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Study of lipids and some biologically active compounds of *Sambucus ebulus* L. spread in Georgia

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Abstract
The goal of the research was to study the fruit of *Sambucus ebulus* L. for the content of lipids and some biologically active compounds. Sums of neutral and polar lipids were obtained from the investigated object with various percentage yields. The basic classes of lipids were established in them and some of their physical-chemical constants were identified. Saturated, unsaturated and polyunsaturated fatty acids were quantitatively and qualitatively identified in the sum of neutral lipids by using the gas-chromatographic method, with high percentage contents of some of them. Phospholipids were identified qualitatively and quantitatively in the sum of polar lipids. Based on the study, the presence of some other biologically active compounds, such as carotenoids and amino acids was identified in the given object. As the obtained results suggest the vegetable oil obtained from the studied object is rich in biologically active compounds, which gives the opportunity to use the oil in medical practice.

Keywords: Lipid, fatty acid, phospholipid

Introduction
Among the biologically active natural compounds, lipids, which are interesting organic substances of practical importance and widely distributed in the plant world, receive special attention. Some of them are an essential component of the plant cell evidencing their vital physiological role.
Lipids are structural components of the cell membrane and connective tissue, which are involved in the regulation of vital processes of an organism, and perform supplying, structural, protective, energetic and regulatory functions. The mechanisms of their actions are diverse: immunotropic, hepatoprotective, biliary, antibacterial, antiviral, anti-inflammatory and cytotoxic; they reduce the risk of atherosclerosis and cardiovascular diseases while strengthening the body's resistance to infectious and malignant diseases [6, 9].
Sambucus ebulus L. - danewort, family: *Caprifoliaceae*, is a perennial shrub common in Europe, Crimea and the Caucasus. It commonly grows on the riverbanks, in forested areas, on mountain slopes and in gardens. The leaves of the plant are long-lanceolate, flowers are yellow umbels and the fruits and seeds are black globular. The plant flowers in June or July and bears fruit from August to October. Its roots and fruits are used for medicinal purposes [5, 14].
The plant contains various biologically active compounds, such as: tannins, flavonoids, coumarins, sterols, anthocyanins, essential oils, alkaloids, and vitamins [2, 4].
The medicinal raw materials of danewort have antimicrobial, antioxidant, diuretic, laxative, sweating and expectorant actions. In medicine it is used to treat the inflammation of the upper respiratory tract, chronic bronchitis and gastrointestinal tract diseases [8, 11, 16].

Material and Methods
Plant material
The fruits of *Sambucus ebulus* L. were collected after the flowering season in Imereti, Georgia in 2020. They were identified in the Department of Pharmacobotany at TSMU I. Kutateladze Institute of Pharmacochemistry. Voucher specimen TBPH#2324 was deposited in the Institute's herbarium. The plant material was dried and powdered for further analysis.
The goal of the present work is to study the content of lipids and some biologically active compounds of the fruit of *Sambucus ebulus* L. - danewort spread in Georgia in order to further use it in medicine and pharmaceutical practice.

Experimental part
Extraction of neutral lipids
A sum of neutral lipids (N/L) of oily consistency was obtained from *S. ebulus* fruits by four-time n-Hexane extraction (1:5) at room temperature and further thickening by a vacuum-rotary apparatus (60 ^{0}C).

Extraction of polar lipids

A sum of polar lipids (P/L) of thick consistency was obtained by four-time extraction of the plant residue remained following the extraction of neutral lipids with chloroform-methanol mixture (2:1) and later thickening it by a vacuum-rotary apparatus (60 ^{0}C).

Analysis of neutral lipids

Neutral lipids were analyzed using TLC with petroleum ether-diethyl ether-ice-acetic acid (85:14:1) as a mobile phase; and TLC silica gel 60 F254 (20 cm × 20 cm, Merck, Darmstadt, Germany) as an immobile phase; detection with iodine vapour and 30% sulfuric acid; determination with color reactions, R_f value and reliable samples.

GC-MS Analysis

Gas chromatographic analysis of fatty acids was done with an Agilent Technologies 7890B GC-MS. The instrument was equipped with a split/spilitless injector. The autosampler was coupled with a capillary column HP-5ms Ultra Inhert (30m×250µm×25µm) and to a mass spectrometer. Injector temperature: 280 °C; detector temperature: 280 °C; initial column temperature: 60°C for 2 min.; 60° - 100 ^{0}C (2,5 C/min); 100 °C for 2 min; 100° - 280 °C (7C/min), 280 ^{0} C for 2min. Transferline temperature: 280 °C. The obtained results were treated with the NIST database to identify the components [3, 12, 13, 15].

Qualitative analysis of phospholipids

The qualitative analysis of phospholipids was provided with a double-side thin-layer chromatography: 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) detection with iodine vapour and the Waskowski reagent; determination with color reactions, R_f value and reliable samples.

Quantitative analysis of phospholipids

The quantification of phospholipids in the total P/L sum of the test object was done with inorganic phosphorus determination by spectrophotometric method (Jasco V-730), wavelength: 620 nm [1, 10].

Amino acid analysis

The amino acids were studied in the 80% ethanol extract of the test object. The analysis was performed with thin-layer chromatography: Silica gel plate TLC Silica gel 60 F254 (20 cm × 20 cm, Merck, Darmstadt, Germany); solvent system: butanol-acetic acid-water (6:2:2); detector: 1% ninhydride solution; determination by color reactions, Rf values and standard samples (amino acid kit from the "Chemreaktivcomplect" factory) [7].

Analysis of carotenoids

The content of carotenoids was determined quantitatively in the N/L sum of the test object with a spectrophotometric method, wavelength: 451 nm [17].

Results and discussion

A sum of neutral lipids was obtained from *S. ebulus* air-dry fruit with a 17% yield. The major classes of the N/L sum were identified: carbohydrates, triglycerides, fatty acids, sterols.

The physical-chemical constants of *S. ebulus* fruit oil were determined: specific weight: d^{20}_4 0,934, refraction index: n_D^{20} 1,479, acidity number: 5,4 mg (KOH), iodine number: 91 I_2.

7 fatty acids were identified qualitatively and quantitatively in *S.ebulus* fruit oil by GC-MS: hexadecane 10.87%, octadecane 2.36%, octadecene 41.63%, 9,12-octadecadene 35.7%, eicosan 0.15%, eicosene 0.21%, and tetracosan 0.18%. Between saturated acids hexadecane acid dominates (10.87%), while octadecene (41.63%) is the dominant unsaturated acid (Fig.1).

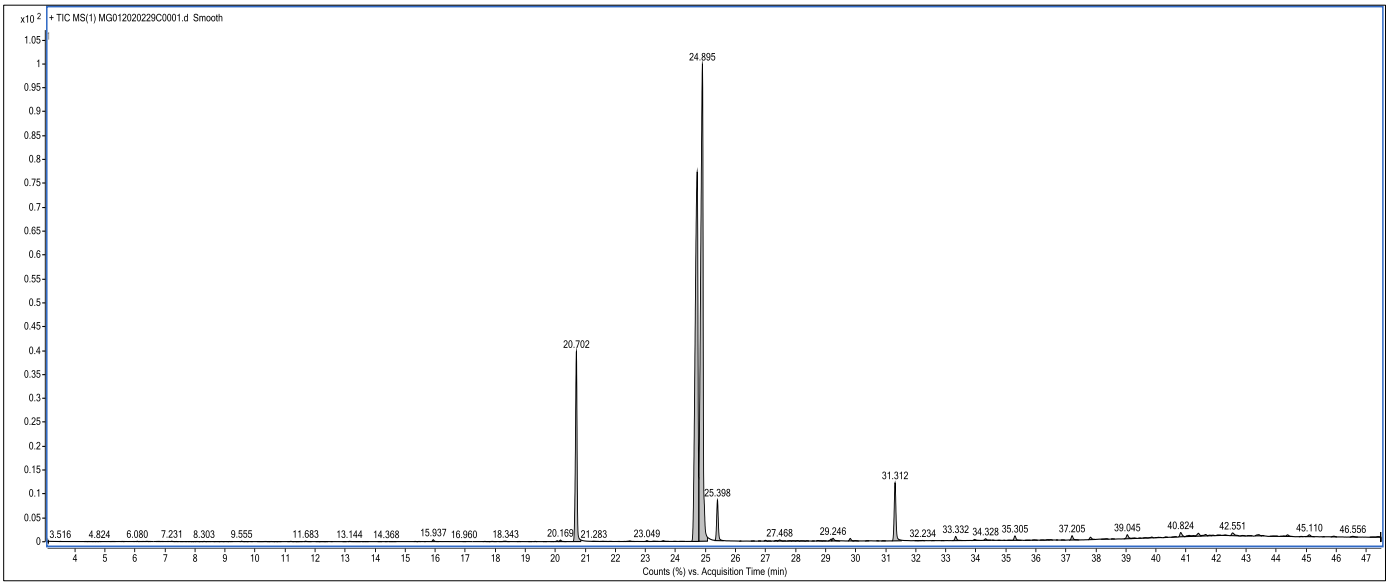

Fig 1: GC-MS profile of free fatty acids from the fruits of *Sambucus ebulus*

A sum of P/L was obtained from the residue after the N/L extraction with a 4,9% yield; 6 phospholipids in the P/L sum were determined qualitatively with the total content of 1,83%. Phosphatidylethanolamine0.37%,phosphatidylcholine0.68%,lysophosphatidylethanolamine 0,27%, N-acylphosphatidylethanolamine 0,31%. The content of carotenoids in *S.ebulus* fruit oil was determined (43.0 mg%). The presence of 4 amino acids: histidine, methionine, alanine, and phenylalanine were qualitatively established in the fruit.

Conclusion

The vegetable oil is obtained from the studied object, which contains saturated, unsaturated and polyunsaturated fatty acids, phospholipids and is rich in various biologically active compounds: carotenoids and amino acids. This allows the creation inexpensive and efficient therapeutic and prophylactic preparations from local raw materials to use practically in medicine and cosmetology.

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