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GAS CHROMATOGRAPHY – MASS SPECTROMETRY ANALYSIS ON ESSENTIAL OIL OF *GERANIUM PHAEUM* L. AND *G. ROBERTIANUM* L. (GERANIACEAE)

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Abstract: In order to continue our previous phytochemical studies concerning the species *Geranium phaeum* L. and *G. robertianum* L. (Geraniaceae), we have analysed by gas chromatography coupled with mass spectrometry two samples of essential oil, extracted using the Neo-Clevenger apparatus, as described in the Romanian Pharmacopoeia Xth Edition. Analyses were performed on a Hewlett-Packard 5890 series II-5972 MSD, the mass spectrograph being tuned using perfluorotributylamine as tuning standard. Following compounds were identified in *G. phaeum* essential oil: β-cubebene, sinularene, germacrene-derivatives and germacrene D, α-humulene, δ-cadinene, viridiflorol and superior hydrocarbons. Following compounds were identified in *G. robertianum* essential oil: trans-caryophyllene, γ-elemene, germacrene D, germacrone and superior hydrocarbons. These are the first GC-MSD Romanian analyses on the essential oil extracted from Geranium species, previous Romanian studies were performed only by thin layer chromatography and concerned *G. macrorrhizum* L. essential oil.

Introduction

Species belonging to the genus Geranium are known as hardy geraniums and a number of them are broadly used as ornamental, medicinal and melliferous plants. The most economically important for its aromatic properties is *G. macrorrhizum* L., which is cultivated in Bulgaria [3]. Many studies were performed in order to establish the chemical composition of its essential oil, which is now well known. But *G. macrorrhizum* L. is not the only Geranium species containing essential oil – *G. robertianum* L. is also known to contain essential oil [1], but its essential oil has not been much studied using performant methods. We have also noticed the presence of essential oil in fresh *G. phaeum* L. herba, while harvesting.

In Romania, only the *G. macrorrhizum* essential oil has been studied by thin layer chromatogtaphy (TLC) [2], while the essential oil of the other two species hasn't been studied at all.

The aim of this paper is to analyse by gas chromatography coupled with mass spectrometry (GC-MSD) the essential oil of Romanian *G. phaeum* L. and *G. robertianum* L.

Material and Methods

We have analysed two samples of fresh aerial part (fresh herba) of *G. phaeum* L. and *G. robertianum* L., respectively, which were submitted to hydrodistillation in a Clevenger–type apparatus. Species were collected as wild plants in Cluj-Napoca (district of Cluj) and were identified in the Department of Pharmaceutical Botany, Faculty of Pharmacy, "Iuliu Haţieganu" University of Medicine and Pharmacy, Cluj-Napoca, where herbarium specimens are identified.

The obtained essential oil was stored dissolved in benzene in sealed glass vials until the analyses moment.

GC-MSD analyses were performed on a Hewlett-Packard 5890 series II-5972 MSD apparatus, using a HP-MS 5 column, 0.26 mm i.d. 30 m, 0.25 mm coating thickness.

The GC was operated under the following conditions:

- manual injection;
- split 1:20;
- injector temperature: 250 ^{o}C;
- carrier gas: He;
- flow: 1 ml min^{-1};
- linear velocity: 36.4 cm s^{-1};
- oven temperature programmed from 60 to 240 ^{o}C at 3 ^{o}C min^{-1};
- detector temperature: 280 ^{o}C;
- time run: 60 min.

The MSD was operated under 70 eV; scan range 41-300 amu.

The MSD was tuned every day using PFTBA (perfluorotributylamine) as tunning standard.

Results were interpretated using the Wiley indirect method database.

Results and Discussions

G. phaeum L. essential oil is a yellow-green semi-solid mass with pleasant odor, reminiscent of clary sage and rose.

The chromatogram for the compounds identified in *G. phaeum* L. essential oil are presented in Fig. 1 and the retention times and quantity (expresses per cent) are shown in table 1.

Germacrene D and its derivatives (we have identified three of its derivatives) are the major components (42.41 %). Other identified compounds: δ-cadinene (3.77%), α-humulene (0.53%), (-)-sinularene (1.25%), β-cubebene (6.04%), viridiflorol (3.75%). Superior hydrocarbons account for at least 13.80 %. We can conclude that the essential oil is mainly sesquiterpenic.

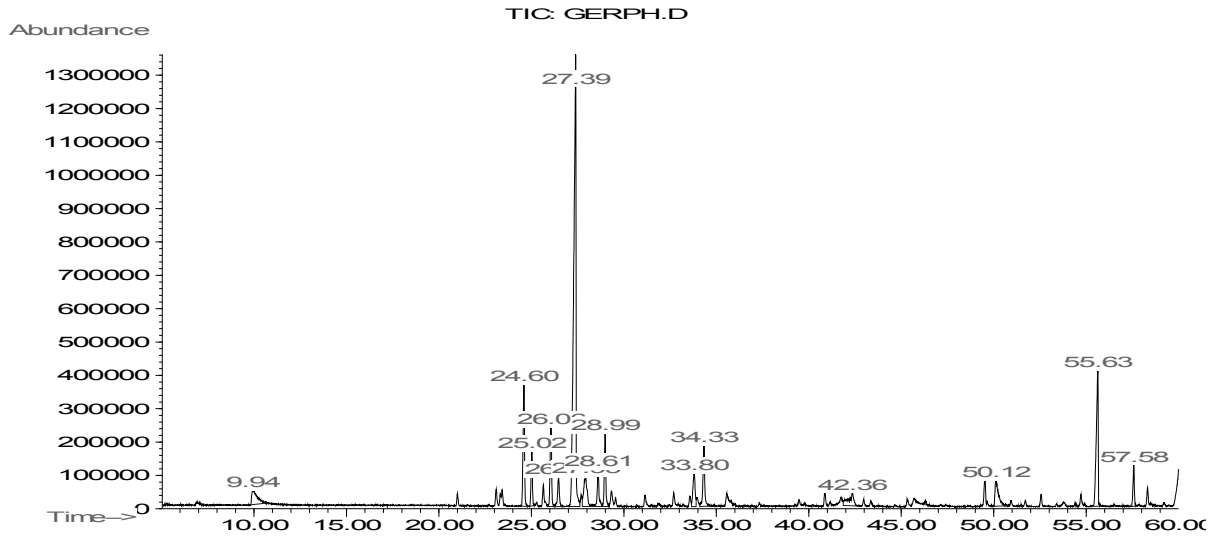

Fig. 1: Chromatogram of *G. phaeum* L. essential oil

G. robertianum L. essential oil is a semi-solid yellow-green mass with characteristic smell, different and less pleasant than the *G. phaeum* L. essential oil.

The chromatogram for the compounds identified in *G. robertianum* L. essential oil are presented in Fig. 2 and the retention times and quantity (expresses per cent) are shown in table 2.

Following compounds were identified in *G. robertianum* L. essential oil: trans-caryophyllene, γ-elemene, germacrene D, germacrone and superior hydrocarbons. Germacren D

is found in small amount (3.50%) in comparison with *G. phaeum* L. oil and the number of identified compounds is also smaller. We can conclude that *G. robertianum* L. essential oil is also mainly sesquiterpenic.

Table 1: Compounds identified in *G. phaeum* L. essential oil

Compound	Retention time	%
β-cubebene	25.598	6.041
germacrene D derivative	25.024	2.558
(-)-sinularene	25.647	1.253
germacrene D	27.39	34.262
α-humulene	27.697	0.538
germacrene D derivative	27.933	3.346
germacrene D derivative	28.609	2.246
δ-cadinene	28.99	3.776
viridiflorol	34.33	3.756
superior hydrocarbons	49.526	1.367
superior hydrocarbons	52.564	0.556
superior hydrocarbons	54.72	0.575
superior hydrocarbons	55.672	8.664
superior hydrocarbons	57.576	1.885
superior hydrocarbons	58.321	0.758

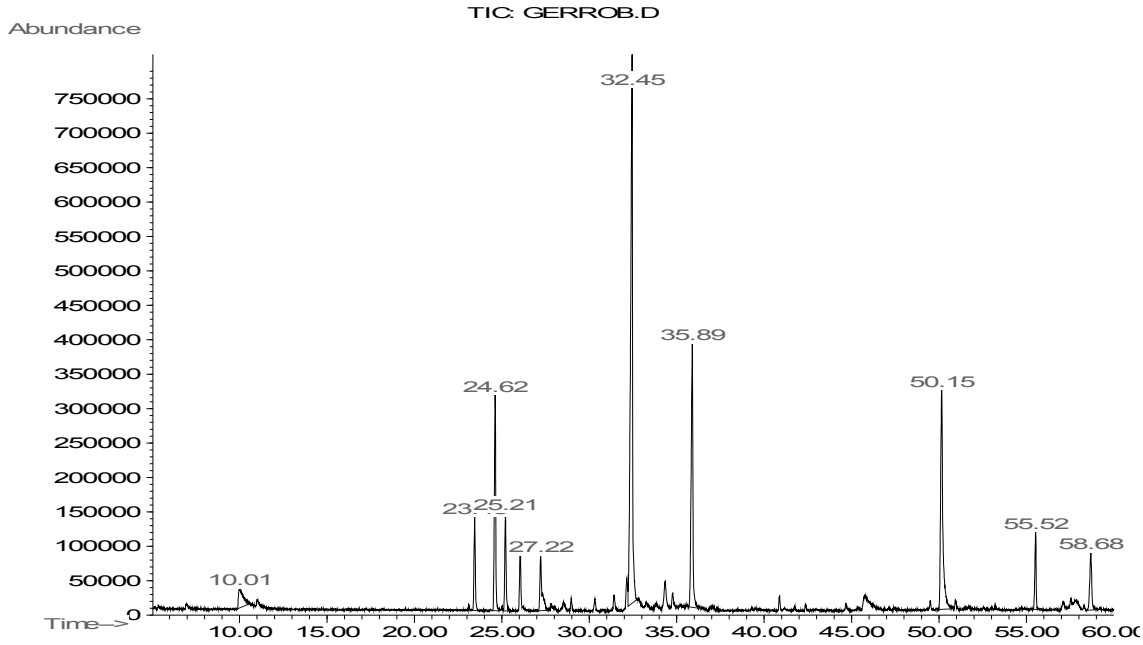

Fig. 2: Chromatogram of *G. robertianum* L. essential oil

Table 2: Compounds identified in *G. robertianum* L. essential oil

Compound	Retention time	%
trans-caryophyllene	24.619	8.856
γ-elemene	25.207	3.988
germacren D	27.22	3.506
germacrone	35.892	42.992
superior hydrocarbons	50.154	14.688

These results must be compared with the literature data concerning the *G. macrorrhizum* L. essential oil, because recent MS-GCD data concerning the essential oil of *G. phaeum* L. and *G. robertianum* L. are missing.

G. macrorrhizum L. essential oil is described as a yellow-green to dark green liquid (above 35 °C) or a semi-solid mass which consists of a mixture of colourless to pale-yellow crystals (stearoptene) with a liquid (eleoptene) at room temperature [4].

The chemical composition of the essential oil extracted from Bulgarian *G. macrorrhizum* L. is well known [4]: it is typically sesquiterpenic, as the major component is germacrone (50-55 %) which constitutes the stearoptene. The other terpenes are mainly ketones: α- and β-elemenone, germazone etc. Hydrocarbons account for 11-13 %, comprising: α- and β-selinene, β-elemene, ar-curcumene, α-santalene, caryophyllene, α-humulene, γ-muurolene, δ-cadinene, calamenene etc. Alcohols make up 10-20 %, including: juniperic camphor, junenol, β-eudesmol, elemol. Monoterpenic compounds amount to 7-10 %, consisting of γ-terpinene, terpinolene, α-pinene, δ-3-carene, α-phellandrene, limonene and borneol. The green colour of the oil is due to azulenes.

Our results show that the chemical compositions of *G. phaeum* L. and *G. robertianum* L. essential oils are partially similar to the composition of *G. macrorrhizum* L. essential oil, all of them being mainly sesquiterpenic and semi-solid at room temperature, but they contain less compounds: both *G. phaeum* L. essential oil and *G. robertianum* L. essential oil contain hydrocarbons but they seem to contain less alcohols and monoterpenic compounds.

Conclusions

We have realised the first Romanian GC-MSD study on essential oil extracted from *G. phaeum* L. and *G. robertianum* L.

We have identified the major components of each of them and we have found partial similarity with the chemical composition of *G. macrorrhizum* L. essential oil (as reported in literature for the Bulgarian product), especially concerning the hydrocarbons.

The nature of the analysed essential oils is mainly sesquiterpenic.

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ANALIZA PRIN GC-MS A ULEIURILOR VOLATILE DE *GERANIUM PHAEUM* L. ŞI *G. ROBERTIANUM* L.

(Rezumat)

În continuarea studiilor fitochimice asupra speciilor *G. robertianum* L. şi *G. phaeum* L., au fost analizate prin gaz-cromatografie cuplată cu spectrometrie de masă probe de ulei volatil, extrase cu ajutorul aparatului Neo-Clevenger, conform tehnicii descrise în Farmacopeea Română Ediția a X-a. A fost folosit un aparat Hewlett-Packard 5890 seria II-5972, iar spectrometrul de masă a fost calibrat folosind perfluorotributilamina ca substanță etalon. Au fost identificați următorii compuşi: β-cubeben, sinularen, derivați de germacren şi germacren D, α-humulen, δ-cadinen, viridiflorol şi hidrocarburi superioare în uleiul de *G. phaeum* L., şi trans-cariofilen, γ-elemen, germacren D, germacronă şi hidrocarburi superioare în uleiul de *G. robertianum* L. Prezentele analize sunt primele analize realizate prin GC-MS asupra uleiului volatil din cele două specii, studiile anterioare româneşti fiind limitate la analizarea prin cromatografie pe strat subțire a speciei *G. macrorrhizum* L.