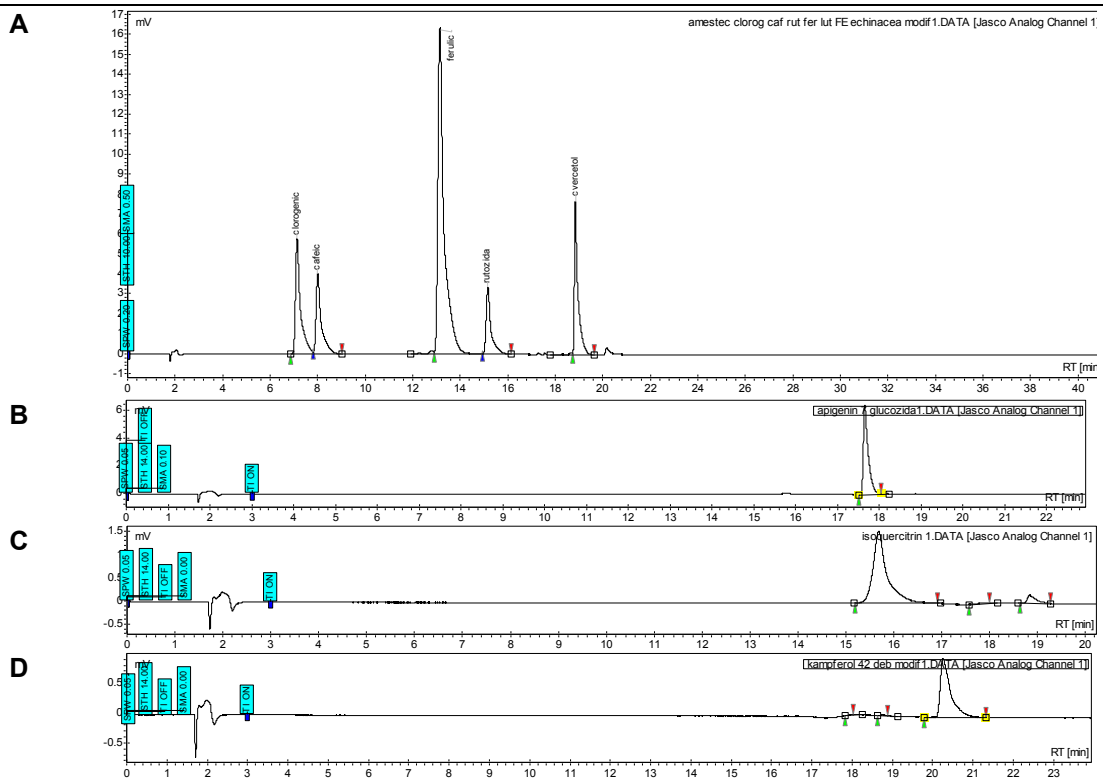


Figure 2.

Chromatograms of standards: A – mixture of chlorogenic acid, caffeic acid, ferulic acid, rutin, quercetin; B – apigenin-7-glucoside; C - isoquercitrin; D – kaempferol

Table III

HPLC analysis of *Peucedani herba* polyphenols

Compound	Sample		
	PHi 70%	**PHh 70%**	**PHi 50%**
Chlorogenic acid	55.310 mg %	-	41.56 mg %
Caffeic acid	-	-	4.310 mg %
Ferulic acid	-	-	+
Rutin	118.36 mg %	-	+
Quercetin	-	+	-
Kaemferol	-	-	-
Apigenin 7-glucoside	-	-	+
Isoquercitrin	+	-	-

Legend: „+" – identified, „-" – not identified

Table IV

HPLC analysis of *Peucedani semen* polyphenols

Compound	Sample			
	PSi 70%	**PSh 70%**	**PSi 50%**	**PSh 50%**
Chlorogenic acid	-	-	8.63 mg %	+
Caffeic acid	-	9.2 mg %	7.07 mg %	6 mg %
Ferulic acid	+	-	+	+
Rutin	-	-	21.7 mg %	-
Quercetin	-	70.96 mg %	-	+
Kaemferol	-	-	-	+
Apigenin-7-glucoside	-	-	+	-
Isoquercitrin	-	-	+	-

Legend: „+" – identified, „-" – not identified

Ferulic acid was identified in both PSi 50% and PSh 50% solutions, so we concluded that it exists in both free, glycolised or esterified forms. Caffeic acid and chlorogenic acid were also found in other *Peucedanum* species: *P. tauricum* (leaves), *P. alsaticum* (flowers), whereas rutin was also identified in aerial parts of P. *japonicum* [18].

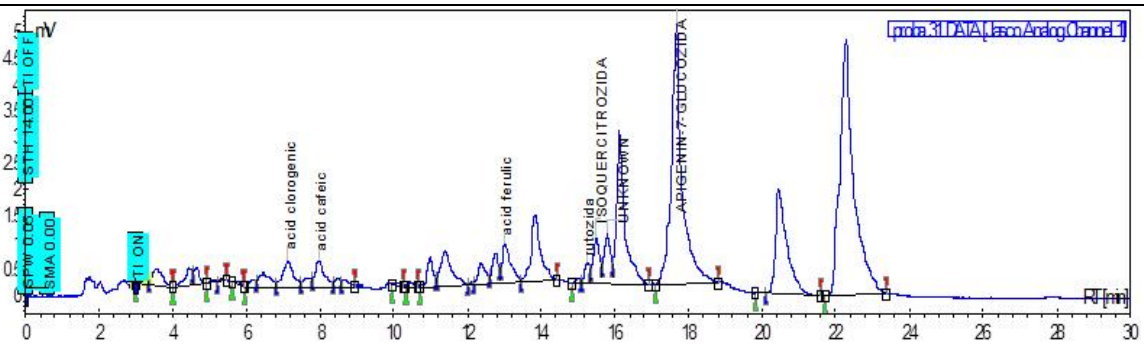
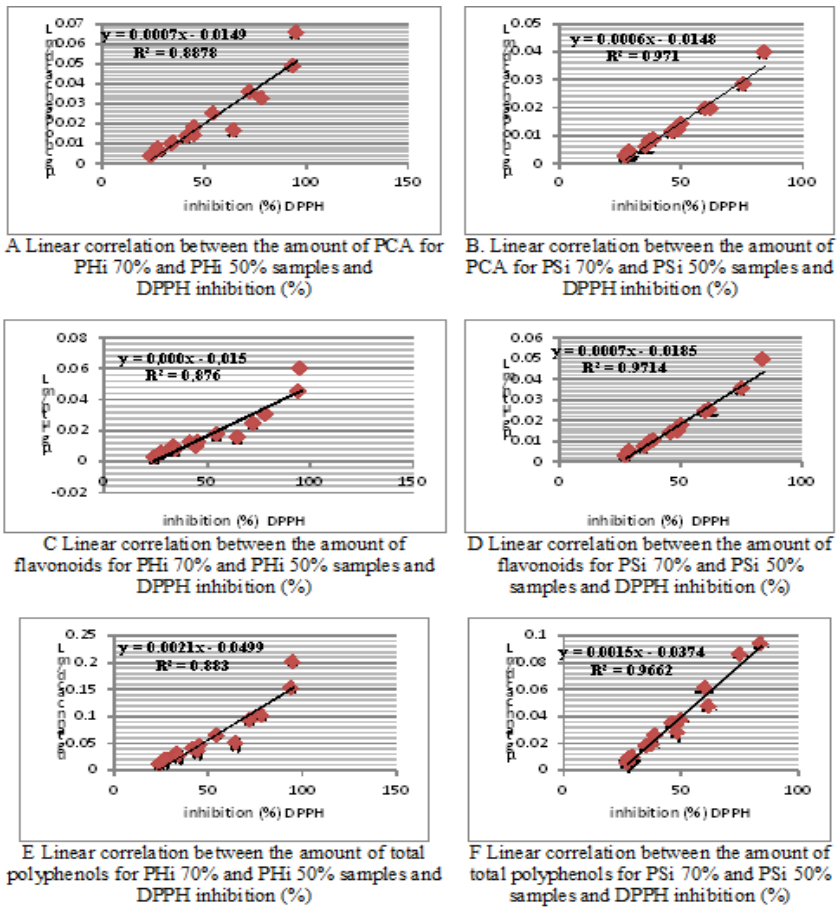


Figure 3.
HPLC chromatogram of PHi 50%

As far as the **antioxidant activity** is concerned, the seeds have a higher scavenger capacity upon DPPH free radical compared to the aerial parts (Table V).

Table V
EC_{50} (mg/mL) values for *Peucedani herba* and *semen* samples

Sample	EC_{50} (mg/mL)
PHi 70%	3.0057 ± 0.1050
PHi 50%	3.0950 ± 0.0028
PSi 70%	2.5429 ± 0.0286
PSi 50%	2.5616 ± 0.2584



A Linear correlation between the amount of PCA for PHi 70% and PHi 50% samples and DPPH inhibition (%)

B. Linear correlation between the amount of PCA for PSi 70% and PSi 50% samples and DPPH inhibition (%)

C Linear correlation between the amount of flavonoids for PHi 70% and PHi 50% samples and DPPH inhibition (%)

D Linear correlation between the amount of flavonoids for PSi 70% and PSi 50% samples and DPPH inhibition (%)

E Linear correlation between the amount of total polyphenols for PHi 70% and PHi 50% samples and DPPH inhibition (%)

F Linear correlation between the amount of total polyphenols for PSi 70% and PSi 50% samples and DPPH inhibition (%)

Figure 4.
Linear correlation between the DPPH scavenger capacity and polyphenols content of *Peucedani herba* and *semen*

We can assume that the higher polyphenols content of seed samples is responsible for a better antioxidant activity. As shown in Figure 4. A-F, one can note a stronger correlation between PCA, flavonoids and total polyphenols contents and inhibition (%) of DPPH activity for PS samples as compared to PH ones.

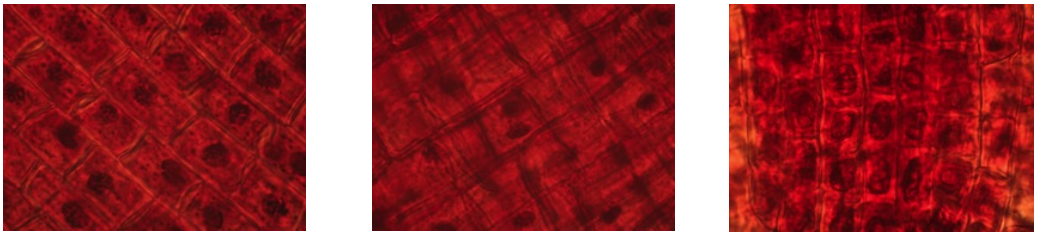
Other authors (Matejić S. & al.) established a strong correlation between antioxidant activity and total polyphenols content of methanolic extracts of *Peucedani herba* [16].

Results for *Triticum* assay are presented in Table VI and Figure 5.

Table VI

Kariokynetic film changes induced by *Peucedani herba* and *semen* samples

Sample	Concentration, g%	Observations
Peucedani herba	5	Rare cellular divisions, nuclei containing 2-3 hypertrophied nucleoli, disorganised nuclear material (5a)
	2.5	Cells with normal phases of mitosis
	0.33	Cells with normal phases of mitosis
Peucedani semen	5	Rare telophases in tropokinesis (5b)
	2.5	Rare cells with hypertrophied nuclei (5c) and telophases in tropokinesis
	0.33	Cells with normal phases of mitosis



a. Hypertrophied nucleoli, cells with disorganised nuclear material

b. Telophases in tropokinesis

c. Nuclei with hypertrophied nucleoli

Figure 5.

Kariokynetic film changes induced by *Peucedani herba* and *semen* samples

Both herbal products showed a modest cytotoxic activity only for the 5% concentration. We assume that coumarins are responsible for the kariokynetic film changes, but other compounds may also be involved. According to Lacy A. & al. (2004), 7-hydroxicoumarin has cytotoxic properties on A427 adenocarcinoma cell line by inhibition of the cell cycle at transition G_1/S, whilst psoralen and its derivates regulate human cervical adenocarcinoma proliferation [14]. Imperatorin, another furanocoumarin found in *Peucedani herba* [18] shows antiproliferative properties against CEM-13 (T-cell leukosis), MT4 (T-cell leukemia) and U-937 (monocytic leukemia) cell lines [7].

Conclusions

Peucedanum officinale L., from S.C. Dacia Plant ecological crops, can be a future candidate for obtaining selective herbal extracts, rich in phenolic compounds with antioxidant activity. Coumarins might be responsible for the cytotoxic activity, observed only at high concentrations.

Future researches will have in view cytotoxicity evaluation (using alternative methods - Lactuca, Artemia salina and Daphnia magna tests), determining the influence on biochemical parameters and establishing the pharmacological profile.

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References

1. Benedec D., Vlase L., Oniga I., Mot A.C., Silaghi-Dumitrescu R., Hanganu D., Tiperciuc B., Crişan G., LC-MS analysis and antioxidant activity of phenolic compounds from two indigenous species of *Mentha*. Note I. *Farmacia*, 2013; 61(2): 262-267.
2. Brand-Williams W., Cuvelier M.E., Berset C., Use of a free radical method to evaluate antioxidant activity. *Lebensmittel-Wissenschaft und Technologie Food Science and Technology*, 1995; 28(1): 25-30.
3. Ciocârlan V., Flora ilustrată a României, pteridophyta et spermathophyta. Ed. Ceres, Bucureşti, 2009; 492-495.
4. Ciulei I., Istudor V., Palade M., Niculete E., Gîrd C.E., Analiza farmacognostică şi fitochimică a produselor vegetale. Ed. Tehnoplast Company SRL, Bucureşti, 1995; vol. II: 409-418.
5. Constantinescu D.Gr., Constantinescu M., Retezeanu M., Olteanu R., Eine phytobiologische methode zur Prufung der cytostatischen Wirkung alkylrerender Verbindungen. *Arzenmittel Forschung*, 1962; 12: 87.
6. Dudonné S., Vitrac X., Coutierré P., Woillez M., Mérillon J.M., Comparative study of antioxidant properties and total phenolic content of 30 plant extracts of industrial interest using DPPH, ABTS,

FRAP, SOD, and ORAC assays. *J. Agric. Food. Chem.*, 2009; 57(5): 1764-1778.
7. Ganbaatar Zh., Shults E.E., Petrova T.N., Shakirov M.M., Otgonsuren D., Pokrovskua A.G., Tolstikov G.A., Batsuren D., Furanocoumarins from *Peucedanum baicalense* of Mongolia flora and their cytotoxic activity. *Chemistry of Natural Compounds*, 2013; 49(1): 99-102.
8. Hiermann A., Schantl D., Zsilavecz M.S., Reiner J., Coumarins from *Peucedanum ostruthium*. *Phytochemistry*, 1996; 43(4): 881-883.
9. https://www.google/ro.wikipedia.org
10. IkeshiroY., Mase I., Tomita Y., Dihydrophyranocoumarins from *Peucedanum japonicum*. *Phytochemistry*, 1993; 33: 1543-1545.
11. Kong L.Y., Min Z.D., Li Y., Pej Z.H., Qianhucoumarin I from *Peucedanum praeruptorum*. *Yaoxue Xuebao*, 1994; 29(1): 49-54.
12. Kong L.Y., Zhi F., Coumarins from *Peucedanum wulongeuse*. *Journal of Asian natural products Research*, 2003; 5(3): 183-187.
13. Kuzmanov B., Andreev N., Kozovska V., Chemotaxonomic study on Bulgarian species of *Peucedanum* L. *Annals del Jardin Botanico*, 1981; 37: 779-781.
14. Lacy A., O'Kennedy R., Studies on coumarins and coumarin related compounds to determine their therapeutic role in the treatment of cancer. *Current Pharmaceutical Design*, 2004; 10: 3797-3811.
15. Makkar H.P.S., Bluemmel M., Borowy N.K., Becker K., Gravimetric determination of tannins and their correlations with chemical and protein precipitation method. *Journal of science Food Agriculture*, 1993; 6(2): 161-165.
16. Matejić J.S., Džnamić A.M., Ćirić A.D., Krivošej Z., Randelović V.N., Marin P.D., Antioxidant and antimicrobial activities of extracts of four *Peucedanum* species. *Digest Journal of Nanomaterials and Biostructures*, 2013; 8(2): 655-665.
17. Movahedian A., Zolfaghari B., Sajjadi S.E., Moknatjou R., Antihyperlipidemic effect of *Peucedanum pastinacifolium* extract in streptozocin induced diabetic rats. *Clinics*, 2010; 65(6): 629-633.
18. Sarkhail P., Traditional uses phytochemistry and pharmacological properties of the genus *Peucedanum*. A review. *Journal of Ethnopharmacology*, 2014; 156: 235-270.
19. Singleton V.L., Rosi J.A., Colorimetry of total polyphenol with phosphomolybdic – phosphotungstic acid reagents. *Am. J. Enol. Vitic.*, 1965; 37: 144-158.
20. Trifan A., Miron A., Aprotosoaie A.C., Hăncianu M., Cioancă O., Gille E., Stănescu U., Phytotoxicity assessment of polyphenolic extracts from *Carum carvi* L. fruits. *Farmacia*, 2013; 61(1): 12-19.
21. Wagner H., Bladt S., Zgainski E.M., Plant drug analysis. A thin layer chromatography atlas. Springer-Verlang, Berlin, 1994; 156.
22. Woźniak-Skalicka K.S., Gtowania K., Quantitative analysis of phenolic acids in extracts obtained from the fruits of *Peucedanum alsaticum* L. and P. cervaria (L.) Lap. *Chromatographia*, 2008; 68(supllement): S85-90.
23. xxx, European Pharmacopoeia, 7th edition, EDQM Council of Europe, Strassbourg, 2011; vol I: 1059, 1071-1072, 1147-1148, 1185-86, 1209.