TALLINNA TERVISHOIU KÕRGKOOL
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Pesticide residues in peppermint, chamomile and bladder herbal teas sold in Estonia.

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INTRO

- The aim of this study was to determine pesticide residues in commercially sold peppermint, chamomile and bladder tea herbs.
- All samples – 11 peppermint, 12 chamomile and 6 bladder teas – were bought from local pharmacies, ecomarkets and supermarkets to include as many producers possible at given time.

METHODS

1. Peppermint and chamomile tea samples were prepared by extraction with organic solvent followed cleaning by silica gel columns. Internal standards (isotope labelled pesticides) were added before sample preparation. Internal standard calibration was used.
2. The chamomile samples in repeated analysis and bladder tea samples were prepared by standard method: „Foods of plant origin - Determination of pesticide residues using GC-MS and/or LC-MS/MS following acetonitrile extraction/partitioning and clean-up by dispersive SPE - QuEChERS-method EN 15662".
3. The analysis was carried out with Agilent Technologies 7890B gas chromatography and Agilent Technologies 5977A mass-selective detector. Confirmatory analysis was carried out with Agilent Technologies 7890B gas chromatography and Agilent Technologies 7000 triple quadrupole mass-selective detector.

4. The qualitative analysis was carried out with Agilent MassHunter Qualitative Analysis B.07.00 and quantitative analysis with Agilent MassHunter Quantitative Analysis B.07.00 programs.

5. The pesticides were selected based on EU Pesticide Database and Statistics Estonia Database.

RESULTS

- Residues of 21 pesticides were detected in 18 samples.
- 8 samples consisted pesticide residues in amounts exceeding allowed limits.
- 11 of the pesticides found in samples are prohibited in EU and member states, one of the pesticides (Quinoxyfen) found in Estonian product has no authorisation in Estonia.
- No pesticide residues were found in 5 chamomile teas, 3 peppermint teas and 3 bladder teas.
- The tea with most residues – 13 diferent pesticides - was not produced in EU.

DISCUSSION

- The origin of pesticide residues in herbal teas is unclear, but the amounts are mostly in trace levels and therefore pose no substantial risk for consumers' health.
- Some herbal teas exceeded EU limits for pesticides and should be avoided.
- It was not assessed how much of the residues end up in infusions, but the amounts are propably in trace levels.

Quantitative analysis and comparison of pesticides in peppermint leaves and teas

Silver Kruus (2017). Tallinn Health Care College, Chair of Assistant Pharmacist.

SUMMARY

The aim of this thesis was to investigate the factors which endanger growth of peppermint, the use of pesticides, the effects of pesticides on peoples health, the use of different adsorbents in column chromatography, the residues of pesticides in peppermint leaves and teas, the detection of pesticide residues remaining in approved limits and to compare results of peppermint leaves and teas.

The data was obtained from evidence-based sources and databases according to the subject. This thesis consists of an analytical study of pesticide residues in peppermint leaves which are grown and/or sold in Estonian retail. The analysis was done by a gas chromatographic method.

The growth of peppermint is endangered by fungal diseases, nematodes and viruses, pesticides are used for controlling various insects, weeds, rodents, fungi, bacteria, snails and slugs, long-term exposure to pesticides can harm a person's life quality and can lead to disturbances in different organ systems. The study found that peppermint teas contain more different pesticide residues than locally grown peppermint leaves that are sold in organic stores and in pharmacies. Most of the detected pesticide residues in the study were within the imposed pesticide limits stated by the European Union, in four different samples the detected pesticide residues exceeded European Union maximum residue limits.

Key words: *Mentha x piperita* L., peppermint, pesticides, pesticide residue limits, quantitative analysis, gas chromatography.

Fig. 1. Analysed peppermint teas.

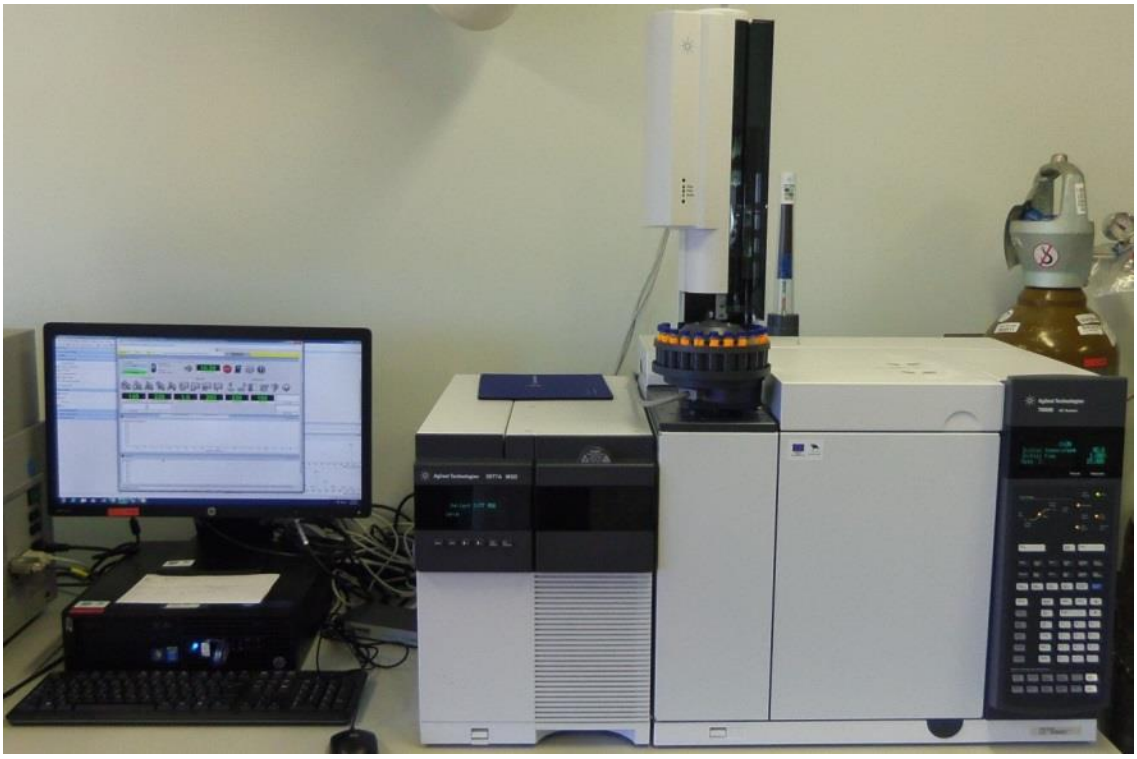

Fig. 2. GC-MS used for analyses.

Overview of the laboratory can be seen at:

https://www.ttk.ee/virtuaaltuur/ Main Building → Pharmaceutical Laboratory Complex → Instrumental-Analysis Laboratory

Quantitative analysis and comparison of pesticides in chamomile herbs and teas.

Tuuli Reiman (2018). Talllinn Health Care College, Medical Technology Education Centre, Curriculum of Assistant Pharmacist.

SUMMARY

In the world chamomile is one of the 5 most popular medicinal herbs. It is frequently used as the first option in treating lighter disorders, so it is very important that the herbs qualify for requirements. The aim of this thesis was to investigate the effect of chamomile to human health, the effect of pesticides to human health, analyse the residues of pesticides in chamomile herbs sold by Estonian pharmacies and teas sold in retail trade and their detection of pesticide residues remaining in approved rates.

The analytical part of this graduation thesis was carried out in Tallinn Health Care College instrumental analysis lab. Gas chromatography was used for analyses. In the term paper glass columns filled with activated carbon and silica gel was used for sample preparations. In graduation thesis sample preparation was made by EN 15662 method. The selection of pesticides based on earlier researches, studied literature and on the Statistics Estonian database. Chamomile herbs and teas were bought from three Tallinn pharmacies, one biomarket and from three different retail markets. Qualitative analyse was carried out with a program Agilent MassHunter Qualitative Analysis B.07.00 (2014 year version) and quantitative analyse with a program Agilent MassHunter Quantitative Analysis B.07.00 (2008 year version). The summary of the graduation thesis affirmed that chamomile teas sold in retail markets were cleaner than chamomile herbs sold in pharmacies. Most of the detected pesticide residues remained under European Union's stated approved rates, except one sample in the first analysis. The second analysis didn't confirm the same amount of pesticide residue.

Keywords: *Matricaria chamomilla* L.*,* chamomile, pesticides, gas chromatography.

Fig. 3. Samples used in the analysis.

Quantitative analysis of pesticides in diuretic herbal tea mixtures.

Maria Semjonova (2019). Tallinn Health Care College, Medical Technology Education Center, curriculum of Assistant Pharmacist.

SUMMARY

The aim of the work was to study the effect of diuretic tea, the use of pesticides in Estonia, the content of pesticides in diuretic herbal teas mixtures and their compliance to the regulations limits. Four of the tea mixtures are originated from Estonia, one from Germany and one had unknown production location. The thesis was compiled as an empirical study, the analytical part was carried out in the instrumental analysis laboratory of Tallinn Health Care College and concluding analysis' were done in Estonian Environmental Research Center's labolatory. Gas chromatography analysis was performed on Agilent Technologies 7890B gas chromatograph with a mass selective detector Agilent Technologies 5977A. The database NIST MS Search 2.2 was used for compound identifying and software Agilent Mass Hunter Quantitative Analysis 7.0 was used for quantitative analysis. Pesticide analysis was performed using the method of gas chromatography and the methodology was based on the final thesis of Silver Kruus and Tuuli Reiman. (Kruus 2017; Reiman 2018).

The diuretic tea is a mixture of different herbs designed to increase urinary excretion. The main components used are: parsley (*Petroselinum crispum*), elk leaf and herb (*Epilobii angustifolii folium um herba*), birch leaf (*Betulae folium*), field horsetail herb (herba Equisetum arvense), chamomile (*Chamomillae flores*) and other aids like St. John's wort (*herba Hyperici*), white dead-nettle herb (*Lamii albi herba*). Teas bought from health stores have a different composition from those that were purchased from pharmacies, but they also affect human body in the same way.

Three tea mixtures were analyzed and 17 pesticides were found, none of which exceeded the limit of quantification. In the confirmatory analysis, 100 pesticides were searched and 4 pesticides were found in the two samples during the first experiment and 5 in one sample during the second experiment. A total of 11 pesticides were found on the check-ups in the confirmatory analysis. Most of them were found in a tea, with unknown origin and probably

brought to Estonia from outside of the European Union. The tea originated from Germany and bought from health store did not contain any pesticide residues.

Keywords: diuretic tea, pesticide, gas chromatography

Fig 4. Urinary tract teas used in analyses.

Table 1. Pesticide classification, authorisation status in EU and producer and purity information of reference substances used in analysis.

Pesticide	Classification	Authorised use	Reference substance producer	Reference purity (%)
Boscalid	Fungicide	AT, BE, BG, CY, CZ, DE, DK, EE, EL, ES, FI, FR, HR, HU, IE, IT, LT, LU, LV, MT, NL, PL, PT, RO, SE, SI, SK, UK	Sigma-Aldrich	99,9
Chlorothalonil*	Fungicide	Not Approved in EU, authorised in AT, BE, BG, CY, CZ, DE, EE, EL, ES, FI, FR, HU, IE, IT, LT, LU, LV, MT, NL, PL, PT, RO, SI, SK, UK	Sigma-Aldrich	99,3
Chlorotoluron	Herbicide	AT, BE, BG, CZ, DE, EE, EL, ES, FR, HR, HU, IE, IT, LV, PL, PT, RO, SI, SK, UK	Sigma-Aldrich	
Dichlobenil	Herbicide	Prohibited in EU and member states	Sigma-Aldrich	
Dichlofluanid	Fungicide	Prohibited in EU and member states	Fluka	99,9
Dimethenamid	Herbicide	Prohibited in EU and member states	Sigma-Aldrich	
Epoxiconazole	Fungicide	AT, BE, BG, CZ, DE, DK, EE, EL, ES, FR, HR, HU, IE, IT, LT, LU, LV, NL, PL, PT, RO, SI, SK, UK	Sigma-Aldrich	
Fenpropidin	Fungicide	AT, BE, BG, CZ, DE, EE, EL, ES, FI, FR, HR, HU, IE, IT, LT, LU, LV, NL, PL, PT, RO, SE, SI, SK, UK	Dr Ehrenstorfer GmbH	99,0
Fenpropimorph	Fungicide	Not Approved in EU, authorised in AT, BE, BG, CZ, DE, EE, EL, ES, FI, FR, HR, HU, IE, IT, LT, LU, LV, NL, PL, PT, RO, SE, SI, SK, UK	Sigma-Aldrich	98,2
Fenvalerate	Insecticide	Prohibited in EU and member states	Sigma-Aldrich	

Hexachlorobenzene	Fungicide	Prohibited in EU and member states	Sigma-Aldrich	96,0
Hexachlorocyclohexane	Insecticide	Prohibited in EU and member states	Dr Ehrenstorfer	
Metolachlor	Herbicide	Prohibited in EU and member states	Sigma-Aldrich	
Pentachlorobenzene	Insecticide	Prohibited in EU and member states	Sigma-Aldrich	96,0
Pirimiphos-methyl	Insecticide	BG, CY, CZ, EE, EL, ES, FR, HR, HU, IT, LT, LU, LV, NL, PL, PT, RO, SI, SK, UK	Sigma-Aldrich	
Prothioconazole-desthio	Fungicide	AT, BE, BG, CZ, DE, DK, EE, EL, ES, FI, FR, HR, HU, IE, IT, LT, LU, LV, NL, PL, PT, RO, SE, SI, SK, UK	Dr Ehrenstorfer	
Tebuconazole	Fungicide	AT, BE, BG, CY, CZ, DE, DK, EE, EL, ES, FI, FR, HR, HU, IE, IT, LT, LU, LV, MT, NL, PL, PT, RO, SE, SI, SK, UK	Sigma-Aldrich	99,3
Tolylfluanid	Fungicide, acaricide	Prohibited in EU and member states	Fluka	99,9
Quinoxyfen**	Fungicide	Not Approved in EU, authorised in AT, BE, CZ, DE, EL, ES, FR, HR, HU, IE, IT, LU, MT, PL, PT, RO, SI, SK, UK	Sigma-Aldrich	99,9
o,p´-DDE	Insecticide	Prohibited in EU and member states	Dr Ehrenstorfer	
p,p´-DDE	Insecticide	Prohibited in EU and member states	Dr Ehrenstorfer	

* Withdrawal authorisations by 20 Nov 2019

** Withdrawal authorisations by 27 Jun 2019

Table 2. Pesticide m/z values in GC-MS and GC-MS/MS analysis and reference substance origin.

Pesticide	**Reference substance origin**	**m/z in GC-MS analysis**		**m/z transitions in GC-MS/MS analysis**	
		Quantitative ion	**Qualitative ion**	**Quantit. MRM transition**	**Qualit. MRM transition**
Boscalid	Sigma -Aldrich	140	112	140,0→112,0	140,0→76,0
Chlorotalonil	Sigma -Aldrich	266	264	263,8→ 229,0	263,8→ 168
Chlorotoluron	Sigma -Aldrich	-	-	167,0→132,0	132,0→77,1
Dichlobenil	Sigma-Aldrich	-	-	171,0→100,0	171,0→136,1
Dichlofluanid	Fluka	123	167	223,9→ 123,1	123,0→ 77,1
Dimethenamid	Sigma-Aldrich	-	-	230,1→154,0	232,1→154,0
Epoxiconazole	Sigma-Aldrich	192	138	192,0→138,1	192,0→111,0
Fenpropidin	Dr Ehrenstorfer	98	145	98,0 →55,1	98,0→70,0
Fenpropimorph	Sigma-Aldrich	128	43	128,1→70,1	128,1→110,1
Fenvalerate	Sigma-Aldrich	-	-	167,0→125,0	197,1→115,2
Hexachlorobenzene	Dr Ehrenstorfer	-	-	283,8→213,9	283,8→248,8
α-Hexachlorocyclohexane	Dr Ehrenstorfer	-	-	216,9→181,0	218,9→183,0
γ-Hexachlorocyclohexane	Dr Ehrenstorfer	-	-	216,9→181,0	181,0→145,0
δ-Hexachlorocyclohexane	Dr Ehrenstorfer	-	-	217,0→181,1	181,0→145,0
Metolachlor	Sigma-Aldrich	-	-	238,0→162,2	162,2→133,2
Pentachlorobenzene	Neochema	-	-	249,9→215,0	248,0→213,0
Pirimiphos-methyl	Sigma-Aldrich	-	-	290,0→125,0	290,0→151,0
Propiconazole	Dr Ehrenstorfer	173	259	173,0→145,0	259,0→69,0
Quinoxyfen	Sigma-Aldrich	237	272	237,0→208,0	271,9→237,1
Tebuconazole	Sigma-Aldrich	125	250	250,0→125,0	252,0→127,0
Tolylfluanid	Fluka	137	238	237,9→137,0	136,9→91,1
o,p´-DDE	Dr Ehrenstorfer	-	-	246,0→176,2	248,0→176,2
p,p´-DDE	Dr Ehrenstorfer	-	-	246,1→176,2	315,8→246,0

Table 3. Herbal teas and found pesticide residues above limit of detection (LOD).

	Sample	Pesticide	Quant. Value (mg/kg)	LOQ (mg/kg)	MRL (mg/kg)
Peppermint teas (supermarkets)	MIĘTA herbatka ziołowa	Dichlofluanid**	0,03	0,01	0,01
		Fenpropimorph*	<0,01	0,01	0,05
	Piparmünditee	Dichlofluanid**	<0,01	0,01	0,01
		Fenpropimorph	<0,01	0,01	0,05
		Tolylfluanid**	<0,01	0,01	0,1
	Piparminttu Tee	Fenpropidin	<0,01	0,01	0,05
	Pfefferminze	Tolylfluanid**	0,29	0,01	0,1
	Peppermint tea	Tolylfluanid**	0,15	0,01	0,1
Peppermint teas (pharmacies)	Elujõu	Tebuconazole	<0,01	0,01	0,05
	Loodusravi	Tolylfluanid**	0,23	0,01	0,1
	Vadi	Tolylfluanid**	<0,01	0,01	0,1
Chamomile teas (supermarkets)	Dilmah	Tebuconazole	<0,01	0,01	0,05
	Greenfield	Dichlofluanid**	<0,01	0,01	0,01
		Tebuconazole	<0,01	0,01	0,05
	Herba	Tebuconazole	<0,01	0,01	0,05
	Rimi	Boscalid	<0,005	0,005	0,01
Chamomile teas (pharmacies)	MK Loodusravi	Tebuconazole	<0,01	0,01	0,05
		Boscalid	<0,005	0,005	0,01
	Elujõud OÜ	Quinoxyfen***	0,098	0,01	0,05
		Chlorothalonil*	<0,01	0,01	0,01
	Kubja Ürditalu	Fenpropidin	<0,01	0,01	0,05
Bladder teas (pharmacies)	Kubja Ürditalu	Metolachlor**	0,006	0,005	0,01
	MK Loodusravi	Pirimiphos-methyl	0,057	0,01	0,05
		Pentachlorobenzene**	<0,001	0,001	0,01
		Hexachlorobenzene**	<0,001	0,001	0,01
Bladder teas (ecomarkets)	Chinese Medicinal herbs Tervise Alkeemia	Tebuconazole	0,18	0,01	0,05
		Chlorotoluron	<0,01	0,01	0,05
		Dichlobenil**	<0,01	0,01	0,05
		Dimethenamid**	<0,01	0,01	0,05
		Epoxiconazole	<0,01	0,01	0,05
		Fenpropimorph*	<0,01	0,01	0,05
		Fenvalerate**	<0,05	0,05	0,1
		α-Hexachlorocyclohexane **	<0,005	0,005	0,01
		γ-Hexachlorocyclohexane **	<0,005	0,005	0,01
		δ-Hexachlorocyclohexane**	<0,005	0,005	0,01
		o,p´-DDE**	<0,005	0,005	0,01
		Pentachlorobenzene	<0,001	0,001	0,01
		p,p´-DDE**	<0,005	0,005	0,01
		Prothioconazole-desthio	<0,01	0,01	0,05