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Analysis of phenolic compounds from *Polymnia sonchifolia* Poepp. & Endl. leaves by HPLC-method

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Abstract
In the present paper, HPLC method was employed to distinguish phenolic compounds (flavonoids, hydroxycinnamic acids, catechins and coumarines) from the other constituents of the yacon leaves. These substances were identified and quantified after comparison with reference standards. The major components were rosmarinic acid (0.97%), quercetin-3-D-glycoside (0.20%), gallocatechin (1.56%) and umbelliferone (0.05%) among the above mentioned groups of biologically active substances.

Keywords: hydroxicinnamic acids, flavonoids, coumarins, catechins, HPLC, *Polymnia sonchifolia* Poepp. & Endl (yacon)

1. Introduction
Currently modern medicine increasingly requires new drugs of natural origin as they play an important role in the treatment of many diseases. Many plants are cultivated with this purpose in Ukraine and yacon is among them.
Yacon (*Polymnia sonchifolia* Poepp. & Endl, Asteraceae) is originated from the Andean highlands and has been cultivated for its tubers throughout South America. Yacon is introducted in New Zealand, USA, Southern Europe, Iran, Japan, Korea, Brazil, Czech Republic, Uzbekistan and Russia [1].
Yacon grows up to a height of two meters, has large opposite sagittate leaves with serrate margins, and multiple yellow-orange flowers 3 cm in size. The plant is distinguished by having two kinds of tuberous roots, a central rhizome with "eyes" for producing new stems, and multiple edible tuberous roots radiating from the rhizome. The brittle, tan to purple, smoothly tapered edible tuberous roots are actually fattened roots that can be up 40 cm in length and weigh two kilos. The edible tuberous roots are crunchy like a crisp, sweet, juicier than any pear [2]. Yacon has been reported to exhibit antihyperglycemic effect [3] due to the presence of fructans (inulin). The potential of yacon tubers to treat kidney problems and for skin rejuvenation, cytoprotective and antioxidative activities of its leaves seems to be related mostly to its phenolic content. However, amount of phenolic compounds (flavonoids, hydroxycinnamic acids, catechins and coumarines) and their variety in *P. sonchifolia* leaves remain uncertain and need to be investigated.
The aim of our research was to indicate the presence and to quantify the content of phenolic compounds (flavonoids, hydroxycinnamic acids, catechins and coumarines) in yacon leaves.

2. Materials and Methods
2.1 Plant material
Yacon leaves were collected at research plots of Taras Shevchenko National University of Kyiv (Kyiv region, Ukraine) in July, 2014. Yacon was introducted in Ukraine by the scientific group of prof. L.T. Mishchenko. The collected samples were dried and stored for further analysis.

2.2 Sample preparation
The extraction method used for dried samples had as follows: 50 ml of 60% aqueous methanol was added to 2.01 g of dried sample and refluxed in a water bath at 90 ºC for 30 min. After this the sample was treated with ultrasound for 10 min, cooled, filtered and made up to 100 ml with 60% methanol solution and injected to HPLC [4-6].

2.3 Chemicals and Methods

All chemical were of analytical grade (> 95% purity). Chlorogenic acid, rosmarinic acid, p-coumaric acid, ferulic acid, caffeic acid, gallic acid, ellagic acid, catechin, gallocatechin, epigallocatechin, epicatechin, epicatechin gallate, coumarin, umbelliferone, scopoletin, quercetin, quercetin-3-D-glycoside, apigenin, luteolin, rutin, kaempferol and hyperoside were purchased from Sigma–Aldrich (USA). Quantification was done via a calibration with standards (external standard method). All standards were prepared as stock solutions in methanol. Working standards were made by diluting stock solutions in 60% aqueous methanol. High performance liquid chromatography (HPLC) method was applied for separation and quantification of flavonoids, hydroxycinnamic acids catechins and coumarines.

2.4 HPLC analysis

The analytical HPLC system employed consisted of a high performance liquid chromatograph Agilent 1200 3 D LC System Technologies, USA coupled with a four-channel vacuum degasser G1354 A, autosampler G1329A, autosampler thermostat G1330 B, column thermostat G1316A, diode array detector (G1315C) in complex with PC software Agilent ChemStation (G2215 BA). The separation was achieved on a Discovery C 18, 250 mm x 4.6 mm x 5 µm (Supelco, № 505129) column with the precolumn of 20 mm at 25 °C temperature. The mobile phase consisted of distilled water with 0,005 N orthophosphoric acid and acetonitrile (solvent A) and 0, 005 N orthophosphoric acid and acetonitrile (solvent B) – analysis of flavonoids, hydroxycinnamic acids and coumarines; 0,1% solution of trifluoroacetic acid, 5% acetonitrile solution and water (solvent A) and 0,1% solution of trifluoroacetic acid with acetonitrile (solvent B) – analysis of tannic substances components. The flow rate was 0.8 ml/min and the injection volume was 10 µm. The monitoring wavelength was 320 and 330 nm for hydroxycinnamic acids; 255 and 340 nm for flavonoids and coumarines; 255 and 280 nm for catechins. The gradient elution parameters used were as given in the table 1.

Table 1: The gradient elution parameters for flavonoids, coumarines, hydroxycinnamic acids and tannic substances components

Flavonoids, coumarines							
Time, min	0	30	33	38	40	41	49-60
Solvent B,%	12	25	25	30	40	80	12
Hydroxycinnamic acids							
Time, min	0	8	15	30	40	41	43-50
Solvent B,%	5	8	10	20	40	75	5
Tannic substances components (catechins)							
Time, min	0	8	10	15	20	25	28	29-40
Solvent B,%	100	12	12	25	25	75	75	100

The identification and quantation of each compound was based on a combination of retention time, peak area and spectral matching.

3. Results and Discussion

Using the aforementioned procedure, the phenolic substances present in yacon leaves plants were separated and quantified. The present method is simple, easy to use, and effective enough for the identification and quantification of major phenolic compounds in plants. Discovery C 18 (Supelco, № 505129) stationary phase, which was used in this study to separate catechins, coumarines, hydroxycinnamic acids and flavonoids in the above mentioned wavelengths showed satisfactory results (see figure 1-3).

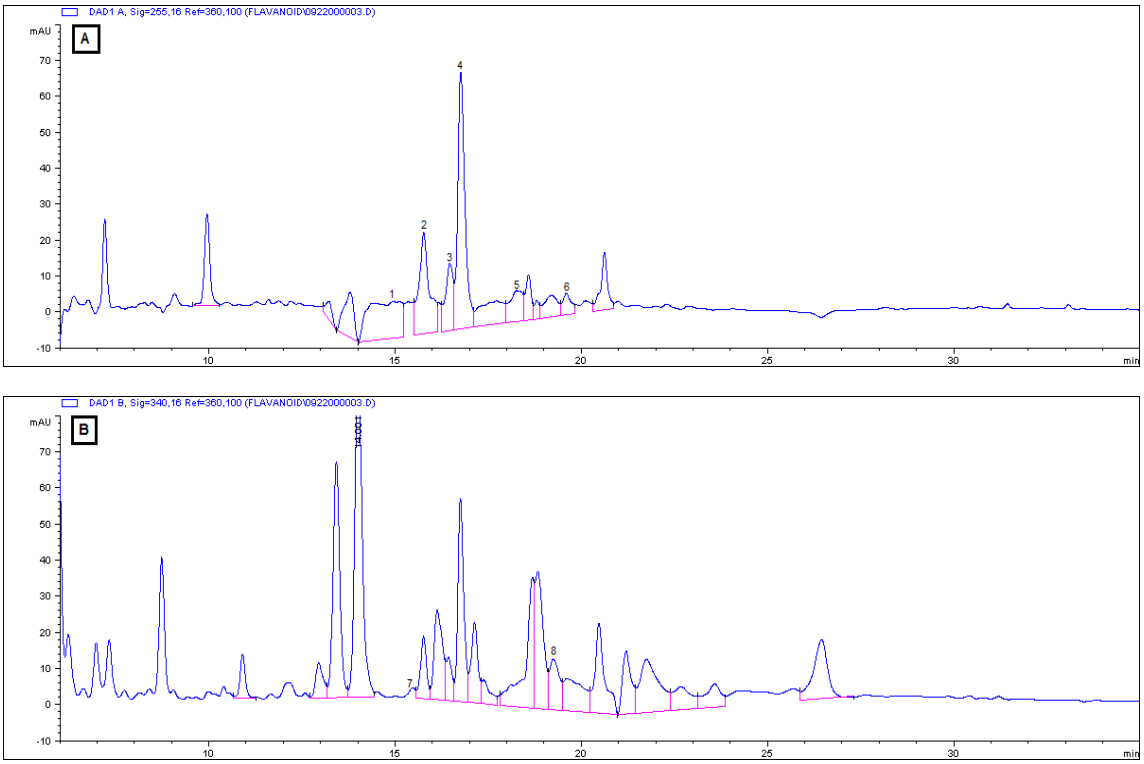

Fig 1: HPLC-chromatograms of flavonoids and coumarines from *P. sonchifolia* leaves: A-under wavelength 255 nm: 1- coumarin, 2- rutin, 3- kaempferol, 4- quercetin-3-D-glycoside, 5- luteolin, 6- quercetin; B- under wavelength 340 nm: 7- scopoletin, 8- apigenin.

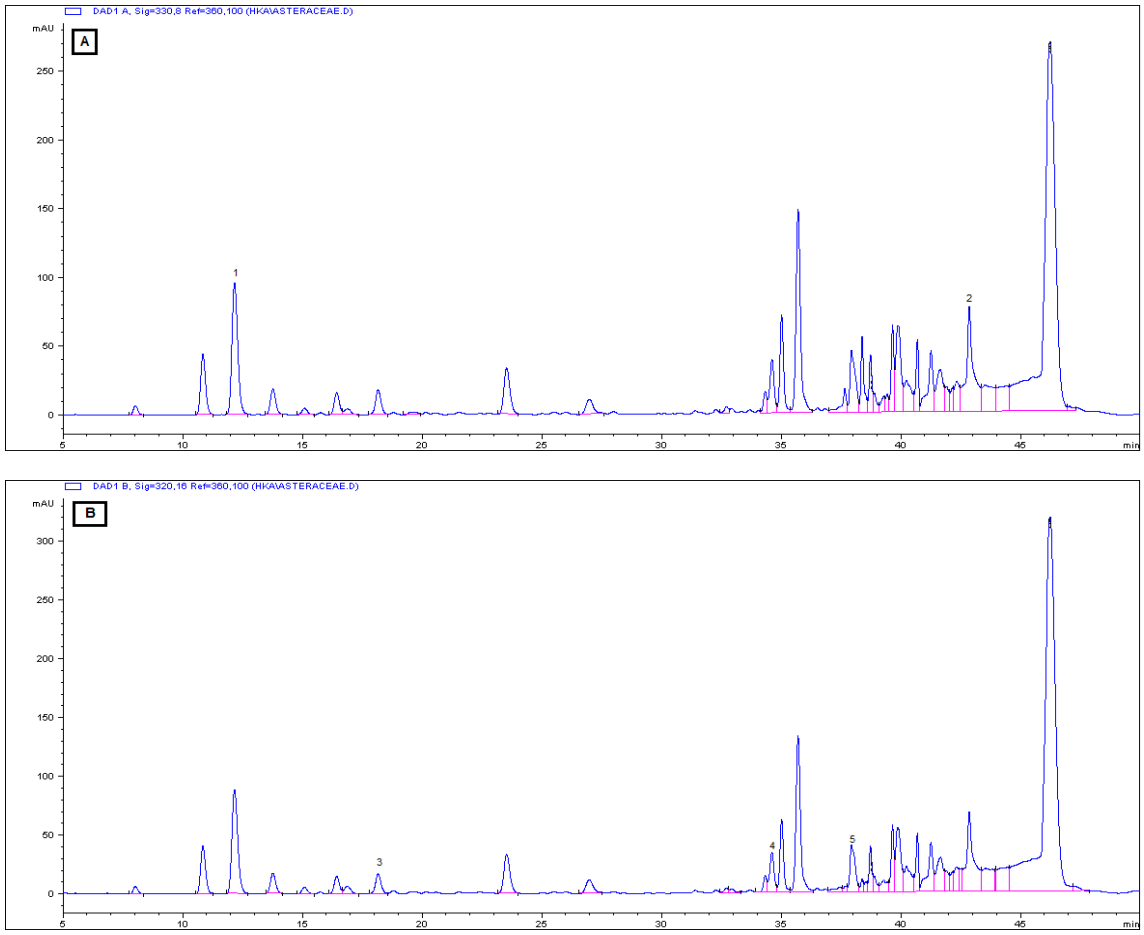

Fig 2: HPLC-chromatograms of hydroxycinnamic acids and coumarines from *P. sonchifolia* leaves: A-under wavelength 330 nm: 1- chlorogenic acid, 2- rosmarinic acid; B- under wavelength 320 nm: 3- caffeic acid, 4- umbelliferone, 5- ferulic acid.

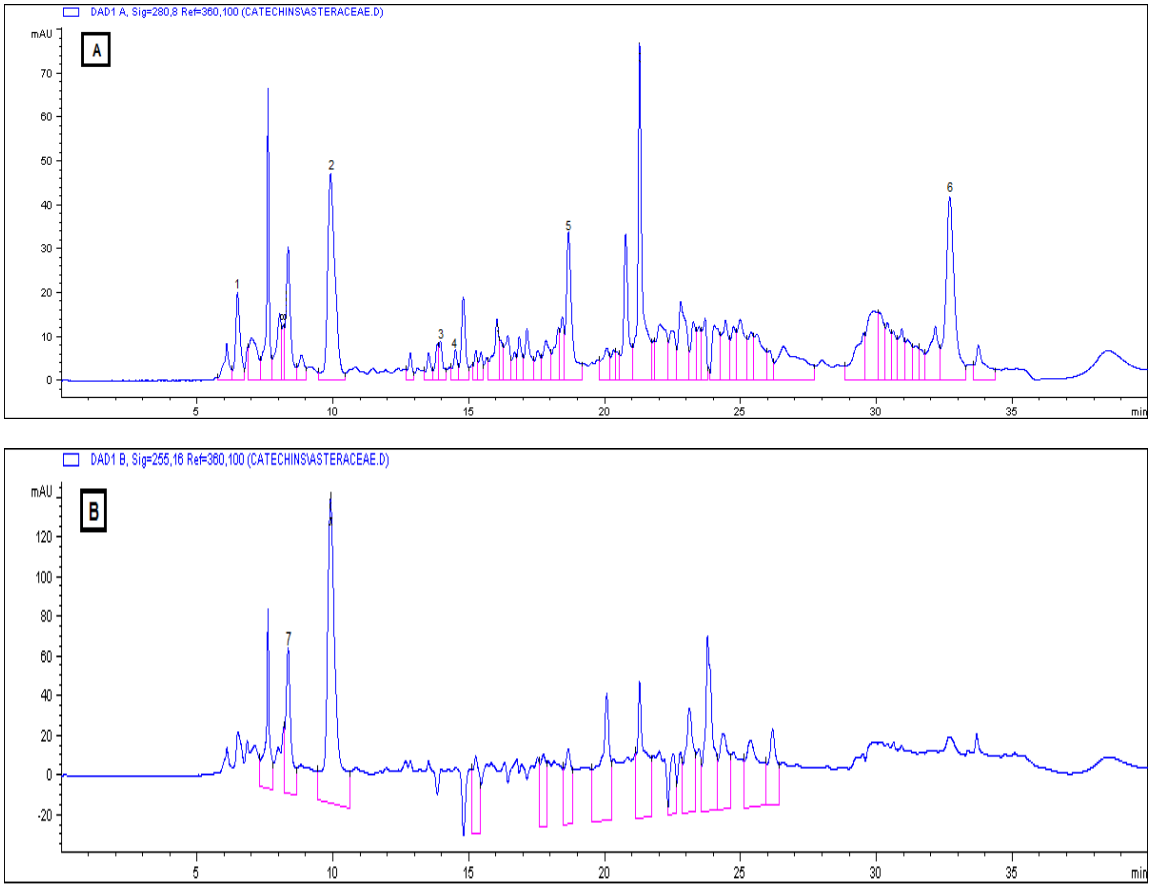

Fig 3: HPLC-chromatograms of catechins from *P. sonchifolia* leaves: A- under wavelength 280 nm: 1- gallic acid, 2- gallocatechin, 3- epigallocatechin, 4- catechin, 5- epicatechin, 6- epicatechin gallate; B- under wavelength 255 nm: 7- ellagic acid

The content of phenolic substances was also determined. Quantification was done via a calibration with standards (external standard method). The amount of hydroxycinnamic acids, flavonoids, catechins and coumarines detected in the yacon leaves is shown in Table 2.

Table 2: Content of phenolic substances in *P. sonchifolia* leaves

№	Hydroxycinnamic acids/Flavonoids/Catechins/Coumarines	Wavelength, nm	Retention time	Content (%)
1	Chlorogenic acid	330	12.15	0.5
2	Rosmarinic acid	330	42.85	0.97
3	Caffeic acid	320	18.15	0.06
4	Ferulic acid	320	37.93	0.06
5	Rutin	255	15.76	0.11
6	Kaempferol	255	16.46	0.07
7	Quercetin-3-D-glycoside	255	16.75	0.20
8	Luteolin	255	18.25	0.02
9	Quercetin	255	19.59	0.02
10	Apigenin	340	19.24	0.01
11	Gallic acid	280	6.48	0.03
12	Gallocatechin	280	9.90	1.56
13	Epigallocatechin	280	13.94	0.3
14	Catechin	280	14.50	0.03
15	Epicatechin	280	18.65	0.17
16	Epicatechin gallate	280	32.68	0.11
17	Ellagic acid	255	8.34	0.02
18	Coumarin	255	14.94	0.006
19	Umbelliferone	320	34.60	0.05
20	Scopoletin	340	15.47	0.006

As shown in Table 2 and in Fig. 1, the identified components of flavonoids and coumarines of yacon leaves were coumarin, rutin, kaempferol, quercetin-3-D-glycoside, luteolin, quercetin, scopoletin and apigenin. The predominant were quercetin-3-D-glycoside (0.20%) and rutin (0.11%). The lowest content was for apigenin (0.01%). Among hydroxycinnamic acids major components were rosmarinic acid (0.97%) and chlorogenic acid (0.5%) followed by caffeic and ferulic acids (0.06% for each) (Fig. 2 and Table 2). We also identified and quantified catechins: gallic acid, gallocatechin, epigallocatechin, catechin, epicatechin, epicatechin gallate and ellagic acid (Fig.3 and Table 2). Major compounds found in yacon leaves were rosmarinic acid, quercetin-3-D-glycoside, gallocatechin and umbelliferone.

4. Conclusion

The phenolic profile of yacon leaves was established using HPLC method. Flavonoids, hydroxycinnamic acids, catechins and coumarines were identified and quantified in the analyzed object. The major components were rosmarinic acid (0.97%), quercetin-3-D-glycoside (0.20%), gallocatechin (1.56%) and umbelliferone (0.05%). *P. sonchifolia* leaves represent a promising source of phenolic constituents, which may be used as natural antioxidants or as ingredients of functional foods.

5. References

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