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Phytochemical study of lipids of *Prunus domestica* L. seeds cultivated in Georgia

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Abstract
The aim of this research was the study of the lipid and biologically active compound composition of the kernels of *Prunus domestica* growing in two different eco-regions of Georgia. Sums of both neutral and polar lipids were obtained from the kernels and their physical-chemical constants were determined. Fatty acid composition was studied using GC-MS and showed the content of 9-hexadecanoic, hexadecanoic, 9,12-hexadecanoic, 9-octadecanoic, octadecanoic, heptadecanoic, and eicosanoic acids, with 9,12-hexadecanoic and 9-octadecanoic being the two dominants acids. The isolated fatty oils of both regions, rich in biologically active compounds, shows the potential of *P. domestica* oil as a cheap and easily accessible regional source for cosmetic and medicinal purposes, with the oil from the more arid Eastern region of Georgia being slightly superior in content.

Keywords: Prunus, plum, lipid, fatty acid, phospholipid

1. Introduction
Among naturally occuring biologically active compounds, lipids attract special attention for their great practical importance. These belong to a group of compounds widespread in the plant kingdom. Some of them are essential components of the plant cell, which indicates their important physiological role. Lipids are structural components of the cell membrane and connective tissue that participate in the regulation of vital processes of the body, perform storage, structural, protective, energetic, regulatory function. Their show varied biological activities, such as immunotropic, hepatoprotective, choleretic, antibacterial, antiviral, anti-inflammatory, cytotoxic, reducing the risk of developing atherosclerosis and cardiovascular disease. They increase the body's resistance to diseases of varied etiology [1-3].
Prunus domestica L. (European Plum), Family: Rosaceae - are large shrubs or small trees around 6 – 12 meters high sometimes somewhat thorny. Leaves are elliptical or obovate with acute or obtuse tips, with crenate or slightly dentate margins and short petioles. Flowers are small, white, sometimes with a greenish hue and have long pedicels. They are solitary or born in umbel-like clusters of 2-5 flowers on short spurs. Stamens are numerous, single pistil with a superior ovary. Fruits are fleshy drupes, oval or round to conical having a smooth surface. Their colour varies depending on the cultivar [4, 5]
P. domestica L has pronounced hypotensive, antihyperlipidemic, antioxidant, antibacterial, anti-inflammatory activity, acts as a laxative on the gastrointestinal tract, is effective in preventing and restoring bone loss. Plum seed oil is used as an emollient, included in moisturizing creams, lotions, and washes [4, 7, 8].
Georgian variety of plum *Prunus domestica* is highly variable with respect to the ecological conditions of the environment, it grows to large-bodied trees, slows down its growth after entering the fruiting stage, is characterized by intense root growth, has a wide, egg-shaped fruit, with bluish-black skin, which is covered with a waxy flake. The pulp is quite dense, juicy and sweet. The average yield per hectare reaches 4-5 tons, but in plum orchards where all agro-recommendations are observed, it is possible to harvest more than 10 tons per hectare [4, 9].
Preliminary phytochemical analysis allows us to find a new plant source and use a new lipid-containing plant resource common in Georgia with medicinal and industrial value. The aim of this research was the study of *Prunus domestica* distributed in two different ecological regions, namely, Western (Imereti, Khoni district) and Eastern (Sagarejo, Kakheti) Georgia.

These geographical regions have distinctly different ecological conditions. Imereti and much of West Georgia are characterized by a moist mild climate, with less pronounced temperature fluctuations, while Sagarejo, as most of East Georgia, are more arid. As a result of the research, lipids and several other biologically active compounds of *Prunus domestica* L. seeds were studied from a medicinal, pharmaceutical and cosmetic-dermatological viewpoint.

Materials and Methods

Plant material

The ripe fruits of *Prunus domestica* were collected from cultivated plants during fruiting season, at the stage of full maturity, in Sagarejo, Kakheti and the Khoni district, Imereti, Georgia in 2020. They were identified in the Department of Pharmacobotany at TSMU I. Kutateladze Institute of Pharmacochemistry. A total of 1000 grams of ripe fruits each were collected. The pyrenes were removed from the fleshy part of the fruit manually, giving around 365 grams. These were cracked to extract the kernels. The latter were dried under ambient temperature outside the reach of direct sunlight, resulting in 57 grams of dry kernels in each batch, and powdered for further analysis.

Extraction and analysis of neutral lipids

An oily sum of neutral lipids (N/L) was obtained from the powdered kernels by four-fold extraction using n-hexane (1:5) at ambient temperature and was further condensed on a vacuum-rotary apparatus (60 °C).

Analysis was carried out using TLC on silica gel 60 F254 (20 cm × 20 cm, Merck, Darmstadt, Germany) plates. Mobile phase petroleum ether-diethyl ether-ice-acetic acid (85:14:1); visualization was performed in iodine vapour and with 30% sulfuric acid; determination was done with color reactions, R_f value and reference samples.

Extraction of polar lipids

A thick sum of polar lipids (P/L) was obtained by four-fold extraction of the powdered kernel residue left after the extraction of neutral lipids using a chloroform-methanol mixture (2:1) and later condensed on a vacuum-rotary apparatus (60 °C).

Fatty acid derivatization

Methyl esters derivates of fatty acids were synthesized using 5% anhydrous hydrogen chloride in methanol. The lipid samples were dissolved in 100 folds hydrogen chloride in methanol and refluxed for 2 hours under 75 °C [10].

Gas chromatography-mass spectrometry (GC-MS)

GC-MS analysis of fatty acids was carried out with an Agilent Technologies 7890B GC-MS instrument equipped with a split/spilitless injector and an autosampler. A HP-5ms Ultra Inhert (30m×250µm×25µm) capillary column was used for sample separation and a a mass spectrometer was used for detection. Injector temperature: 280 °C; detector temperature: 280 °C; initial column temperature: 150 °C for 2 min.; 1500° - 195 °C (15 C/min); 195 °C for 5 min; 195° - 205 °C (4 C/min), 205^0 C for 5 min. 205° - 211 °C (4 C/min), 211 C for 5 min. Transferline temperature: 280 °C. Split value 200. The obtained results were treated with the NIST database to identify the components [11, 12].

Qualitative analysis of phospholipids

Isolated phospholipids were qualitatively analyzed with double-sided TLC: mobile phase: 1. Chloroform-methanol-25% ammonia (65: 30: 5). 2. Chloroform-methanol- acetic acid-water (170:25:25:6); immobile phase: TLC Silica gel 60 F254 (20 cm × 20 cm, Merck, Darmstadt, Germany); visualization was performed in iodine vapour and the Waskowski reagent; determination was done with color reactions, R_f value and reference samples. Quantitative analysis of the total phospholipid sum was performed using inorganic phosphorus determination by UV-Vis spectrophotometry (Jasco V-730), wavelength: 620 nm [13].

Amino acid analysis

Amino acids were extracted from the powdered kernels using 80% ethanol and analyzed using thin-layer chromatography: silica gel plates: 60 F254 (20 cm × 20 cm, Merck, Darmstadt, Germany); mobile phase: butanol-acetic acid-water (6:2:2); visualizing agent: 1% ninhydride solution; determination by color reactions, Rf values and standard samples (amino acid kit from the "Chemreaktivcomplect") [14].

Analysis of carotenoids

The quantitatively content of carotenoids was determined in the neutral lipid sum using UV-Vis spectrophotometry at 451 nm [15].

Results and Discussion

The sum of neutral lipids (n/l) was obtained from the seeds of *Prunus domestica* L. commonly cultivated in Western and Eastern Georgia by five-fold extraction with n-hexane, yielding 37% and 38% respectively. The main classes included in the mentioned totals are established: hydrocarbons, diglycerides, triglycerides, fatty acids, sterol.

The physical-chemical constants of the studied plum seed oil have been determined: in the first case - specific gravity 0.910, refractive index 1.463, acidity number 0.38, iodine number 92.3, solubility number 197.2. In the second case - specific gravity 0.916, refractive index 1.468, acidity number 0.41, iodine number 93, sulfur number 198.12.

Using GC-MS, the following fatty acids were qualitatively and quantitatively identified in the plum kernel oil of both regions: 9-hexadecanoic, hexadecanoic, 9,12-hexadecanoic, 9-octadecanoic, cctadecanoic, heptadecanoic, and eicosanoic acids.

After removal of neutral lipids, total polar lipids (p/l) were obtained from plant whey of both objects with a yield of 0.75% and 0.78%, 5 phospholipids included in them were qualitatively determined, with a total content of 0.15 and 0.16%: lysophosphatidylcholine, lysophosphatidylinositol, phosphatidylinositol, phosphatidylcholine, phosphatidylethanolamine. The quantitative content of lipids is given in table 1.

Vitamin E was detected and quantified at 35.1 mg% and 39.4 mg% and carotenoid content was 5.24 mg% and 7.5 mg% in the Western and Eastern material respectively. Plum seeds contain 6 amino acids: asparagine, serine, leucine, alanine, cysteine, phenylalanine.

Table 1: Comparisson of lipid content of *P.domestica* from Eastern and Western Georgia

Plant material	*P. domestica* from Eastern Georgia %	*P. domestica* from Western Georgia %
Fatty acids (neutral lipids)		
9-hexadecenoic acid	2,21	0,62
Hexadecanoic acid	5,67	6,06
9,12-octadecadienoic acid	17,85	16,43
9-octadecenoic acid	72,02	74,33
Octadecanoic acid	1,7	1,63
Heptadecanoic acid	< 0.1	< 0.1
Eicosanoic acid	< 0.1	< 0.1
Total polar lipids	0.78	0.75
Total phospholipids	0.16	0.15

Conclusion

The study of oil composition of Georgian *Prunus domestica* showed the presence of saturated fatty acids (hexadecanoic, heptadecanoic and octadecanoic acid) and unsaturated fatty acids (9-hexadecanoic, 9, 12-hexadecanoic, 9-octadecenoic and eicosanoic acid). The highest proportion of the plum kernel oil is composed of 9-octadecenoic and 9, 12-hexadecanoic acid. According to the results obtained of pharmacological tests plum oil shows pronounced hepatoprotective, moderate anti-inflammatory and gastroprotective activity. Based on the abovementioned, in the future it will be possible to determine the creation of a cheap, effective medicinal-prophylactic and cosmetic products based on local raw materials, which will have practical use in pharmacy, medicine and cosmetics. The results of this scientific research will make a significant contribution to the creation of both affordable and competitive pharmaceutical products.

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