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**# GENERAL INFORMATION**

**1. Title of Dataset**

Dataset: Genetic diversity, reproductive capacity and alkaloids content in three endemic Alkanna species

**2. Creator Information**

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### 1. Abstract for the dataset

This data includes studies on three endemic species of Alkanet, *Alkanna* (*Alkanna primuliflora*, *A. stribrnyi* and *A. graeca*) from the family Boraginacea, distributed in Bulgaria. We have presented raw data (photographs, chromatograms and graphs) from embryological, genetic and phytochemical studies. The three *Alkanna* species are Balkan endemics and have a limited distribution on the Balkan Peninsula. The limited distribution of the species is often associated with problems in the reproductive capacity of the species, which is why the embryological examination was performed. The morphological features that distinguish the three species vary, they are unclear, and sometimes transitional forms are observed between the three species, which is why DNA extraction and Inter-simple sequence repeat (ISSR) amplification product analysis were used. The data included GC of the crude mixtures of alkaloids of *Alkanna primuliflora*, *A. graeca* and *A. stribrnyi* and MS spectra of the identified pyrrolizidine alkaloids (PAs).

**1)** For the embryological examination (male and female generative sphere, pollen viability and seed germ) a classical paraffin methodology was applied. For the embryological study, we collected flowers and buds at different stages of development from the three species from different populations. The material was processed according to classical methods and documented with photographic material. The main embryological structures and processes in the male and female generative sphere were established after observations using LM "Olympus" CX2. The microphotographs were made using an "Infinity lite" digital camera 1.4 Mpx. The presented photos are unprocessed and are used to describe the male and female and male generative spheres of the three species. The boards in the article are based on these photos. A description is provided to the attached photos in this document.

**2)** DNA extraction and Inter-simple sequences repeat (ISSR) amplification product analysis of the collected leaves of the three species and applied ISSR patterns of 15 *Alkanna* individuals belonging to *Alkanna primuliflora* (1-5), *Alkanna graeca* (6-10) and *Alkanna stribrnyi* (11-15) generated by primers (AC) 8YT (left) and (AG) 10C (right), respectively. The DNA marker is GeneRuler 100 bp plus. Based on statistical data processing (Principal Coordinate Analysis (PCoA) and Hierarchical Analysis of Molecular Variance (AMOVA) in GenALEX software) were conducted to show the genetic relationships among the *Alkanna* populations on the basis of Nei's genetic distances. The DNA fragments were visualized under UV light and further analyzed with a video image analyzer (BioImaging Systems, Cambridge, UK).

3) Extraction of plant material and qualitative analysis of alkaloids. From GC-MS Analysis of alkaloids identified from *Alkanna primuliflora*, *A. graeca* and *A. stribrnyi*, chromatograms were used, which were not used in the article. They represent a crude alkaloid mixture of the three species. Based on mass spectral fragmentation with standard reference spectra from the database, alkaloids were identified in all three species.

## 2. Context of the research project that this dataset was collected for.

Assessment of the genetic diversity and reproductive potential of the three *Alkanna* species. Determination of alkaloid content.

## 3. Date of data collection:

2018, From April to June.

## 4. Geographic location of data collection:

- *Alkanna primuliflora* collected - Thracian lowland, Plovdiv, Bulgaria; Sienite rocks, dry places; GPS: 42°08'59.9"N 024°45'48.8"E 107masl;

- *A. stribrnyi* collected - Thracian lowland, above Asenovgrad town, Sv. Petka, Bulgaria; dry stone places, metamorphic rocks, The soil cover is very thin. GPS: 41°59'35.8" N 024°52'20.1" E 310masl;

- *A. graeca* collected - Above Asenovgrad town, dry stone places, metamorphic rocks, Bulgaria; The soil cover is very thin. GPS: 41°59'13.8" N 024°52'17.1" E 388masl.

## 5. Funding sources that supported the collection of the data:

The authors are grateful for the financial support provided by the "Program for young scientists and post-doctoral students" of the Bulgarian Academy of Sciences in Sofia, and the financial support from Agricultural University, Plovdiv, Bulgaria, grant number 17-12.

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SHARING/ACCESS INFORMATION  
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### 1. Licenses/restrictions placed on the data:

This work is licensed under a Creative Commons Attribution 4.0 International License  
<https://creativecommons.org/licenses/by/4.0/>

**2. Links to publications related to the dataset:**

Semerdjieva, I., Petrova, G., Yankova-Tsverkova, E., Doncheva, T., Kostova, N., Nikolova, R., Zheljazkov, V.D. (2020) Genetic diversity, reproductive capacity and alkaloids content in three endemic Alkanna species PLOS ONE doi: 10.1371/journal.pone.0233516

**3. Recommended citation for the data:**

Semerdjieva, I., Petrova, G., Yankova-Tsverkova, E., Doncheva, T., Kostova, N., Nikolova, R., Zheljazkov, V.D. (2020) Dataset: Genetic diversity, reproductive capacity and alkaloids content in three endemic Alkanna species (Version 1) [Dataset] Oregon State University <https://doi.org/10.7267/8623j475z>

**4. Dataset Digital Object Identifier (DOI):**

<https://doi.org/10.7267/8623j475z>

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VERSIONING AND PROVENANCE  
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**1. Last modification date**

2020, May, 15

**2. Links/relationships to other versions of this dataset:**

There are no other versions of this data

**3. Was data derived from another source? No**

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METHODOLOGICAL INFORMATION  
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**1. Description of methods used for collection/generation of data:**

*Alkanna primuliflora* one population, *Alkanna graeca* one population and *Alkanna stribrnyi* three populations were subject to this study. Flowers and buds are collected during the period from April to June to investigate the reproductive potential of the three plants species. Also, seeds from the studied populations were collected at the end of June. For genetic research, leaves of the three species were collected during flowering. Leaves and stems were collected for alkaloid analysis.

## **2. Methods for processing the data:**

**1) Localization of the populations for research** - collection of information from the literature, field research; establishing exact coordinates; Collection of material.

**2) Embryological studies** - Young and open flowers were collected and fixed in a mixture of FAA (5 parts formalin: 5 parts glacial acetic acid: 90 parts of 70% ethanol). The fixed plant material was dehydrated in ascending ethanol series, embedded in paraffin according to the classical paraffin methods (Sundara, 2000). Consequently, the embedded in paraffin material was cut into 6-12  $\mu\text{m}$  sections with a rotary microtome. The sections were stained with Heidenhain's haematoxylin (Romeis, 1948) and embedded in Entellan, in order to develop permanent slides.

**3) DNA extraction** - This study attested that the individual plants within the populations of the three *Alkanna* species in Bulgaria had a very limited number. Therefore, leaf samples were collected carefully from a few plants to minimize the potential damage to the populations. We performed the sampling depending on the density of each population; plants were randomly chosen for sampling. For genetic analysis, we used the leaves of the three species at flowering (in May) as follow: *A. primuliflora* - ten samples; *A. graeca* - eight samples; *A. stribrnyi* - six samples.

**Inter-simple sequences repeat (ISSR) amplification product analysis** - First, the polymorphism of 40 markers was tested and then 10 polymorphic and reproducible ISSR primers (Microsynth, Balgach, Switzerland) were selected and used (Table 2). Polymerase chain reactions (PCR) were performed according to the method described previously [28]. PCR reactions were carried out in a Techne TC-5000 gradient thermal circler (Techne, Staffordshire, UK). The reproducibility of the technique was tested by replicating each amplification reaction twice. Amplification products were separated on 2% agarose gels stained by incorporating 1% GelRed (Biotium Inc., USA) at 1.5h, 135V along with 100 bp Plus DNA Ladder (Thermoscientific, Vilnius, Lithuania). The DNA fragments were visualized under UV light and

further analyzed with a video image analyzer (BioImaging Systems, Cambridge, UK).

4) **Alkaloids extraction** - The GC / MS analysis was performed with GC Hewlett Packard 6890 (Palo Alto, California, U.S.A.) using MS detector Hewlett Packard 5973 fitted with a HP-5 MS column (30m x 0.25mm x 0.25  $\mu$ m). The program was as follows: injector T 250 ° C; the temperature program was 100 ° C (2 min) to 280 ° C, 5 ° C / min, isothermal at 280 ° C for 20 min. Split ratio 1: 30, carrier gas (He), constant flow 0.8 mL / min.

The GC of each crude mixture of alkaloids was presented into file GC-MS data.docx (Raw material).

The alkaloids were identified by comparison of their mass spectral fragmentation with standard reference spectra from database Wiley 275 and the literature data. The MS data of the alkaloids was presented into file GC-MS data.docx (Raw material).

### **3. Instrument- or software-specific information needed to interpret the data:**

We used the following research instrumentation and equipment:

1) **For GPS coordinates of the populations - GPS Oregon 750.**

2) **Embryological studies** - The main embryological structures and processes in the male and female generative sphere were established after observations using LM "Olympus" CX2. The microphotographs were made using "Infinity lite" digital camera 1,4 Mpx; Rotary microtom.

3) **DNA analysis and ISSR** - The DNA fragments were visualised under UV light and further analysed with a video image analyser (BioImaging Systems, Cambridge, UK); PCR reactions were carried out in a Techne TC-5000 gradient thermal circler (Techne, Staffordshire, UK).; Analysis of Molecular Variance (AMOVA) in GenALEx software were conducted to show the genetic relationships among investigated Alkana populations based on Nei's genetic distances.

4) **Alkaloids extraction** - The GC/MS analysis was performed with GC Hewlett Packard 6890 (Palo Alto, California, U.S.A.) using MS detector Hewlett Packard 5973 fitted with a HP-5 MS column (30m x 0.25mm x 0.25  $\mu$ m).

### **4. Standards and calibration information, if appropriate:**

The alkaloids were identified by comparison of their mass spectral fragmentation with standard reference spectra from database Wiley 275 and the literature data. The MS data of the alkaloids was presented into file GC-MS data.docx (Raw material).

#### **5. Environmental/experimental conditions:**

Field work and sample collection was performed in sunny and quiet, warm weather; T ° 25-30 ° C. The remaining activities Embryological research, DNA analysis and extraction of alkaloids were performed in laboratory conditions under controlled conditions.

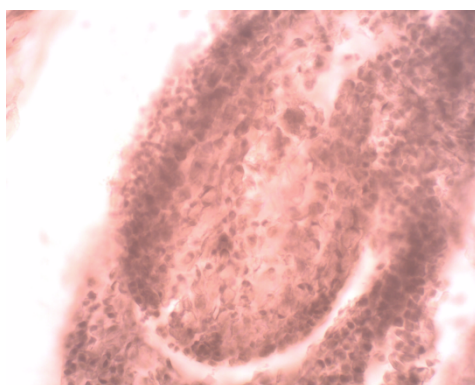
#### **6. People involved with sample collection, processing, analysis and/or submission:**

Semerdjieva, I., G. Petrova, E. Yankova-Tsvetkova, T. Doncheva, N. Kostova, R. Nikolova, and V.D. Zheljazkov

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**DATA & FILE OVERVIEW**  
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#### **1. File List A - - Embryological data (row data)**

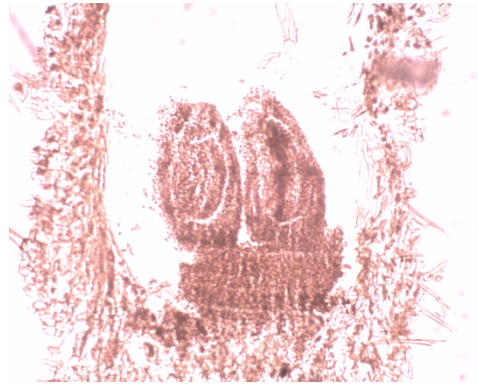
File name. **Picture\_1\_Alkanna\_primuliflora.bmp;**



**Short description** - anatropous ovule with one-celled archesporium in *A. primuliflora*;

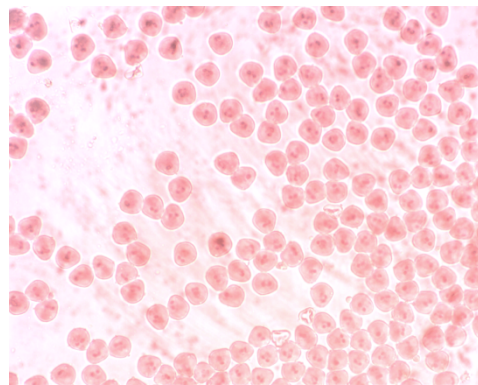
**File name** **picture\_2\_Alkanna\_stribrnyi.bmp**





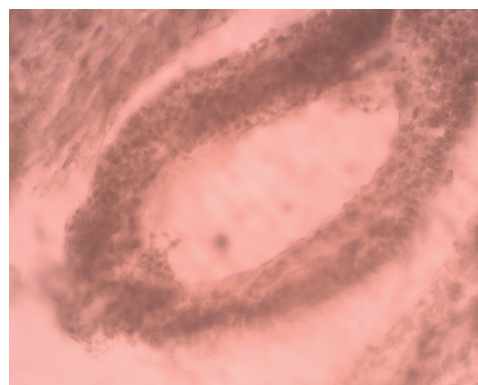
**Short description** - two-loculate ovary in *A.stribrnyi*;

**File name** picture\_3\_ *Alkanna\_primuliflora.bmp*;



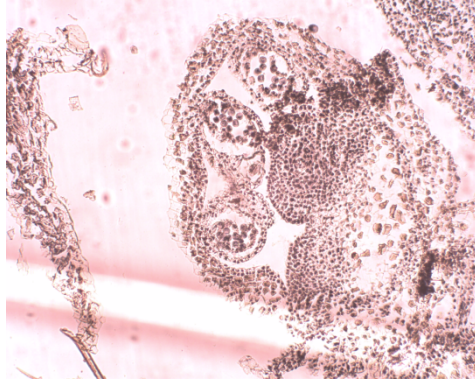
**Short description** - two-celled mature pollen grains in *A. primuliflora*;

**File name** picture\_4\_ *Alkanna\_graeca.bmp*



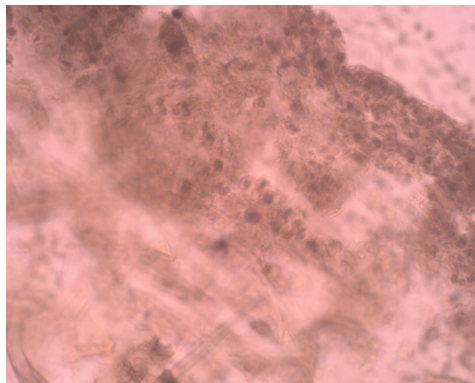
**Short description** - mature embryo sac with postament in the chalazal part in *A graeca*;

**File name** picture\_5\_ *Alkanna\_primuliflora.bmp*



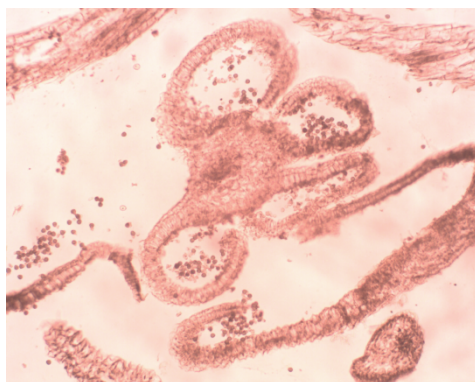
**Short description** - proterandry in *A. primuliflora* (x40 for fig H);

**File name** *picture\_6\_Alkanna\_sribrnyi.bmp*



**Short description** - globular embryo in *A. sribrnyi*;

**File name** *picture\_7\_Alkanna\_primuliflora.bmp*;



**Short description** - tetrasporangite anthers in *A. primuliflora*;

**File name** *picture\_8\_Alkanna\_primuliflora*;

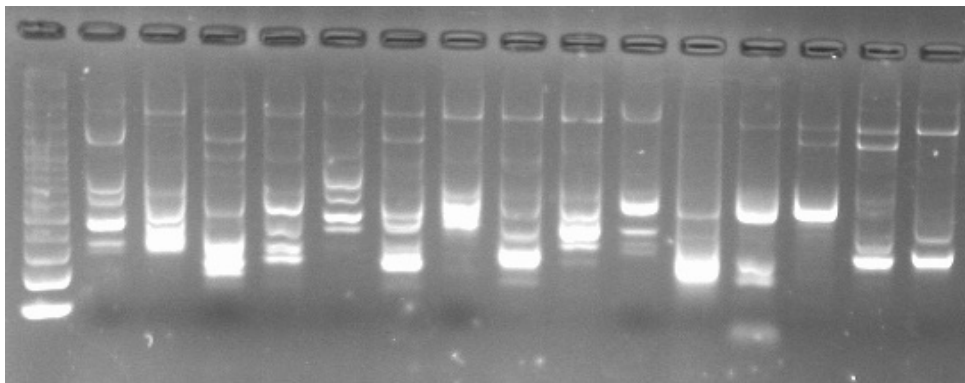


**Short description** - mature embryo with part of endosperm and seed coat in *A. primuliflora*;

## 2. File List B. DNA analysis

Filename: image009\_ISSRpatterns\_primersAG10C.png

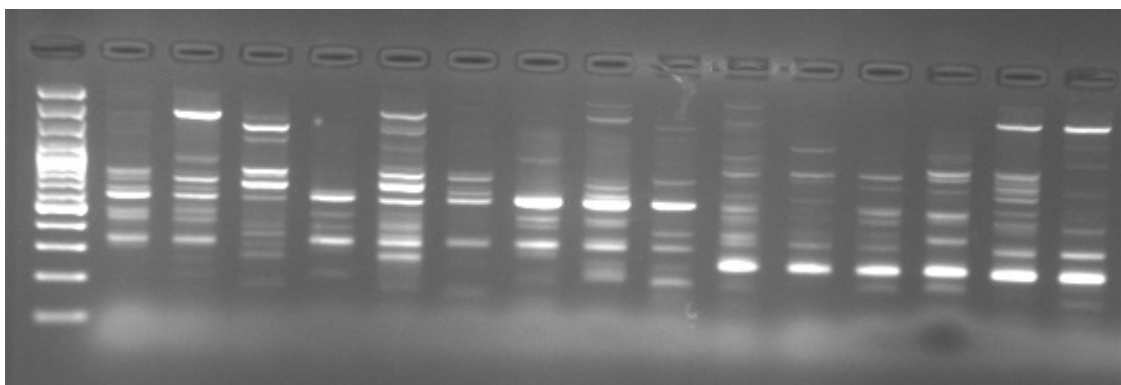
ISSR patterns of *Alkanna primuliflora*, *Alkanna graeca* and *Alkanna sribrnyi*



**Short description:** ISSR patterns of 15 *Alkanna* individuals belonging to *Alkanna primuliflora* (1-5), *Alkanna graeca* (6-10) and *Alkanna sribrnyi* (11-15) generated by primer (AG)10C. The DNA marker is GeneRuler 100 bp plus.

File name: image010\_ISSRpatterns\_primersAC8YT.png

ISSR patterns of *Alkanna primuliflora*, *Alkanna graeca* and *Alkanna sribrnyi*

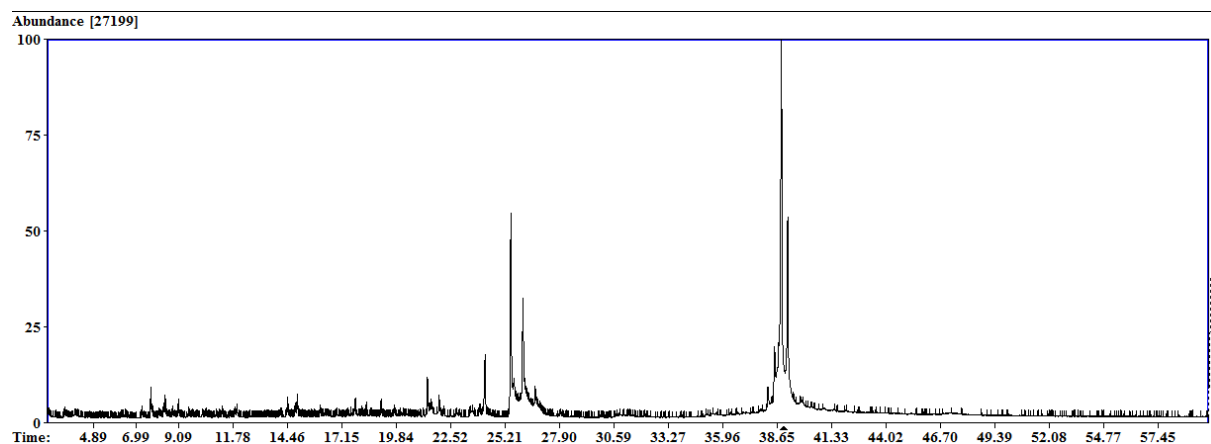


**Short description:** ISSR patterns of 15 *Alkanna* individuals belonging to *Alkanna primuliflora* (1-5), *Alkanna graeca* (6-10) and *Alkanna stribrnyi* (11-15) generated by primer (AC)8YT. The DNA marker is GeneRuler 100 bp plus.

**3. File List C. GC-MS Analysis of alkaloids identified from *Alkanna primuliflora*, *A. graeca* and *A. stribrnyi*.**

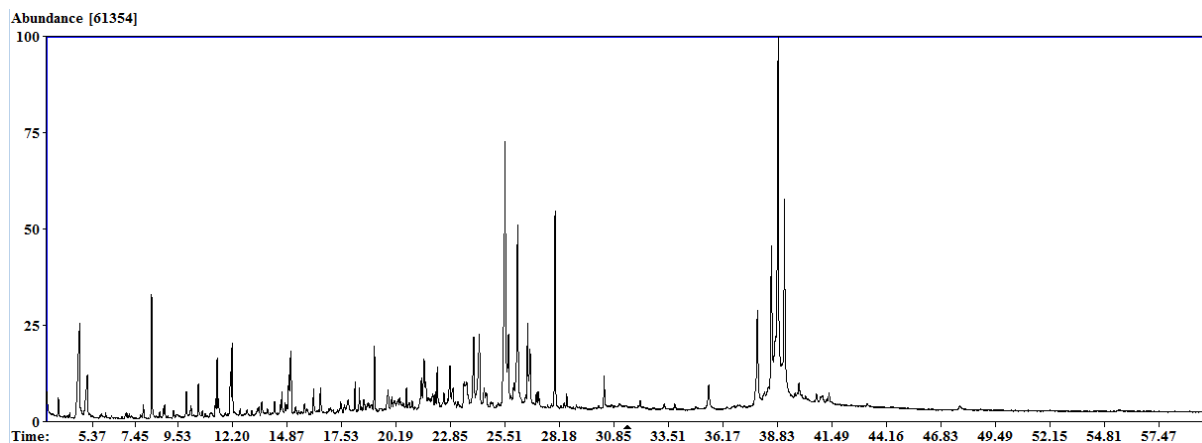
**File name: image011\_GC\_A\_primuliflora\_flowering.png**

GC of the crude alkaloid mixture of *A. primuliflora* /in flowering stage/



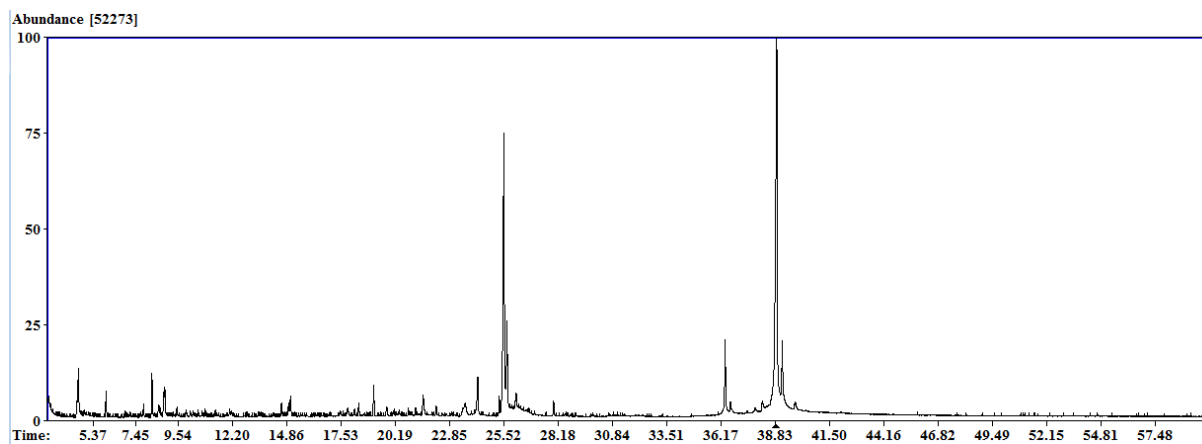
**File name: image012\_GC\_A\_primuliflora\_afterflowering.png**

GC of the crude alkaloid mixture of *A. primuliflora* /after flowering/



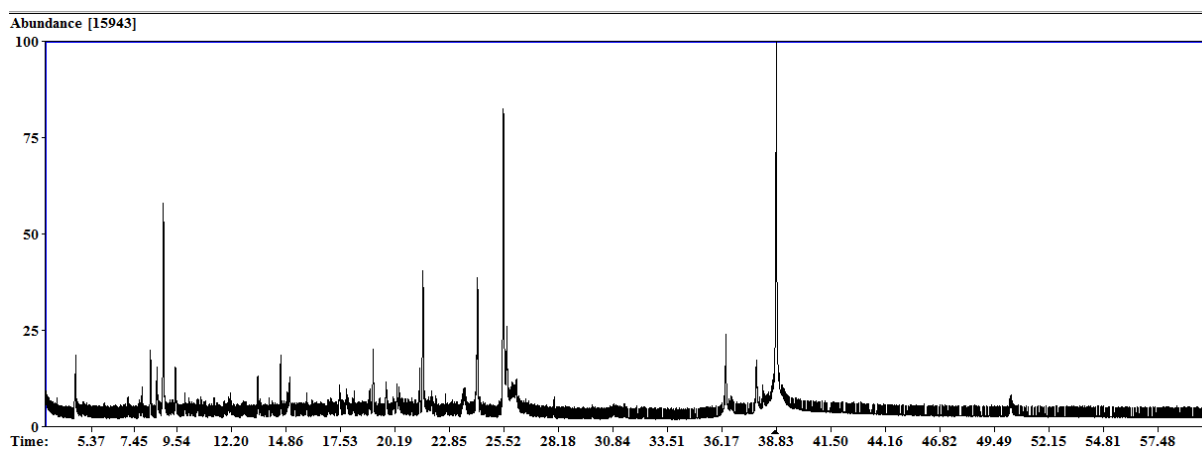
File name: image013\_GC\_A\_graeca.png

GC of the crude alkaloid mixture of *A. graeca*



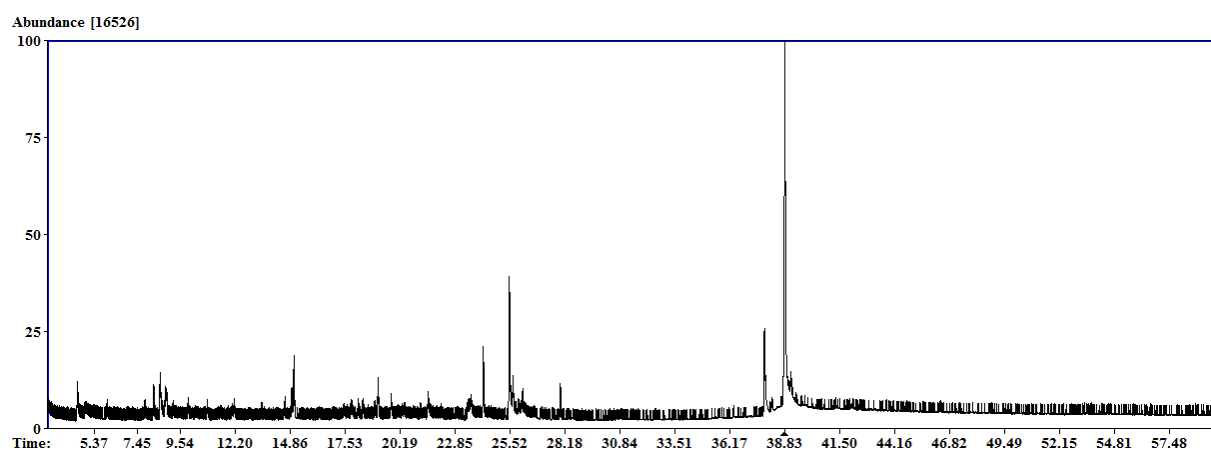
File name: image014\_GC\_A\_stribrnyi\_1.png

GC of the crude alkaloid mixture of *A. stribrnyi 1*



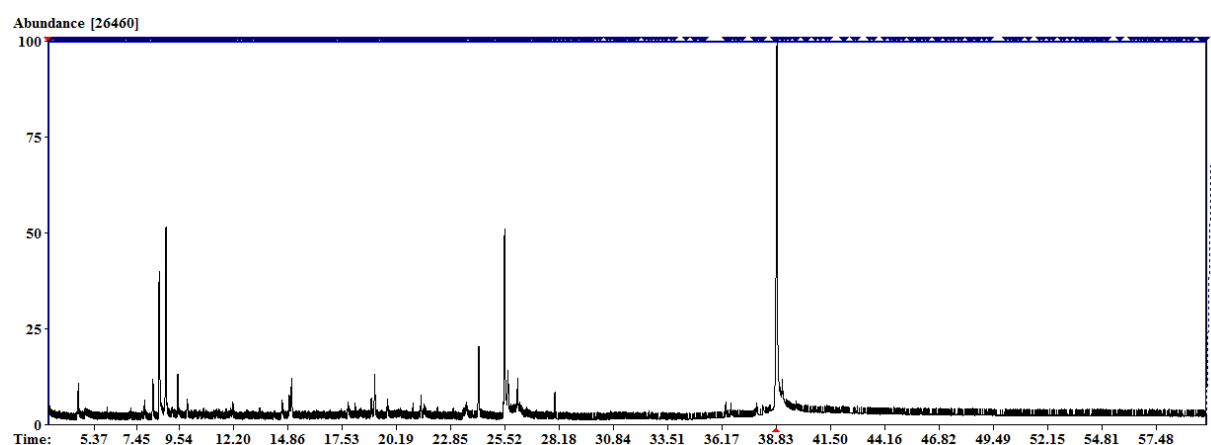
File name: image015\_GC\_A\_stribrnyi\_2.png

GC of the crude alkaloid mixture of *A. stribrnyi* 2



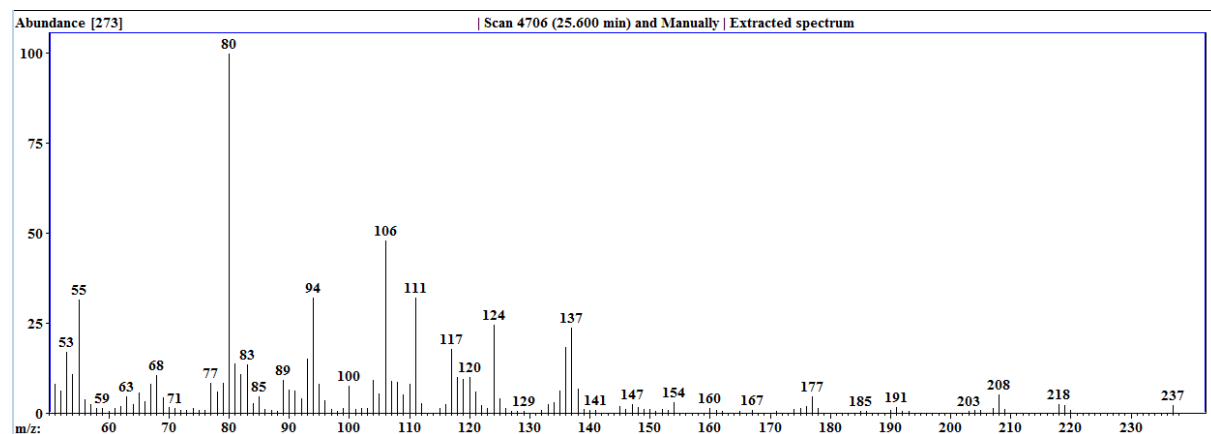
File name: image016\_GC\_A\_stribrnyi\_3.png

GC of the crude alkaloid mixture of *A. stribrnyi* 3.



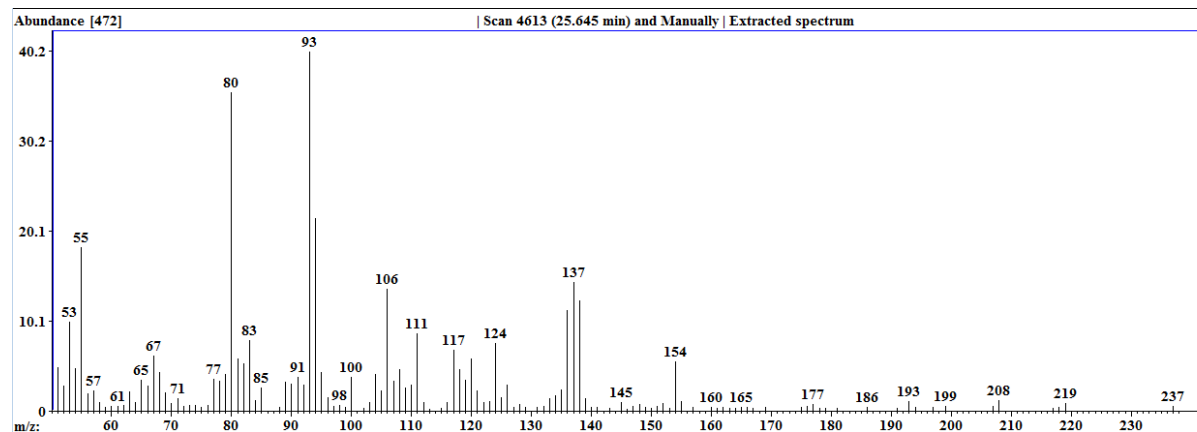
File name: image017\_MS\_7\_angeloylretronecine.png

MS of 7-Angeloylretronecine (1)



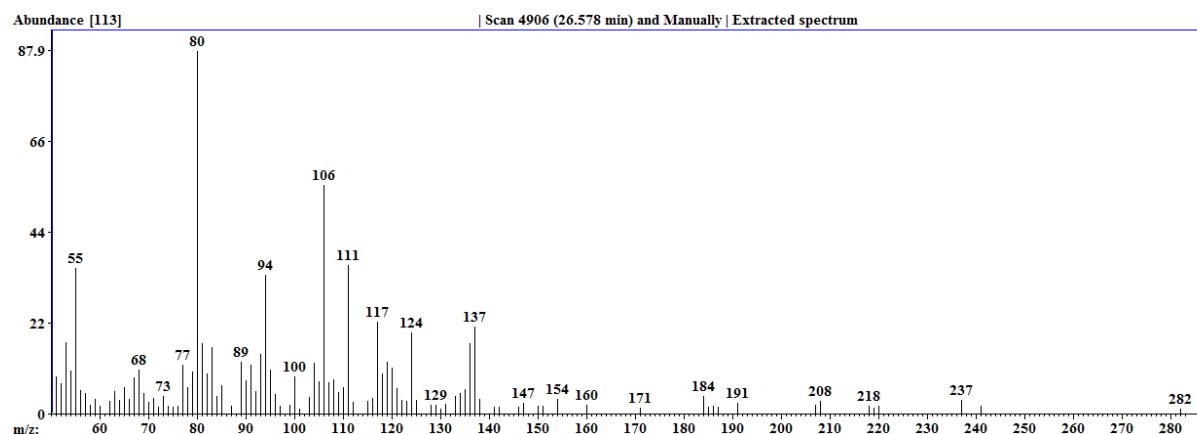
**File name: image018\_MS\_9\_angeloylretronecine.png**

MS of 9-Angeloylretronecine (2)



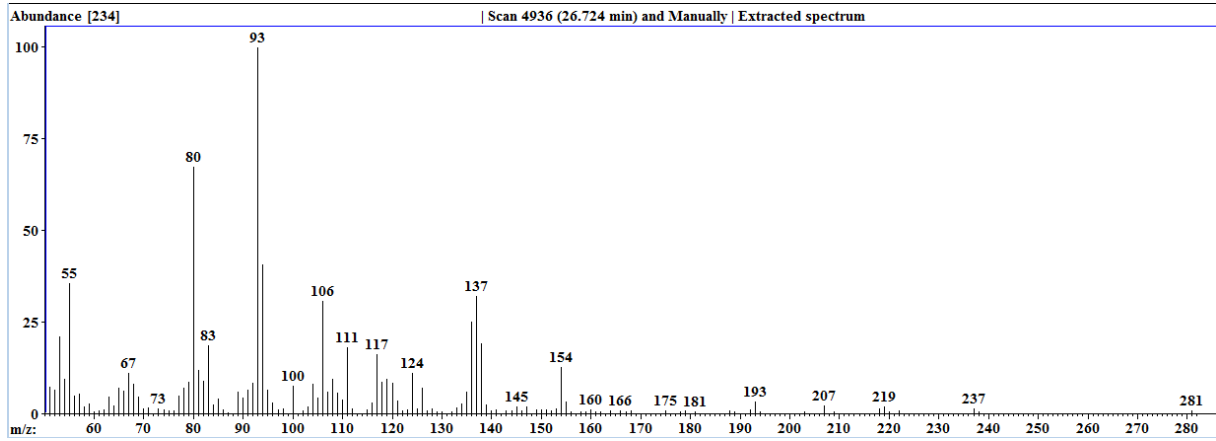
**File name: image019\_MS\_7\_tigloylretronecine.png**

MS of 7-Tigloylretronecine (3)



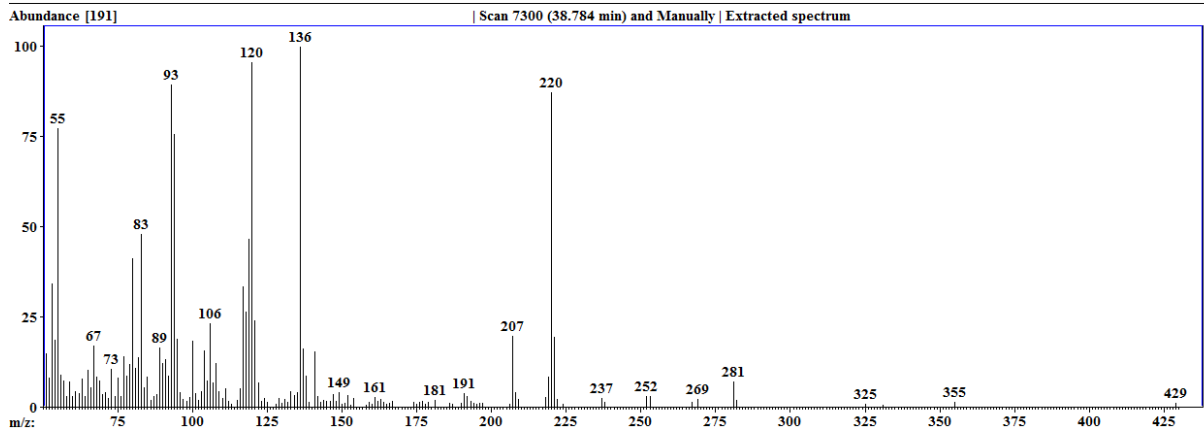
**File name: image020\_MS\_9\_ticloylretronecine.png**

MS of 9-Tigloylretronecine (4)



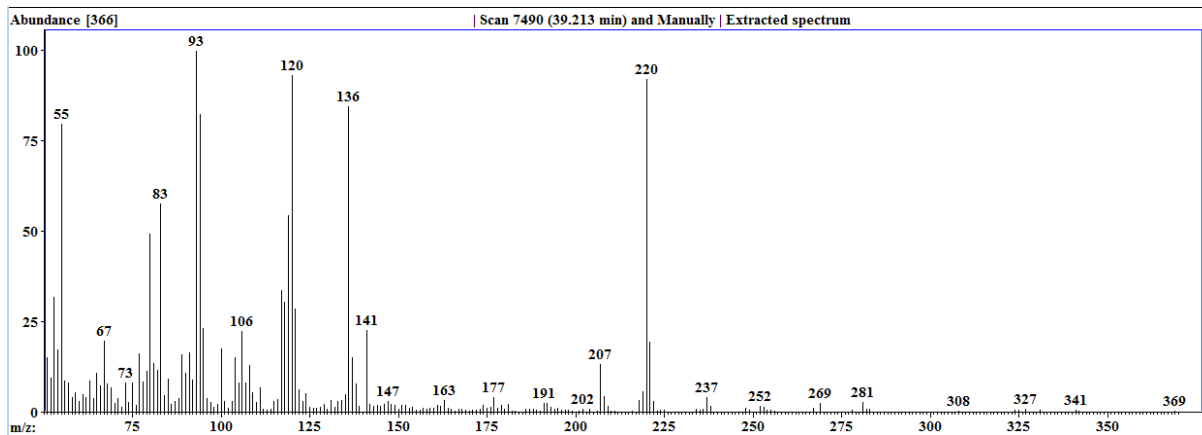
File name: image021\_MS\_triangularine.png

MS of Triangularine (5)



File name: image022\_MS\_triangularicine.png

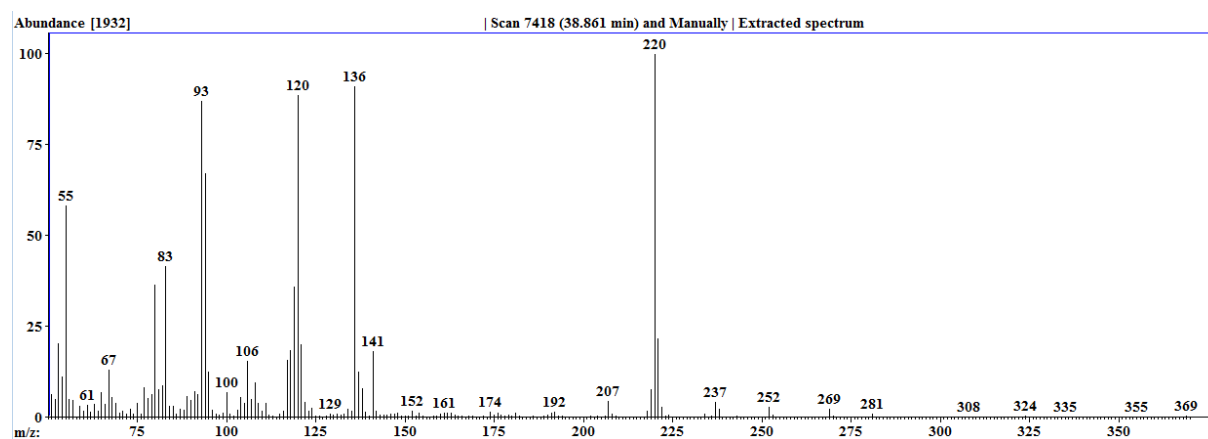
MS of Triangularicine (6)



File name: image023\_MS\_dihydroxytriangularine.png



MS of Dihydroxytriangularine (7)



File name: image024\_MS\_dihydroxytriangularicine.png

MS of Dihydroxytriangularicine (8)

