GC-MS/MS STUDY OF LIPIDOMIC PROFILES OF GRASSHOPPER'S ABDOMINAL SECRETION

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INTRODUCTION:

In the era of pathogens resistant to commonly prescribed medicines, there is a need of constant search for more effective drugs. New biologically active substances are sought, i. e. new compounds which could be introduced into therapy as active agents or as "*lead compounds*", serving next as primary structures for derivatives with optimum biological activities. In such a situation, it seems reasonable to continue studies based on etnopharmacological premises, which would be focused on the search for potential drugs. Research trends dedicated to discovery of active substances of natural origin have been known since centuries. Recently, numerous studies have been focused on insects due to discovery of high levels of compounds with different biological activities. Biomass of insects contains mainly hydrocarbons, free fatty acids, triacylglicerols, alcohols, waxes, ethyl esters, proteins and sterols. The particular interest paid to insects recently lays in identification of peptides with potential antifungal, antibacterial and myotropic activities. According to the etnopharmacological observations, an ointment-like material squeezed out from abdomen of grasshoppers was used by villagers of the West-Central Poland to facilitate healing of wounds and scars. A characteristic feature of this material is its exquisite rheological property, which probably encouraged testing it as a natural drug.

AIMS of STUDY

1. Development of a reliable analytical procedure with GC-MS/MS separation of compounds extracted from grasshopper's abdominal secretion (*Chorthippus spp.)*
2. Analysis of lipidomic profiles of insects samples (n=30)
3. Statistical analysis of the obtained data

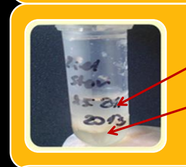
RESULTS

SAMPLE PREPARATION

Obtaining grasshoper's secretion

Bligh& Dyer extraction

1. 100 µL chloroform : methanol (1:1) mixture used for 10 mg of biological material
2. Chloroform
3. Aqua



Aqueous fraction

Organic fraction

SPE extraction

1. Hexan
2. $CHCl_3$:Hexan
3. $CHCl_3$
4. $CHCl_3$:MeOH
5. Metanol
6. Izopropanol

Derivatization

BSTFA:TMCS (99:1), Sigma Aldrich

60 min

97°C

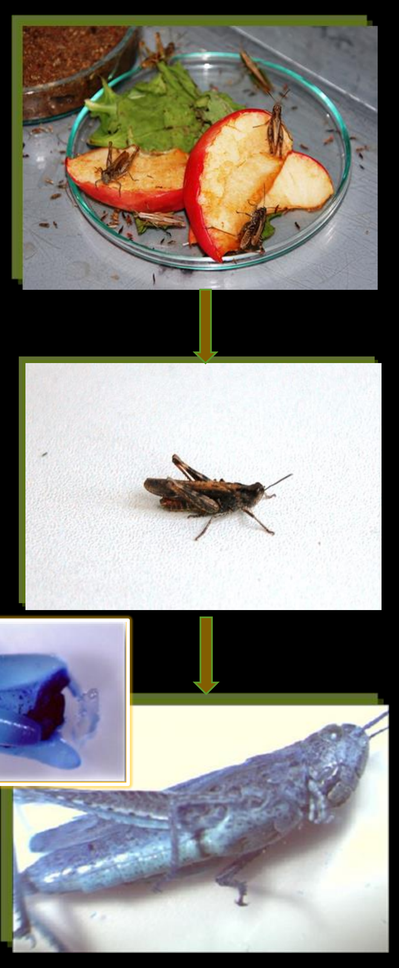


Fig. 1 Macro- and microscopic insects filing and identification

EQUIPMENT

•Instrumentation:

GC-MS-TQ 8030 (Shimadzu, Japan)

•Column:

Zebron ZBB-5MS (30m lenght, 0.25 mm I.D.; df=0.25 µm film thickness)

CHROMATOGRAPHIC and SPECTROMETRIC PARAMETERS

GAS CHROMATOGRAPHY		MASS SPECTROMETRY	
Injection Temp.	320°C	Interface Temp.	320°C
Column Oven Temp.	45°C (5min) (3°C/min)	Ion Source Temp.	220°C
	80°C (0 min) (8°C/min)	Tuning Mode	Normal
	320°C (15 min)	Acquisition Mode	SCAN
Injection Mode	Splitless	Scan Mass Range	m/z 10-1000
Injection Volume	1µL	Scan Speed	10 000 u/sec

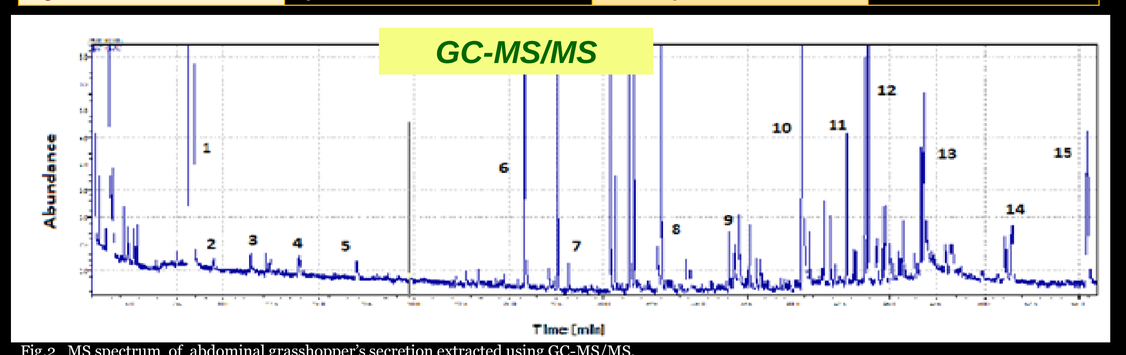


Fig.2 MS spectrum of abdominal grasshopper's secretion extracted using GC-MS/MS.
1: styrene, 2: toluene, 3: phenylglyoxal, 4: benzyl chloride, 5: pentachloroethane, 6: benzene, (2,2-dichloro-1-methylcyclopropyl), 7: 9H-fluorene, 2-methyl-, 8: benzene, 1,1'-(1-butene-1,4-diyl)bis-, (Z), 9: fumaric acid, di(3-phenylpropyl) ester, 10: (2,3-diphenylcyclopropyl)methyl phenyl sulfoxide, *trans*, 11: tetratetracontane, 12: benzene, 1,1'-(1-chloro-3-iodo-1,3-propanediyl)bis, 13: 2,6-dimethyl-4-nitro-3-phenyl-cyclohexanone, 14: tetrapentacontane, 15: benzene, 1,1'-(2-butene-1,4-diyl)bis

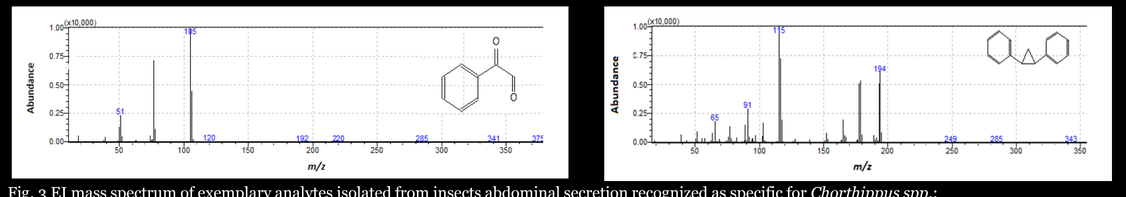
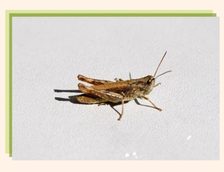
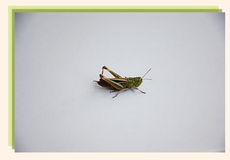


Fig. 3 EI mass spectrum of exemplary analytes isolated from insects abdominal secretion recognized as specific for *Chorthippus spp.*:
A) Phenylglyoxal B) 1,2 diphenylcylopropane

Compounds specific for the studied species

Chorthippus biggutulus	*Chorthippus montanus*	*Chorthippus parallelus*
1. Benzaldehyde 2. Ethylene, 1,1-diphenyl-	1. Hydrocarbons: Ethylene, 1,1-diphenyl- 2. Fatty acids: *alpha*.-Linolenic acid, Pentadecanoic acid 3. Alcohols: 1-Methylcyclohexanol 4. Aldehydes: 2-Phenylpropenal	1. 1,3,5,7-Cyclooctatetraene 2. 10,11-Dihydro-5H-dibenzo(a,d)cycloheptene 3. Acetic acid, cyano- 4. Benzene, 1,1'-(2-butene-1,4-diyl)bis- 5. Benzoylformic acid 6. Benzylcyclobutane 7. *cis*-Stilbene 8. Methane, oxybis[dichloro-





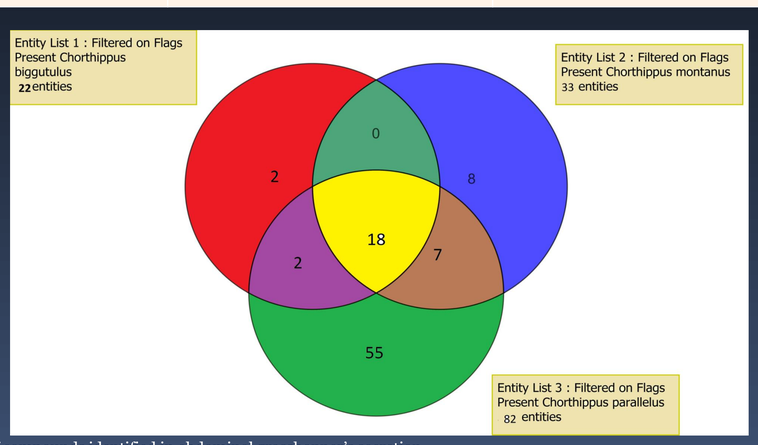


Fig. 4 Venn diagram of compounds identified in abdominal grasshopper's secretion

Compounds identified in all the samples obtained from *Chorthippus spp.*

Compound	EI- MS ions (m/z)	t_R [min]	MW (Daltons)	Compound	EI- MS ions (m/z)	t_R [min]	MW (Daltons)
(2,3-Diphenylcyclopropyl) methyl phenyl sulfoxide, trans-	91, 117, 193, 207	40.43	332	Benzene, 1,1'-(1-chloro-3-iodo-1,3-propanediyl)bis	91, 125, 193, 231	43.97	356
1,1-Diphenylcyclopropane	115, 194, 91, 65, 178, 165	30.29	194	Benzene, 1,1'-(3-methyl-1-propene-1,3-diyl)bis	51, 91, 115, 193, 208	40.65	208
1,2-Diphenylcyclopropane	115, 194, 91, 65, 51, 179	30.60	194	Bicyclo[4.2.1]nona-2,4,7-triene, 7-phenyl	39, 115, 165, 179, 194	40.49	194
1,2-Propanediol, 3-benzyloxy-1,2-diacetyl-	91, 117	46.77	266	Cyclopropylphenylmethane	51, 91, 132	31.43	132
2-Cyanosuccinonitrile	40, 78, 104	8.35	105	Ethane, 1,1-diethoxy-	45, 73, 103, 117	3.43	118
4a,9a-Methano-9H-fluorene	40, 89, 152, 165, 180	28.12	180	Phenylglyoxal	51, 77, 105	11.37	134
Alpha-phenyl-alpha-tropylacetaldehyde tosylhydrazone	91, 115, 117, 179, 194	33.07	378	Styrene	51, 63, 78, 104	8.34	104
Benzene,(2,2-dichloro-1-methylcyclopropyl)-	51, 77, 91, 115, 129, 163	25.9	200	Toluene	39, 65, 91	4.07	92
Benzene, 1,1'-(1,3-propanediyl)bis-	51, 92, 117, 196	30.35	196	Triethyl borate	45, 73,117	4.66	146

CONCLUSIONS:

1. The proposed method of isolation and identification of fatty compounds are useful in the study of lipidomic profiles of grasshopper's abdominal secretion.
2. Analysis of samples obtained from different insect species demonstrated the presence of specific compounds in the metabolome of individual species.
3. The method, developed in this project, may be helpful in the future in identification of *Chorthippus spp*.
4. It was found that the location of insects harvest and insects species have an impact on its lipidomic profile.

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REFERENCES:

1.Van Huis A., Insect as food in Sub-Saharan Africa, Insect Science Application, (2003), Vol.23, No.3, 163-185.
2.Gołębiowski M., Malinski E., Bogus M., Kumirska J., Stepnowski P. The cuticular fatty acids of *Calliphora vicina, Dendrolimus pini* and *Galleria mellonella* larvae and their role in resistance to fungal infection. Insect Biochemistry and Molecular Biology 38, 619-627 (2008).
3. Buszewska-Forajta M., Siluk D., Struck-Lewicka W., Raczak-Gutknecht J., Markuszewski M. J., Kaliszan R., Identification of lipid fraction constituents from grasshopper *(Chorthippus spp.)* abdominal secretion with potential activity in wound healing with the use of GC-MS/MS technique, submitted for publication