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GS/MS analysis of fatty acids in flowers and leaves of *Chrysanthemum×hortorum Bailey Belgo* and *Pectoral'* variants

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Abstract
The represented study describes fatty acids' qualitative compositions and quantitative content in flowers and leaves of *Chrysanthemum×hortorum Bailey Belgo* and *Pectoral'* variants determined by GC/MS method. Fatty acids, Lipophilic alcohol and Lipophilic hydrocarbon were identified and quantified after comparison with reference standards. The predominant components were unsaturated fatty acids (in leaves and flowers of *Chrysanthemum×hortorum Bailey Belgo* variant) and saturated fatty acids (in *Pectoral'* variant's flowers). Linolenic alcohol and eicosane were studied: both investigated samples of leaves of *Chrysanthemum×hortorum Bailey* are characterized by linolenic alcohol presence; eicosane was identified in leaves of *Chrysanthemum×hortorum Bailey Pectoral'* variant.

Keywords: *Chrysanthemum×hortorum Bailey Belgo* and *Pectoral'* variants, flowers, leaves, fatty acids, esters of fatty acids, GS/MS analysis

1. Introduction
Perennial garden chrysanthemum *(Chrysanthemum×hortorum Bailey, Asteraceae* family*)* is well-known ornamental plant. Species of *Chrysanthemum* originate in China, but today species and variants are widely distributed (cultivated) in Eastern and Central Asia, Europe, Northern Africa and Northern America. In Chine, where the plants can be found in wild nature, species of *Chrysanthemum* are used as constituents of spices and tea formulas; Chinese medicine recommends there flowers and herb as medicinal plant raw materials with antibacterial, antiviral, fungicide, carminative and depurative remedy [1, 2]. At the same time allergenic properties of *Chrysanthemum* are described in literature and well known for gardeners [1].
Belgo and *Pectoral'* variants are commonly used in landscape design in Ukraine. Flowering period occurs in autumn (October). Literature data on important pharmacological activities of *Chrysanthemum* indicates availability of it's detailed pharmaceutical and pharmacological researches. Chemical composition of the plant raw material of *Chrysanthemum×hortorum Bailey Belgo* and *Pectoral'* variants have to be studied due to possibilities of new phytomedicines' introduction to medical practice.

Fig 1: *Chrysanthemum×hortorum Bailey Belgo* variant

Fig 2: *Chrysanthemum×hortorum Bailey Pectoral'* variant

Fatty acids belong to primary plants' metabolites and are important constituents of plant organisms. Plant originated fatty acids also are normal components of human diet performing several important functions – from energy supporting to antioxidant and cholesterol-normalizing (unsaturated or essential ones) [6, 7]. In addition, fatty acids and oils obtained from plant raw materials make an influence on pharmacokinetic and common pharmacological effect of phytomedicines.

2. Materials and Methods

2.1 Plant Raw Materials: herbs of *Chrysanthemum×hortorum Bailey Belgo* and *Pectoral'* variants (Fig. 1 and 2) were collected during mass flowering period (in September and October, 2016) in Western Ukraine (Ternopil region) and Central Ukraine (Vinnytsya region). Leaves and flowers were separated and dried at temperature 35±5 ^{0}C in electric dryer.

2.2 Sample Preparing: The sample of plant raw material was grinded into a powder by laboratory mill, then about 0.5 g (accurately mass) was selected and placed into the glass vial and 3.3 ml of reacting mixture (methanol: toluene: sulfusic acid (44:20:2 v/v)) and 1.7 ml of internal standard solution (undecanoic acid heptane solution) were added. The sample was maintained at 80 °C for 2 hours, cooled and centrifuged for 10 minutes at 5000 rpm. 0.5 ml of the upper heptane phase was taken, then the heptane phase containing methyl esters of fatty acids.

2.3 Chemicals and Methods: The method is based on the production of methyl esters of fatty acids, followed by analysis by gas-liquid chromatography and mass spectrometry [3]. Chromatographic separation was performed on gas chromate-mass-spectrometric system (GC/MS method) (*Agilent* 6890N/5973inert, *Agilent Technologies*, USA), the column is capillary HP-5MS (30m×0,25mm×0,25mkm, *Agilent Technologies*, USA) The evaporation temperature 250 °C, the interface temperature 280 °C. The separation was carried out in the programming mode of temperature – the initial temperature 60 °C was maintained for 4 minutes, later raised with a gradient of 4 °C / min to 250 °C, held for 6 minutes, then the temperature was raised to 300 °C with a gradient of 20 °C, and kept for 5 minutes. A sample of 1 µl was injected in a 1:20 split mode. Detection was performed in SCAN mode in the range (38-400 m / z). The gas flow rate of the carrier through a column of achieved as 1.0 ml / min [5].

2.4 GC/MS Analysis: Methyl esters of fatty acids were identified after retention time in comparison with retention time for methyl esters of fatty acids standard mixture for bacteria (Supelco, USA) and using NIST 02 mass-spectra library. Quantification of fatty acids methyl esters was determined by the internal standard addition to the sample analyzed. Undecanoic acid solution was used as the internal standard [4].

3. Results and Discussions

Flowers and leaves of two variants of *Chrysanthemum×hortorum Bailey* have been analyzed for fatty acids' qualitative composition and quantitative content by GC/MS method. Comparative results of identification and quantification of fatty acids are represented in table 1. GS/MC chromatograms of fatty acids of the investigated plant raw materials are shown on figures 3-6.

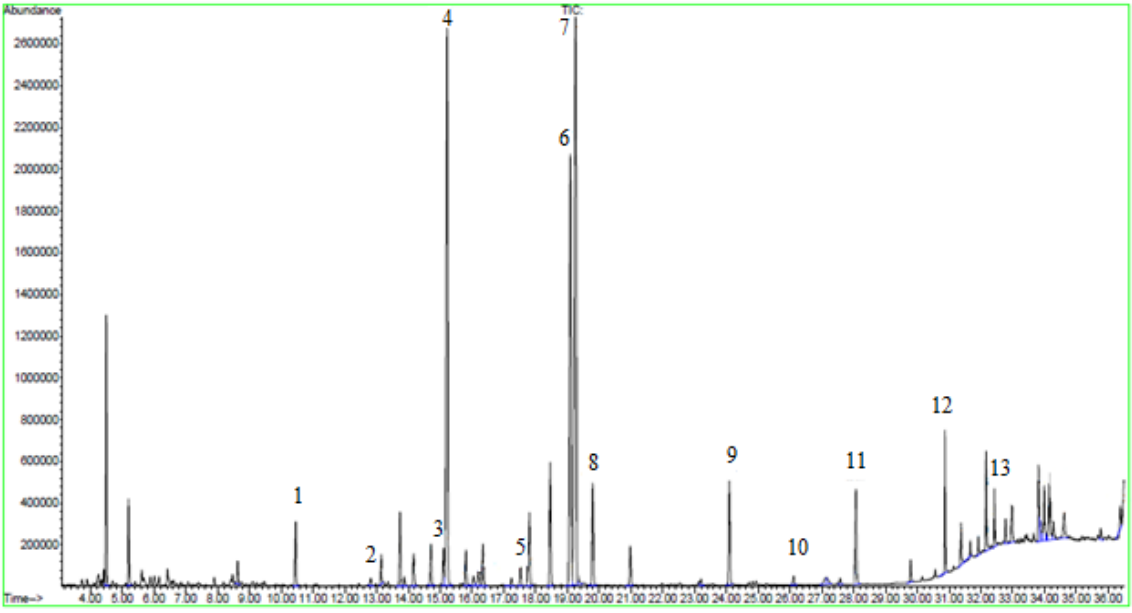

Fig 3: GS/MS chromatogram of fatty acids from leaves of *Chrysanthemum×hortorum Bailey Pectoral'* variant: 1 – myristic acid, 2 – pentadecanoic acid, 3 – palmitoleic acid, 4 – palmitic acid, 5 – heptadecanoic acid, 6 – linoleic acid, 7 – linolenic acid, 8 – linolelaidic acid, 9 – arachidic acid, 10 – heneicosanoic acid, 11 – behenic acid, 12 – lignoceric acid, 13 – cerotic acid

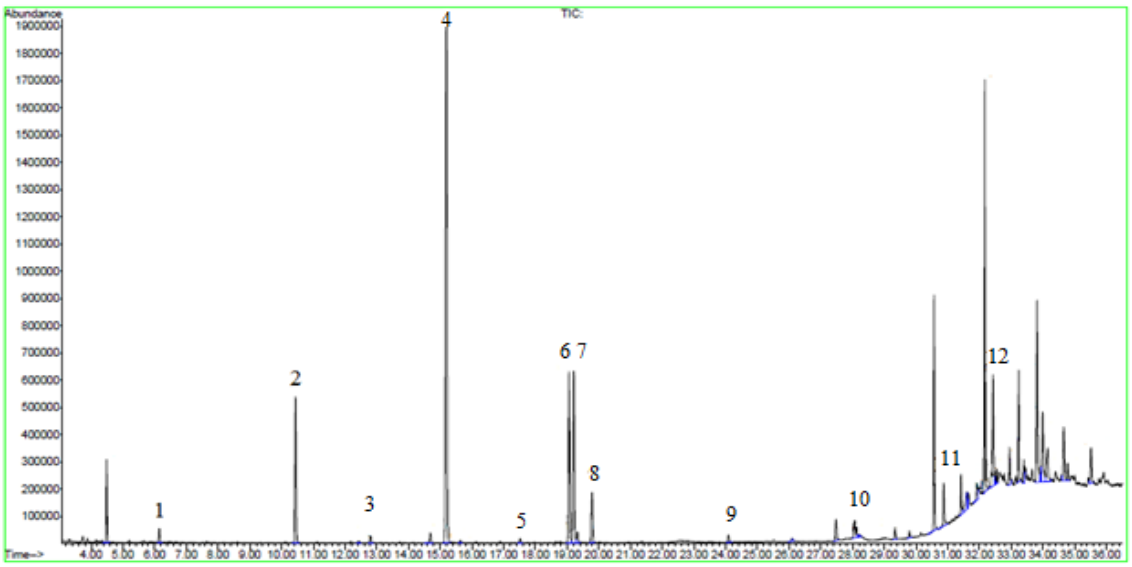

Fig 4: GS/MS chromatogram of fatty acids from flowers of *Chrysanthemum×hortorum Bailey Pectoral'* variant: 1 – lauric acid, 2 – myristic acid, 3 – pentadecanoic acid, 4 – palmitic acid, 5 – heptadecanoic acid, 6 – linoleic acid, 7 – linolenic acid, 8 – linolelaidic acid, 9 – arachidic acid, 10 – behenic acid, 11 – lignoceric acid, 12 – cerotic acid

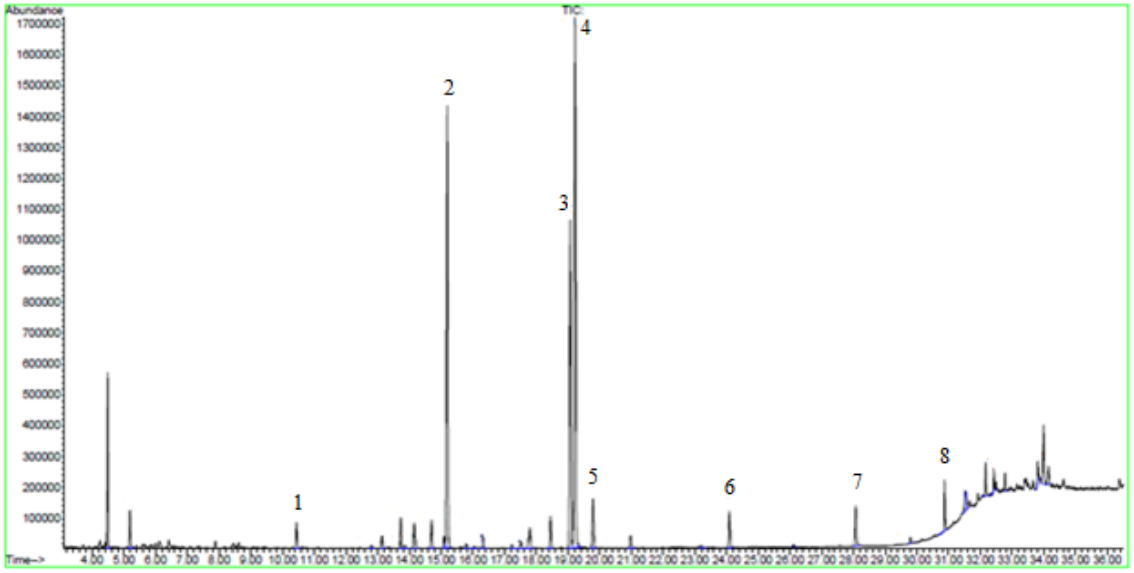

Fig 5: GS/MS chromatogram of fatty acids from leaves of *Chrysanthemum×hortorum Bailey Belgo'* variant: 1 – myristic acid, 2 – palmitic acid, 3 – linoleic acid, 4 – linolenic alcohol, 5 – linolelaidic acid, 6 – arachidic acid, 7 – behenic acid, 8 – lignoceric acid

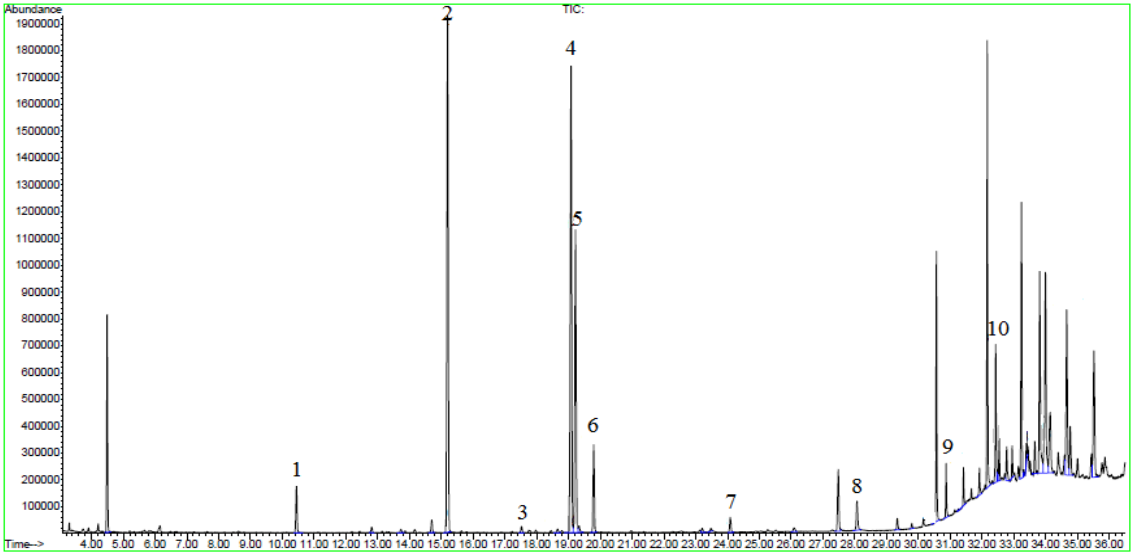

Fig 6: GS/MS chromatogram of fatty acids from flowers of *Chrysanthemum×hortorum Bailey Belgo'* variant: 1–myristic acid, 2–palmitic acid, 3–margaric acid, 4–linoleic acid, 5 –linolenic acid, 6–linolelaidic acid, 7–arachidic acid, 8–behenic acid, 9–lignoceric acid, 10 – cerotic acid

Table 1: The Fatty Acids Profile of flowers and leaves of *Chrysanthemum×hortorum Bailey Belgo* and *Pectoral'* variants (GS/MS)

S. No	Names of Compounds		Compounds' content in plant raw materials, mg/kg			
	Fatty Acid/Compound	Identified after GC/MS Analysis	*Belgo*		*Pectoral'*	
			Leaves	Flowers	Leaves	Flowers
Unsaturated fatty acids						
1.	Linoleic acid	9,12-Octadecadienoic acid methyl ester	5.67	5.91	4.86	4.10
2.	Linolenic acid	9,12,15-Octadecatrienoic acid, methyl ester	9.32	3.71	7.43	4.42
3.	Palmitoleic acid	9-Hexadecenoic acid, methyl ester	-	-	0.34	-
4.	Linolelaidic acid	Octadecanoic acid, methyl ester	0.95	0.98	0.95	1.27
Totally			*15.94*	*10.6*	*13.58*	*9.79*
Saturated fatty acids						
5.	Arachidic acid	Eicosanoic acid, methyl ester	0.57	0.18	0.98	0.18
6.	Margaric acid	Heptadecanoic acid, methyl ester	-	0.15	0.15	0.11
7.	Lauric acid	Dodecanoic acid	-	-	-	0.31
8.	Myristic acid	Methyl tetradecanoate	0.39	0.53	0.56	3.29
9.	Palmitic acid	Hexadecanoic acid, methyl ester	7.91	6.32	6.13	0.24
10.	Heneicosanoic acid	Heneicosanoic acid, methyl ester	0.10	-	0.10	-
11.	Behenic acid	Docosanoic acid, methyl ester	0.68	0.38	0.91	0.53
12.	Lignoceric acid	Tetracosanoic acid, methyl ester	0.51	0.41	0.73	0.86
13.	Cerotic acid	Hexacosanoic acid, methyl ester	-	1.11	0.34	0.34
Totally			*10.16*	*9.08*	*9.9*	*14.78*
Lipophilic alcohols						
14.	Linolenic alcohol	9,12,15-Octadecatrien-1-ol	9.32	-	7.43	-
Lipophilic hydrocarbons						
15.	Eicosane	Eicosane	-	-	0.57	-

49 Lipophilic components have been detected and 15 of them have been identified. Fatty acids (saturated and unsaturated), lipophillic alcohol (linolenic alcohol) and Lipophilic hydrocarbon (eicosane) were studied.

Unsaturated fatty acids predominate in the composition of Lipophilic compounds of both investigated samples of leaves and in the composition of *Chrysanthemum×hortorum Bailey Belgo* variant's flowers; *Pectoral'* variant's flowers were characterized by predomination of saturated fatty acids.

Among unsaturated fatty acids linoleic, linolenic and linolelaidic acids have been detected and quantified in all investigated plant raw materials. Palmitoleic unsaturated fatty acid was found in leaves of *Chrysanthemum×hortorum Bailey Pectoral'* variant.

Arachidic, margaric, lauric, myristic, palmitic, heneicosanoic, behenic, lignoceric and cerotic saturated acids were determined. Arachidic, myristic, palmitic, behenic and lignoceric acids are constituents of lipoids of all investigated samples. Margaric and cerotic acids were identified in all investigated samples except the leaves of *Chrysanthemum×hortorum Bailey Belgo'* variant. Heneicosanoic acid was detected in both samples of *Chrysanthemum×hortorum Bailey* leaves. Lauric acid was found in *Chrysanthemum×hortorum Bailey Pectoral'* variant.

Linolenic alcohol was detected in both investigated leaves of *Chrysanthemum×hortorum Bailey*.

Eicosane was identified in leaves of *Chrysanthemum×hortorum Bailey Pectoral'* variant.

4. Conclusions

Perennial garden chrysanthemum (*Chrysanthemum×hortorum Bailey*) appears to be of interest not only as ornamental plant but also as promising medicinal plant. Investigation of primary metabolites was the first step to the investigation of the plant raw materials of perennial garden chrysanthemum. Fatty acids' profile of *Chrysanthemum×hortorum Bailey Belgo* and *Pectoral'* variants have been determined and analyzed in this study. Among identified fatty acids unsaturated ones predominated in leaves of two investigated variants of chrysanthemum, higher content of saturated fatty acids was determined in flowers of *Chrysanthemum×hortorum Bailey Pectoral'* variant.

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