

## RESEARCH ARTICLE

# Study of Biologically Active Compounds in *Prunus persica* Leaves Extract

Taras V. Upyr<sup>1</sup>, Iliia S. Jelev<sup>2</sup>, Larysa V. Lenchyk<sup>\*1</sup>, Mykola A. Komisarenko<sup>1</sup>,  
Abderrahim Ayaou<sup>1</sup>, Olena G. Poghosyan<sup>1</sup>, Galina, I. Dimova<sup>2</sup>, Hanna O. Yeromina<sup>1</sup>

<sup>1</sup>National University of Pharmacy, Kharkiv, Ukraine

<sup>2</sup>Medical University-Varna, Varna, Bulgaria

\*Corresponding Author E-mail: larysa.lenchyk@gmail.com

## ABSTRACT:

Qualitative composition and content of biologically active compounds in the peach leaves and 50% ethanol extract obtained from peach leaves have been studied. The total content of phenolic compounds, determined by spectrophotometry in peach leaves was as  $3,17 \pm 0,15$  %; tannins –  $1,02 \pm 0,05$  %; amounts of flavonoids –  $2,54 \pm 0,12$  % and the amounts of hydroxycinnamic acids –  $1,59 \pm 0,07$  %. The qualitative composition and content of phenolic compounds in the 50% ethanol extract was studied with HPLC using an Agilent Technologies chromatograph (model 1100) equipped with a continuous-flow vacuum degasser G1379A, a 4-channel low-pressure gradient pump G13111A, an automatic injector G1313A, a column thermostat G13116A, a diode array detector G1316A. Nine phenolic substances were found, including five hydroxycinnamic acids and four flavonoids. The dominant substances of all determined compounds were kaempferol-3-O- glucoside (46,70 %) and rutin (21,59 %). Among flavonoids, kaempferol glycosides dominated over quercetin glycosides in a 3: 1 ratio. Among hydroxycinnamic acids, the predominant component was chlorogenic acid, its part was 57.65 % of the sum of hydroxycinnamic acids. Free and linked monosaccharides, such as glucose, galactose, rhamnose, arabinose and ribose, were identified in the extract, and their amount was determined by HPLC. Arabinose was identified just in linked form. Obtained results will be used for standardization of the peach leaves and extract and for further pharmacological research.

**KEYWORDS:** Peach, leaves, extract, phenolics, flavonoids, hydroxycinnamic acids, sugars.

## INTRODUCTION:

Peach (*Prunus persica* L.), family *Rosaceae* is widely cultivated in many countries around the world, including Ukraine and Bulgaria, for the tasty fruits. Peach fruits contain carotenoids, organic acids, polysaccharides and phenolic compounds, among which flavonoids, anthocyanins were found, [1]. Fatty oil (*Oleum Persicorum*) is obtained from the seeds of the fruits. Peach oil is used in medicine as a laxative, choleric, reparative agent, the basis for injection preparations and as part of cosmetics, whereas the peach leaves extract showed an immunotropic and anti-inflammatory effect [1, 2].

Peach leaves polyphenols are promising for research, since this class of substances has a wide range of pharmacological activity. For example, cherry shoots extract was rich on flavonoids and showed anti-inflammatory effect in a dose of 100 mg/kg [3]. Extract from plum leaves contained hydroxycinnamic acids and flavonoids and showed high antioxidant activity [4].

Therefore, the aim of present work was to obtain extract from peach leaves and study the composition and content of phenolic constituents and sugars in leaves and obtained extract before and after hydrolysis.

## MATERIAL AND METHODS:

*Prunus persica* leaves were harvested in July 2018 in Varna (Bulgaria). For selection of the optimal extractant 30 %, 50 %, 70 % ethanol extracts were obtained and qualitative and quantitative determination of phenolics was carried out.

For studying the composition and content of phenolic compounds 20.0 g of powdered dry raw material was poured 200 ml of appropriate extractant, taking into

account the coefficient of absorption of raw materials. Extraction was carried out at room temperature for 7 days. Extraction was performed twice using a new portion of the solvent, the extracts were combined and concentrated to 20 ml.

For identification of the biologically active substances of the extracts, such standard methods as qualitative reactions, paper chromatography (PC) and thin layer chromatography (TLC) were used. Flavonoids and hydroxycinnamic acids were studied by one- and two-dimensional TLC and PC with reliable samples of substances. The following solvent systems were used: glacial acetic acid - water - ethyl acetate (20:20:60), n-butanol - acetic acid - water (4:1:2) and 15 % acetic acid. The obtained chromatograms were treated with a solution of amino ether of diphenylboronic acid in methanol, a solution of macrogol and ammonia solution. The obtained chromatograms were viewed before and after treatment with reagents in daylight and UV light [2, 5].

Quantitative determination of the content of phenolic compounds in peach leaves was carried out spectrophotometrically. The content of tannins, the amount of phenolic compounds in terms of pyrogallol, the amounts of flavonoids in terms of quercetin, hydroxycinnamic acids in terms of rosemary acid were determined [5].

For hydrolysis, 300 µl of a 4 % alcohol solution of 50 % ethanol peach leaves extract was placed in a 2 ml vial, and 300 µl of 6N hydrochloric acid solution in ethanol (1:1 by volume) was added. Hermetically sealed vial kept in a heating chamber at 100°C for 1 hour. After cooling, the contents of the vial were centrifuged and used for analysis.

The qualitative composition and content of phenolic compounds in 50% ethanol peach leaves extract was studied with HPLC using an Agilent Technologies chromatograph (model 1100) equipped with a continuous-flow vacuum degasser G1379A, a 4-channel low-pressure gradient pump G13111A, an automatic injector G1313A, a column thermostat G13116A, a diode array detector G1316A. For the analysis, a chromatographic column ZORBAX-SB C-18, 2.1 × 150 mm, filled with an octadecyl-silyl sorbent with a particle size of 3.5 µm, was used.

The analysis was carried out under the following conditions: the column thermostat temperature was 35 °C; the sample volume was 2 µl, the feed rate of the mobile phase was 0.25 ml/min, the working pressure of eluent was 240-300 kPa. The detection parameters were as follows: measurement scale 1.0; scan time 0.5 sec; Spectrum removal parameters – each peak was 190-600 nm; wavelength 280, 313, 350, 371, 254 nm.

Identification of phenolic compounds was performed by retention time of standards and spectral characteristics. Quantitatively flavonoids, hydroxycinnamic acids were determined by calibration with standards.

For the analysis, the following gradient chromatography was established: solutions A (0.1 % H<sub>3</sub>PO<sub>4</sub>, in water) and B (MeOH) were used as a mobile phase in a ratio of 90:10 (first 8 minutes), 70:30 (from 8 to 25 minutes) and in the ratio of 20:80 (25 minutes). From 26 to 30 minutes, only solution B was used and from 30 to 35 minutes, solutions A and B were used again in a ratio of 90:10. These conditions are related to the well-known method and was carried out with some changes in the gradient [6, 7].

Study content of monosaccharides in the peach leaves extract before and after hydrolysis was carried out by HPLC using a chromatograph Agilent Technologies (model 1100) equipped with a flowing vacuum degasser G1379A, 4-channel pump G13111A low pressure gradient, automatic injector G1313A, columns thermostat G13116A, refractometric detector G1362A. For the analysis, a carbohydrate chromatographic column 7.8 × 300 mm “Supelcogel-C610H” was used. The following chromatography regime was established for the analysis: the feed rate of the mobile phase was 0.5 ml/min; aqueous eluent – 0.1 % H<sub>3</sub>PO<sub>4</sub> solution; operating pressure of eluent 33-36 kPa; column thermostat temperature 30 °C; sample volume 5 µl; detection - refractometric. The identification of sugars was performed according to the retention times of the standards.

## RESULTS:

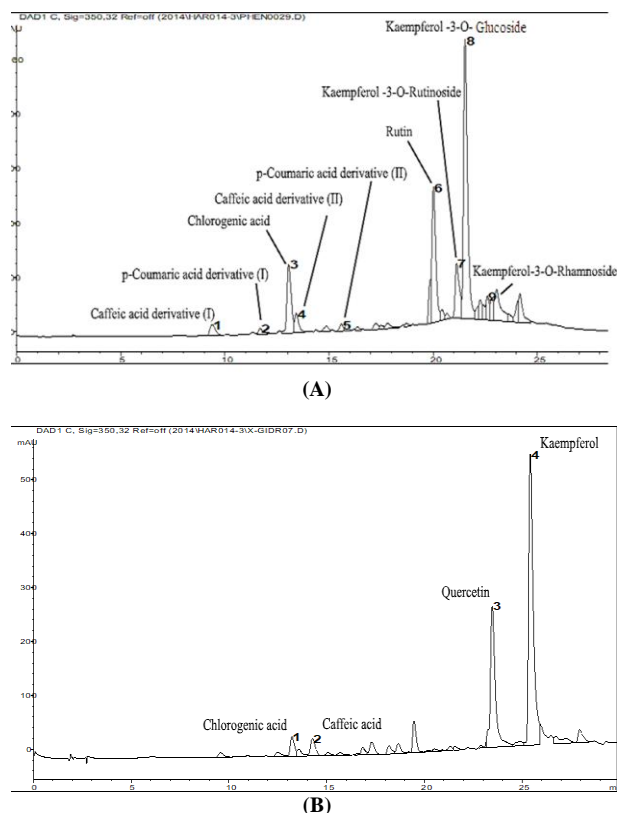
As the result of our preliminary study of biologically active substances in the peach leaves extracts with TLC and PC with authentic samples, the presence of hydroxycinnamic acids and flavonoids, including rutin, was established in all obtained extracts. The comparative study of content of hydroxycinnamic acids and total phenolics reveal that extract obtained by 50% ethanol was the richest on hydroxycinnamic acids and didn't differ in the total content of phenolics from 70% ethanol extract, but exceed 30 % ethanol extract. So 50% extract was chosen for further investigation with HPLC.

The total content of phenolic compounds, determined by spectrophotometry in the peach leaves was as 3, 17±0, 15 %, tannins–1, 02±0, 05 %, amounts of flavonoids – 2, 54±0, 12 % and the amounts of hydroxycinnamic acids – 1, 59±0, 07 %.

The results of the HPLC study of phenolic compounds in the 50% ethanol extract from peach leaves are presented in Table 1 and Figure 1. Statistical processing of the results was carried out using the Statistica 6.0 package. The statistical error did not exceed 5 %.

**Table 1. The content of phenolic compounds in the extract of peach leaves before and after hydrolysis (n = 3)**

Compound	Before hydrolysis		After hydrolysis	
	Retenti on time, minutes	Content, (mg/L)	Retenti on time, minutes	Content, (mg/L)
Caffeic acid derivative (I)	9.38	106,1±1,75	-	-
<i>p</i> -Coumaric acid derivative (I)	11.70	44,8±0,21	-	-
Chlorogenic acid	13.07	478,5±4,88	13.14	139.6±6.18
Caffeic acid derivative (II)	13.42	143,6±1,21	-	-
<i>p</i> -Coumaric acid derivative (II)	15.58	57,0±0,21	-	-
Rutin	20.02	930,0±7,02	-	-
Kaempferol -3-O-Rutinoside	21.13	405,7±3,54	-	-
Kaempferol -3-O- Glucoside	21.53	2011,2±19,79	-	-
Kaempferol -3-O- Rhamnoside	22.61	129,6±1,18	-	-
Caffeic acid	-	-	14.20	63.5±0.12
Quercetin	-	-	23.52	695.4±9.89
Kaempferol	-	-	25.46	1239.3±27.02

**Fig. 1. Graphical results of HPLC investigation of phenolic compounds in peach leaves extract before (A) and after hydrolysis (B)**

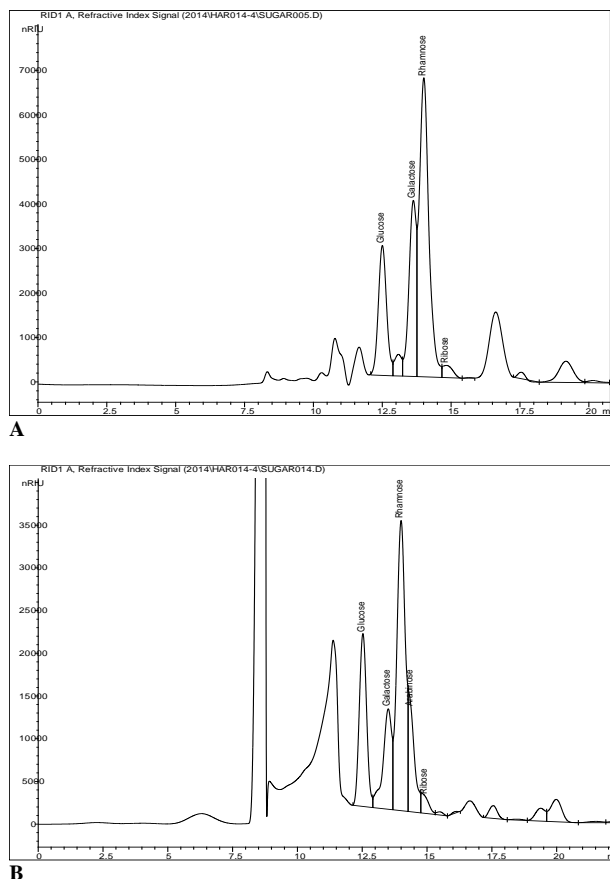
As a result of study of the peach leaves extract, 9 substances derived from hydroxycinnamic acids and flavonoids were found.

A study of the monosaccharide composition of the peach leaves extract before and after hydrolysis revealed four monosaccharides before hydrolysis and five – after (Table 2).

**Table 2: Monosaccharide composition of the extract of peach leaves before and after hydrolysis (n = 3)**

Monosaccharide	Content, %, g/L		Difference
	Before hydrolysis	After hydrolysis	
Glucose	7,04±0,05	11,09±0,07	+4,05
Galactose	8,80±0,07	8,41±0,07	-0,39
Rhamnose	23,08±0,10	24,72±0,10	+1,64
Arabinose	-	5,90±0,05	+5,90
Ribose	0,9±0,02	1,8±0,02	+0,9

Graphical result of determination of monosaccharides is shown on figure 2.

**Fig. 2. Graphical results of HPLC investigation of monosaccharides in peach leaves extract before (A) and after hydrolysis (B).**

## DISCUSSION:

According to result of spectrophotometric determination of amount of biologically active compounds flavonoids exceed of hydroxycinnamic acids content in the peach leaves in 1.6 times.

The total content of detected by HPLC phenolic compounds was 4.31 %; including the amount of hydroxycinnamic acid – 0.83 %, and flavonoids – 3.48 %. Content of flavonoids was 4 times higher than the content of hydroxycinnamic acids.

The dominant compound was kaempferol-3-O-glycoside, which content was 46.70 % of the sum of all phenolics and 57.79 % of the amount of flavonoids.

Among flavonoids, kaempferol glycosides dominated over quercetin glycosides in a 3: 1 ratio. Among hydroxycinnamic acids, the predominant component was chlorogenic acid, its part was 57.65 % of the sum of hydroxycinnamic acids.

It is known that kaempferol and its glycosides possess a broad spectrum of pharmacological activity, including antioxidant, anti-inflammatory, antimicrobial, antitumor, cardioprotective, neuroprotective, antidiabetic, anti-osteoporotic, anxiolytic, anesthetic, and antiallergic [8]. The content of rutin in the extract was 26.75 % of the sum of all flavonoid glycosides. It is known that rutin has a wide spectrum of action. It is usually used as an antimicrobial, antifungal and antiallergic agent. Modern studies have shown its pharmacological potential for the treatment of various chronic diseases such as cancer, diabetes, hypertension and hypercholesterolemia [9]. Moreover, little but important evidence can be retrieved from the clinical and nutritional scientific literature, where flavonoids are investigated as major anticancer compounds [10].

Chlorogenic acid is one of the most common polyphenolic compounds in medicinal plant raw material. For example, its content is rather high in coffee. Various studies have confirmed its antibacterial, antioxidant and anticarcinogenic activity, as well as a special role in the metabolism of glucose and lipids [11]. In addition, it has been demonstrated *in vitro* and *in vivo* that chlorogenic acid has an anxiolytic effect in combination with antioxidant activity [12].

Therefore, the high content of kaempferol glycosides and chlorogenic acid in a 50% alcoholic extract of peach leaves, established by HPLC, makes it promising for further study and creation of new phyto medicines with possible antimicrobial, anti-inflammatory and anticancer activity.

The total amount of free sugars before hydrolysis in the extract was  $39.82 \pm 0.08$  g/L, and after hydrolysis –  $51.92 \pm 0.08$  g/L. Although the content of free rhamnose was the highest in the extract, after hydrolysis, it increased by only 7.11 %, while the glucose content increased by 57.52 %. Thus, it was found that rhamnose dominates in the free form in the extract, arabinose is present only in the glycoside composition, and glucose and ribose were found in both the free and bound forms. Galactose was not found in a bound form, although it was present in the free form in the extract and its content was 22.10 % of the sum of all free sugars.

## CONCLUSIONS:

The study of the chemical composition of the peach leaves extract was carried out before and after hydrolysis by HPLC. The prospects of creating a new medicine

were confirmed. Obtained results will be used for standardization of the peach leaves extract and for further pharmacological research.

## CONFLICT OF INTEREST:

Authors declared no the conflict of interest.

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