INTERNATIONAL JOURNAL OF ADVANCES IN PHARMACY, BIOLOGY AND CHEMISTRY

Research Article

# Determination of Content of Flavonoids, Hydroxycinnamic acids and Volatile compounds in Plum leaves

**L. V. Lenchyk**

Department of Chemistry of Natural Compounds,

National University of Pharmacy, Kharkiv, Ukraine.

**ABSTRACT**

**Background:** *Prunusdomestica* L. is widely cultivated in Ukraine. Plum leaves extract was obtained and pronounced antioxidant activity of it has been ascertained. It was proved that the extract showed a higher antioxidant activity than α-tocopherol inhibiting the processof spontaneous lipid peroxidation *invitro*. In addition, by further research, it was found that the extract is not toxic and $LD_{50}$ of it was 3980-5000 mg/kg. Among compounds responsible for such effect phenolic compounds as flavonoids, hydroxycinnamic acids play role. The aim of our research was determination of content of flavonoids, sum of phenolic compounds and hydroxycinnamic acids in plum leaves and investigation of qualitative composition and content of volatile compounds there for further standardization raw material and development new drugs at its base.

**Methods:** Quantitative determination of hydroxycinnamic acids, flavonoids, and polyphenolic compounds was performed by spectrophotometric method. The study of qualitative composition and quantitative determination of volatile compounds in plum leaves was carried out bygas chromatography with mass spectrometricdetection (GC-MS). For quantitative calculations the internal standard method was used.

**Results:** Content of total phenolic compounds (5.26 ± 0.19%), hydroxycinnamic acids (2.11 ± 0.08%) and flavonoids (0.61 ± 0.01%) was determined. Composition and content of some lipophilic compounds were investigated by the GC-MS. 42 substances were established. The total amount of lipophilic compounds was 452.62 mg/kg. Among them 22 mono- and sesquiterpenes, 8 fatty acids, 6 hydrocarbons, 2 tocopherols and 4 steroids were established. Squalene and α-tocopherol were determined in the greatest quantity. The total amount of essential oil component was 130.73 mg/kg. α-Thujone has the greatest amount among them. Fatty acids were present in plum leaves in trace amounts. The total amount of tocopherols was 111.24 mg/kg. Total amount of stygmasta-3,5-dien derivatives and isomers were 38.27 mg/kg.

**Conclusion:** Hydroxycinnamic acids, flavonoids, terpenoids, and other substances were investigated in plum leaves and their content was determined by spectroscopy. Presence and content of volatile components were studied by GC-MS.

**Keywords:** plum leaves, hydroxycinnamic acids, flavonoids volatile compounds, spectroscopy and gas chromatography.

## INTRODUCTION

Plum *Prunus domestica* L. is widely cultivated in Ukraine as horticultural crops. It is very popular among the local population and is eaten fresh or processed as juice, jams and compotes[1]. In ethnoscience medical practice fresh or dried plum leaves are used to small wounds and ulcers that accelerate their healing[2]. Pronounced antioxidant activity of the plum leaves extract has been ascertained at the Biochemistry department National University of Pharmacy under Professor Andrey L.

Zagayko supervision[3]. It was proved that the extract showed a higher antioxidant activity than α-tocopherol inhibiting the process of spontaneous lipid peroxidation *in vitro*. In addition, by further research, it was found that the extract is not toxic and $LD_{50}$ of it was 3980-5000 mg/kg[4]. Among compounds responsible for such effect phenolic compounds as flavonoids, hydroxycinnamic acids and other, play role. Composition and content of such phenolic compounds in extract has been investigated[5]. Determination of content of phenolic and other biologically active compounds in plum leaves is necessary for further standardization of plant raw material and preparing project of Ukrainian Pharmacopoeia monograph for *Prunus domestica* leaves. In addition, composition of volatile compounds of Ukrainian plume lives has not been studied in detail yet.

The aim of our research was determination of content of flavonoids, sum of phenolic compounds and hydroxycinnamic acids and investigation of qualitative composition and content of volatile compounds in plum leaves for further standardization raw material and development new drugs at its base.

## MATERIAL AND METHODS

In September 2014 *Prunus domestica* leaves were collected in the farm garden, in a village called Liptsy, Kharkiv region of Ukraine. Plant raw material was dried and grinded. The study of phenolic compounds was carried out by one- and two-dimensional paper chromatograms (PC) in solvent systems: n-butanol-acetic acid anhydrous-water (4:1:2), 15% acetic acid. Chromatogram was viewed in UV light before and after treating by ammonia solution, 1% alcoholic solution of aluminum chloride and 3% solution of iron chloride.

Quantitative determination of hydroxycinnamic acids, flavonoids, and polyphenolic compounds was performed by spectrophotometric method. Absorbance was measured in the cuvette with a layer thickness of 10 mm on a spectrophotometer Hewlett Packard 8453. The measurements were carried out 5 times. Statistical analysis of the results was performed according to the requirements of SPU[6].

Quantitative determination of hydroxycinnamic acids was calculated as chlorogenic acid equivalent in % of dried plant raw material. Maximum absorption of chlorogenic acid reference solution occurred at 327 nm, so the measurements were carried out at this wavelength by modified method[7].

About 1.00 g (accurate weight) *Prunus domestica* leaves was placed in 100 ml flask and 70 ml of 20% alcohol was added. The flask was joined to condenser and heated on a water bath for 1 hour. Extraction was carried out twice. Extracts were cooled, filtered through a paper filter, quantitatively transferred to a 200 ml volumetric flask. The volume of filtrate in a volumetric flask was leaded to the mark by 20% alcohol and mixed (solution A).

3 ml of solution A were placed in 50 ml volumetric flask and was leaded to the mark by 20% alcohol. Absorbance of the solution was measured on Hewlett Packard 8453 spectrophotometer at a wavelength of 327 nm against 20% ethanol blank. Content of hydroxycinnamic acid (X, %) was calculated by a formula:

$$X = \frac{A \cdot 200 \cdot 50 \cdot 100}{E_{1см}^{1} \cdot m \cdot 3 \cdot (100 - W)}$$

Where,

A – absorption of the studied solution; $E^{1\%}_{1см}$ – specific absorption rate of chlorogenic acid, which is equal to 531; m – weight of the plant material, g; W – the loss in weight at drying, %.

To choose the method of quantitative determination of flavonoids in plum leaves, we have analyzed the spectrum of 70% alcohol extract of the plum leaves. It was found that according to absorption maxima of the plum leaves extract due to overlaying of more intense absorption bands by accompanying substances determination of content of flavonoids by direct spectrophotometry inappropriate. By using spectrophotometric method based on the reaction of complexation flavonoid aglycone with aluminum chloride, there was a Bathochromic shift of the absorption band of flavonoids from 330-350 to 390-420 nm. Adding alcohol solution of aluminum chloride allowed to exclude the impact of accompanying substances. Maximum of absorption band of flavonoids appeared at 417 nm, which allowed carrying out analysis at this wavelength. Thereby quantitative determination of flavonoids in plum leaves was carried out by known method of State Pharmacopoeia of the USSR XI ed and calculated as rutin equivalent in % of dried plant raw material[8].

The determination of phenolic compounds in plum leaves calculated as gallic acid equivalent was performed by modified method[9].

10,0 g of plum leaves, accurately weighed was placed into flask, 150 ml of water was added and condenser was connected. Extraction was carried out during 2 hours on the water bath twice with new portion of water. Extracts were cooled, filtered through a paper filter, united and concentrated to 200-250 ml and filtered in volumetric flask capacity 250 ml. The volume of filtrate in a volumetric flask was leaded to the mark by water (solution A).

1,0 ml of the solution A was placed to a volumetric flask capacity 25 ml and leaded to the mark by 40% ethanol. 1 ml of the obtained solution was placed to a volumetric flask capacity 25 ml and was leaded to the mark by the same solvent.

Absorbance of the solution was measured at a wavelength of 271 nm against 40% ethanol blank. The content of phenolic compounds in % was calculated by a formula:

$$X = \frac{A \cdot 250 \cdot 25 \cdot 25 \cdot 100}{540 \cdot m \cdot 1 \cdot 1 \cdot (100 - w)},$$

Where, A – absorption of the studied solution; m – weight of the plant material, g; *w*– the loss in weight at drying, %; 540 – coefficient of specific absorbance of 40% alcohol gallic acid solution at a wavelength of 271 nm.

The study of qualitative composition and quantitative determination of volatile compounds in plum leaves was carried out by gas chromatography with mass spectrometric detection (GC-MS). For quantitative calculations the internal standard method was used[10].

Accurately weighed sample of dried plum leaves (0.05 g) was placed in a 2 ml vial and the internal standard (50 µg of tridecane in hexane) was added and filled up with 0.6 ml of methylene chloride. The sample was incubated for 3 hours at a temperature 50°C in ultrasonic extractor. The extract was decanted into a 2 ml vial, and concentrated by blowing (100 ml/min) ultra-pure nitrogen until the residual volume of extract was 10 µl.

Injection of sample (3 µl) in a chromatographic column was carried out splitless. Analysis was carried out on Agilent Technologies 6890 chromatograph with mass spectrometric detector 5973; chromatography column - DB-5, capillary external diameter - 0.25 mm and a length - 30 m; the rate of carrier gas (helium) was 1.2 ml/min; heater temperature to sample introduction was 250°C; temperature of thermostat was programmed from 50 to 320 °C with a speed of 4 °C/min.

For components identification library of mass spectra NIST05 and WILEY 2007 with a total number of spectra more than 470,000 in conjunction with programs for the identification the AMDIS and NIST was used. Calculation of components content C (mg/kg) was carried out by the formula:

$$C = P_1 \cdot 50 \cdot / P_2 \, m \cdot$$

Where is:

$P_1$ - The peak area of the tested substance; $P_2$ - the peak area of the standard; 50 – mass of internal standard (µg), injected into the sample; m– sample mass (g).

The statistical processing of results was carried out using package Statistica 6.0. The error does not exceed 5%.

## RESULTS

After investigation of 50% alcohol plum leaves extract by one- and two-dimensional paper chromatography spots of brown, yellow, light blue, light violet colors were observed, which after treating by ammonia solution acquired a yellow-brown, bright yellow, bright blue, light blue, bright violet fluorescence, indicating the presence of flavonoids, hydroxycinnamic acids and other phenolic compounds. By the one-dimensional PC with reference solution of rutin and chlorogenic acid presence of these phenolic compounds in plum leaves was confirmed.

Values of flavonoids, phenolic compounds, and hydroxycinnamic acids were calculated. The content of flavonoids was 0.61 ± 0.01%, polyphenols – 5.26 ± 0.19% and hydroxycinnamic acid – 2.11 ± 0.08%.

Results of determination of volatile compounds are showed in the table1. Graphical result can be seen on figure 1.

## DISCUSSION

Biologically active compounds of plum leaves were investigated by PC method. Presence of flavonoids, hydroxycinnamic acids was established and among them rutin and chlorogenic acid were identified as the most abundant. Contents of hydroxycinnamic acid, flavonoids and polyphenols compounds were determined. It will be used to further standardization of the plum leaves as medicinal raw material. The total phenolic compounds and, among them, flavonoids and hydroxycinnamic acids, may play important role in determined antioxidant activity of extract obtained from plum leaves[3]. Antioxidant properties of flavonoids and hydroxycinnamic acids may be explained by highly mobile electrons. Experimental evidence suggests a direct relationship between antioxidant activity of flavonoids and phenolic OH groups in their molecules[11,12]. In addition, the antioxidant activity of phenolic compounds can be considered as a possible mechanism through which such biological effects as detoxification, adaptogenic, immunomodulatory and antitumor implemented[11, 13]. Hydroxycinnamic acids, may influence on glucose and lipids metabolism, and rutin has wide range of pharmacological properties [14, 15].

**Table 1**
**Results of determination of volatile compounds in *Prunus domestica* leaves**

| № | Retention time | Name of the compound | Content, mg/kg |
|---|---|---|---|
| 1 | 6.42 | Sabinene | 1.18 |
| 2 | 7.81 | Isopropylbenzene | 0.82 |
| 3 | 7.95 | 1,8-Cineole | 2.78 |
| 4 | 8.02 | Limonene | 0.76 |
| 5 | 10.10 | α-Thujone | 49.02 |
| 6 | 10.44 | β-Thujone | 4.65 |
| 7 | 11.11 | Camphor | 11.15 |
| 8 | 16.60 | trans-Pinocarvl acetate | 9.49 |
| 9 | 18.09 | Terpinyl acetate | 6.41 |
| 10 | 18.40 | α-Cubenene | 3.34 |
| 11 | 20.41 | β-Elemene | 0.48 |
| 12 | 20.73 | trans-Caryophyllene | 0.42 |
| 13 | 21.02 | α- Caryophyllene | 6.20 |
| 14 | 21.35 | α-Farnesene | 0.83 |
| 15 | 22.63 | Germacrene B | 1.14 |
| 16 | 23.40 | δ-Cadinene | 11.31 |
| 17 | 23.47 | Caryophyllene oxide | 4.63 |
| 18 | 24.72 | Spatulenol | 6.02 |
| 19 | 25.01 | Bisabolene epoxide | 1.57 |
| 20 | 25.27 | 1,5,5,8-Tetramethyl-12-oxa bicyclo[9.1.0]dodeca-3,7-diene | 1.73 |
| 21 | 29.47 | Myristic acid, | 5.11 |
| 22 | 30.79 | 6,10,14-Pentadec-2-one | 1.05 |
| 23 | 31.14 | Pentadecanoic acid | 3.60 |
| 24 | 31.72 | Pharnesyl acetone C | 5.75 |
| 25 | 32.63 | Palmitoleic acid | 0.62 |
| 26 | 32.70 | Palmitic acid | 5.84 |
| 27 | 34.85 | Linoleic acid | 1.11 |
| 28 | 34.85 | Linolenic acid | 0.45 |
| 29 | 34.86 | Oleic acid | 2.99 |
| 30 | 34.95 | Stearic acid | 0.60 |
| 31 | 36.65 | Tricosane | 3.86 |
| 32 | 37.71 | Pentacosane | 4.21 |
| 33 | 38.71 | Hexacosane | 3.94 |
| 34 | 40.60 | Heptacosane | 3.95 |
| 35 | 41.63 | Squalene | 124.24 |
| 36 | 42.36 | Nonacosane | 11.86 |
| 37 | 43.65 | γ- Tocopherol | 3.26 |
| 38 | 43.73 | Stygmasta-3,5-dien (isomer) | 2.10 |
| 39 | 44.03 | Stygmasta-3,5-dien | 32.71 |
| 40 | 44.32 | α-Tocopherol | 107.98 |
| 41 | 45.93 | Stygmasta -4,22- dien -7-on | 2.41 |
| 42 | 46.01 | Stygmasta -4-en-3-on | 1.05 |

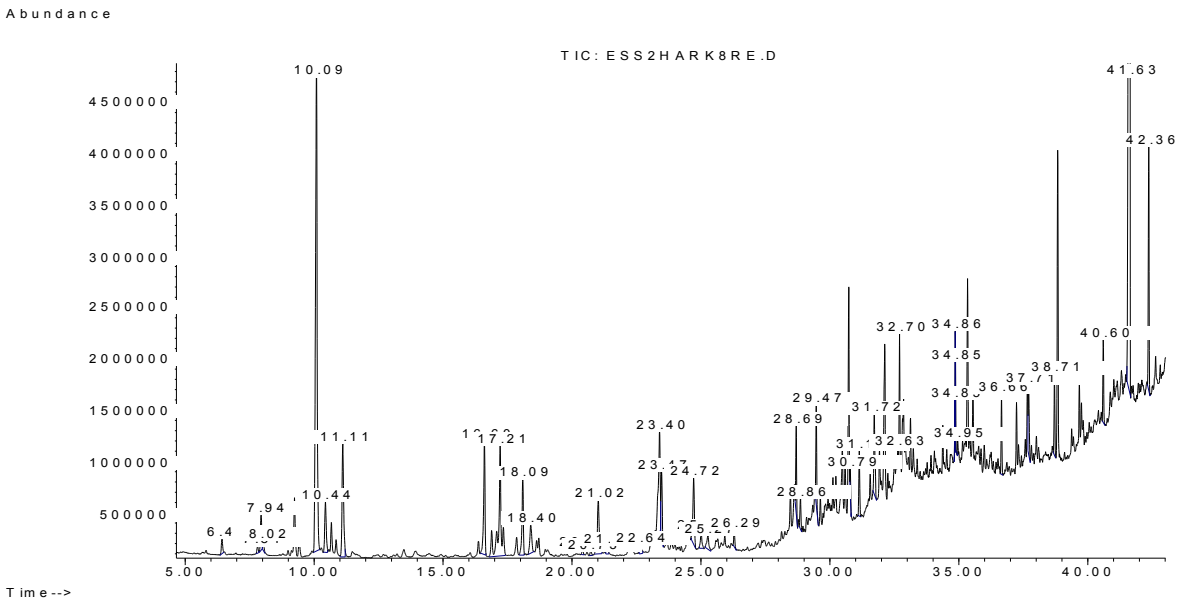


**Figure 1.**
**Graphical result of GC-MS investigation of volatile compounds in *Prunus domestica* leaves**

By the GC-MS composition and content of some lipophilic compounds were investigated. 42 substances were established. The total amount of lipophilic compounds was 452.62 mg/kg. Among them 22 mono- and sesquiterpenes, 8 fatty acids, 6 hydrocarbons, 2 tocopherols and 4 steroids were established. Squalene and α-tocopherol were determined in the greatest quantity. The total amount of essential oil component was 130.73 mg/kg. α-Thujone has the greatest amount among them. This is toxic component and we should avoid presence of it in dry extract. Fatty acids were present in plum leaves in trace amounts. The total amount of tocopherols was 111.24 mg/kg. Presence of such compounds may play role in antioxidant activity of plum leaves extract. Total amount of stygmasta-3,5-dien derivatives and isomers were 38.27 mg/kg. Nowadays determination of Stygmasta-3,5-dien in some fatty oils (for example, olive oils) is used to ascertain the authenticity of oils, the European Community Regulation requires the Stigmasta-3,5-diene and wax ester contents to be determined[16].

## CONCLUSION

1. Hydroxycinnamic acids, flavonoids, terpenoids, and other substances were investigated in plum leaves.
2. Content of total phenolic compounds, hydroxycinnamic acids, flavonoids was determined by spectroscopy.
3. Presence and content of volatile components were studied by GC-MS.

## ACKNOWLEDGEMENTS

This material is based on work supported by the National University of Pharmacy, Ukraine, Department of Chemistry of Natural Compounds and Department of Biochemistry**.**

## REFERENCES

1. Wyk, B-E. van Food plants of the World: identification, culinary uses and nutritional value / B.-E. van Wyk. – Gauteng: Briza Publications, 2005. – P. 308.
2. Upyr LV. Plum: in book Pharmaceutical encyclopedia / Chairman Ed. Council VP. Chernykh. – 2-nd ed. – K.: «MORION» 2010. – P.1290.
3. Zagayko AL, Senyuk IV, Lenchyk LV, Galimullin RM. Study of antioxidant activity of the extract from plum ordinary leaves. Ukr Biochem J, 2014; 30 (1): 25-28.
4. Lenchyk LV, Senyuk IV, Ali Sahlani BJ. Determination of acute toxicity of the extract from *Prunus domestica* leaves and organic acids in raw materials. Ukr Biochem J, 2016; 42(1): 66-71.
5. Lenchyk L. Determination of phenolic compounds in *Prunus domestica* leaves extract. Scripta Scientifica Pharmaceutica, 2015; 2 (2): 35-39.
6. The State Pharmacopoeia of Ukraine / State Enterprise “Ukrainian scientific Pharmacopoeial center”. – 1-st edd. Supplement 2– Kharkiv: RIREG, 2008. – 620 pp.

7. Koyro OO, Stepanova SI, Shtrygol SYu. Hydroxycinnamic acids assay in the ground elder plant drugs. 2009. Ukrainian Journal of Clinical and Laboratory Medicine. 4 (2): 52–55.
8. The State Pharmacopoeia of USSR. 11 edd. Issue 2. General methods of analysis. Medicinal herbs / Ministry of health of USSR. – M. : Medicina, 1990. – 400 pp.
9. Upyr DV, Martynov AV, Kyslychenko VS. Study of the of phenolic compounds content of the mistletoe raw material. Collection of scientific works of staff member of P. L. Shupyk NMAPE, 2012;18 (2): 368-373.
10. Upyr TV, Komissarenko AN, Koshevoy ON. Terpenoid composition of *Ledum palustre* herb essential oil. Collection of scientific works of staff member of P. L. Shupyk NMAPE, 2014; 23(4): 408–412.
11. Zajjchenko AV, Sharifov HSh, Stahorskaiy MA, Haleeva EL, Navruzova GF. Phytochemical justification of pharmacological effects of phenolic compounds of peach (review). Fitoterapiya-chasopis, 2014; 4:71-74.
12. Baraboy V.A. Bioantioxydants. M.: Kniga-plus: 2006. - 462.
13. Solomko ESh, Stepanova EV, Abramov ME. Angiogenesis inhibitors from plants origin: perspective for clinical usage. Russian journal of biotherapy, 2010; 9(4): 3-10.
14. Shengxi Meng et al. Roles of Chlorogenic Acid on Regulating Glucose and Lipids Metabolism: A Review. Hindawi Publishing Corporation. Evidence-Based Complementary and Alternative Medicine, 2013.
15. Al-Dhabi NA, Arasu MV, Park CH, Park SU. Letter to the editor: An up-to-date review of rutin and its biological and pharmacological activities. EXCLI Journal, 2015; 14:59-63
16. Mauro Amelio, Renzo Rizzo, Flavio Varazini. Separation of stigmasta-3,5-diene, squalene isomers, and wax esters from olive oils by single high-performance liquid chromatography run. J. of the American Oil Chemists' Society, 1998; 75(4): 527-530.