Chemistry and biological activity of secondary metabolites of plants *Allium saxatile* and *Allium ponticum* widespread in Georgia

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Abstract

The genus *Allium* belongs to family Alliaceae, and consists of 1233 species, mostly growing in northern hemisphere. Among them, 70 species grow in the Caucasus region and 38 species are described in Georgia, among which, 3 species are endemic to the Caucasus region and 5 species are endemic to Georgia.

Species of *Allium* contain several classes of biologically active compounds, such as: Sulfuric compounds, Flavonoids, Coumarins, Alkaloids, Steroidal saponins, Organic acids, Vitamins (C, B1, B2), Carotenoids, micro- and macroelements.

The objects of the PhD research were plants of genus *Allium*, growing in Georgia: *Allium saxatile* Bieb and *Allium ponticum* Miscz, ex Grossh.

The aim of the research is to study the morphology and the anatomy of vegetative and generative organs of the plants, phytochemical study of secondary metabolites, determination of biological activity of secondary metabolites (Antioxidant, Cytotoxic, Anti-protozoal, Anti-inflammatory, Analgesic and gastroprotective), Determination of chemical structure of biologically active compounds.

During the research anatomy and morphology of *A. saxatile* and *A. ponticum* were studied and their microstructural characteristics were defined. Optimal condition for extraction, fractionation and isolation of individual compound was elaborated. Chemical structure of isolated individual compounds was determined.

One saponin: (3β,25R)-Spirost-5-en-3-yl-O-α-L-rhamnopyranosyl-(1→2)-O-[β-Ds-glucopyranosyl-(1→4)]-β-D-glucopyranoside (Deltonin) and two flavonoids: kaempferol 3-O-glucoside (Astragalin) and Kaempferol 3-O-neohesperidoside were isolated from *A. saxatile.*

Using *in vitro* and *in vivo* methods, biological activity of crude extract, fractions and individual compounds was evaluated.

Cytotoxic activity against Melanoma (A2058) and breast adenocarcinoma (MDA-MB-231) cells was evaluated. Crude extract of *A. saxatile* showed an IC_{50} value of 37.6 μg/ml, while that of *A. ponticum* was >50 μg/ml. The activity of the fractions was ranging between 4.04 μg/ml and 35.6

µg/ml. For individual **compound 3** activity was 3.83 µg/ml. Presence of saponins in 100% methanolic fractions may explain observed cytotoxic activity.

Antioxidant activity of compounds was evaluated with TBARS test on macrophage cells (RAW 264.7). Ranging between 3.5 µg/ml and 42.16 µg/ml for crude extracts of both plants. Antioxidant activity of fractions was ranging between 2.93 µg/ml and 24.53 µg/ml. Presence of flavonoids in fractions may explain observed antioxidant activity.

Using *in vivo* models, anti-inflammatory, analgesic and gastroprotective effect of the crude extracts and fractions was studied.

Results of analgesic activity have shown that the activity of *A. saxatile* depends on the time, while *A. ponticum* has stronger analgesic effect, but with short duration. Fractions of both plants have shown similar tendency, but their activity is dose dependent.

Results of the anti-inflammatory activity showed that crude extract of *A. saxatile* has better anti-inflammatory activity than crude extract of *A. ponticum* 32.7%. Among fractions, A.S.F3 has the highest anti-inflammatory activity.

Gastroprotective effect was only observed in crude extract of *A. saxatile,* in comparison with untreated control.

Throughout our research, we developed methods to quantify the content of secondary metabolites.

The results of dosage of steroidal saponins, showed that plant material of *A. saxatile* contains 1,69% of furostanolic saponins and *A. ponticum* contains _ 0,37%; In crude extracts the yield of furostanolic saponins was increased to 37.15% for *A. saxatile* and 11.54% for *A. ponticum*. Loss on drying ranged between 7.21% and 7.77%. Total ash content was ranging between 4.9% and 8.09%, for *A. saxatile* and *A. ponticum*.

In conclusion, the result of research suggests that both plants *A. saxatile* and *A. ponticum* growing in Georgia are rich with biologically active compounds and can be considered as perspective plants for medicinal use.

Résume

Le genre *Allium* appartient à la famille des *Alliaceae* et comprend 1233 espèces, qui poussent principalement dans l'hémisphère nord. Parmi elles, 70 espèces poussent dans la région du Caucase et 38 espèces sont décrites en Géorgie, parmi lesquelles 3 espèces sont endémiques à la région du Caucase et 5 espèces sont endémiques à la Géorgie.

Les espèces d'*Allium* contiennent plusieurs classes de composés biologiquement actifs, tels que : composés sulfurés, flavonoïdes, coumarines, alcaloïdes, saponines stéroïdiques, acides organiques, vitamines (C, B1, B2), caroténoïdes, micro- et macro-éléments.

Les plantes ayant fait l'objets de la recherche étaient des plantes du genre *Allium*, poussant en Géorgie : *Allium saxatile* Bieb et *Allium ponticum* Miscz, ex Grossh.

L'objectif de la recherche sont d'étudier la morphologie et l'anatomie des organes végétatifs et génératifs des plantes, l'étude phytochimique des métabolites secondaires, la détermination de l'activité biologique des métabolites secondaires (antioxydant, cytotoxique, anti-protozoaire, anti-inflammatoire, analgésique et gastroprotecteur), la détermination de la structure chimique des composés biologiquement actifs.

Au cours de la recherche, l'anatomie et la morphologie d'*A. saxatile* et d'*A. ponticum* ont été étudiées et leurs caractéristiques microstructurales ont été définies. Les conditions optimales pour l'extraction, le fractionnement et l'isolement des composés individuels ont été élaborées. La structure chimique des composés individuels isolés a été déterminée.

Une saponine : (3β,25R)-Spirost-5-en-3-yl-O-α-L-rhamnopyranosyl-(1→2)-O-[β-Ds-glucopyranosyl-(1→4)]-β-D-glucopyranoside (Deltonin) et deux flavonoïdes : Kaempferol 3-O-glucoside (Astragalin) et Kaempferol 3-O-neohesperidoside ont été isolés à partir d'*A. saxatile.*

L'activité biologique de l'extrait brut, des fractions et des composés individuels a été évaluée à l'aide de méthodes *in vitro* et *in vivo*.

L'activité cytotoxique contre les cellules de mélanome (A2058) et d'adénocarcinome du sein (MDA-MB-231) a été évaluée. L'extrait brut d'*A. saxatile* a montré une valeur d'IC50 de 37,6

µg/ml, tandis que celle d'*A. ponticum* était >50 µg/ml. L'activité des fractions était comprise entre 4,04 µg/ml et 35,6 µg/ml. Pour le composé individuel 3, l'activité était de 3,83 µg/ml. La présence de saponines dans les fractions 100 % méthanoliques peut expliquer l'activité cytotoxique observée.

L'activité antioxydante des composés a été évaluée par le test TBARS sur des cellules macrophages (RAW 264.7). Les résultats varient entre 3,5 µg/ml et 42,16 µg/ml pour les extraits bruts des deux plantes. L'activité antioxydante des fractions était comprise entre 2,93 µg/ml et 24,53 µg/ml. La présence de flavonoïdes dans les fractions peut expliquer l'activité antioxydante observée.

Les effets anti-inflammatoires, analgésiques et gastroprotecteurs des extraits bruts et des fractions ont été étudiés à l'aide de modèles *in vivo*.

Les résultats de l'activité analgésique ont montré que l'activité d'*A. saxatile* augmente avec le temps, tandis qu'*A. ponticum* a un effet analgésique plus fort, mais de courte durée. Les fractions des deux plantes ont montré une tendance similaire, mais leur activité dépend de la dose.

Les résultats de l'activité anti-inflammatoire ont montré que l'extrait brut d'*A. saxatile* a une meilleure activité anti-inflammatoire que l'extrait brut d'*A. ponticum* 32,7%. Parmi les fractions, A.S.F3 a l'activité anti-inflammatoire la plus élevée.

L'effet gastroprotecteur n'a été observé que dans l'extrait brut d'*A. saxatile*, en comparaison avec le contrôle non traité.

Tout au long de nos recherches, nous avons développé des méthodes pour quantifier le contenu des métabolites secondaires.

Les résultats du dosage des saponines stéroïdiennes ont montré que le matériel végétal d'*A. saxatile* contient 1,69% de saponines furostanoliques et *A. ponticum* en contient 0,37% ; dans les extraits bruts, le rendement des saponines furostanoliques a été porté à 37.15% pour *A. saxatile* et à 11.54% pour *A. ponticum*. La perte à la dessication se situe entre 7,21% et 7,77%. La teneur en cendres totales était comprise entre 4,9 % et 8,09 % pour A. saxatile et *A. ponticum*.

En conclusion, les résultats de la recherche suggèrent que les deux plantes *A. saxatile* et *A. ponticum* poussant en Géorgie sont riches en composés biologiquement actifs et peuvent être considérées comme des plantes prometteuses pour un usage médicinal.

Introduction

Biologically active compounds of natural origin are an important source for the development of medicinal products. Wide and versatile biological activities of medicinal plants is based on their phytochemical composition, based on important classes of secondary metabolites, such as: alkaloids, terpenes, phenolic compounds, cardiac glycosides, saponins and etc. They possess a significant role in improving the state of human health, because they are active components of remedies for various diseases.

Many countries are paying special attention to the development of new medicines based on plant materials, which will be effective, safe, high quality and available for the patients. In this regard, it is still relevant for modern medicine to identify new medicinal plant resources, identify biologically active compounds in them and develop new medicines based on them.

The genus *Allium*, which includes plants such as onions, garlic, and chives, is known for its wide range of biological activities and the presence of various phytochemical compounds. However, despite the extensive literature available on Allium species, there are still many species within the genus that have not been thoroughly studied.

In the context of the research, the focus is on two specific *Allium* species found in Georgia: *Allium saxatile* Bieb and *Allium ponticum* Miscz, ex Grossh. These species are of interest because they have not received significant attention or investigation in terms of their biological activities and phytochemical composition.

When studying these *Allium* species, research will typically explore their biological activities, which can include cytotoxic, antioxidant, analgesic, gastroprotective and anti-inflammatory properties. These activities are often attributed to the presence of various bioactive compounds, such as saponins, flavonoids, phenolic compounds, etc., which are commonly found in *Allium* plants.

To investigate the biological activities and phytochemical composition of *Allium saxatile* and *Allium ponticum*, research would typically employ various scientific methods and

techniques. These can include extraction of the sum of bioactive compounds, their fractionation, characterization and identification of phytochemicals using techniques like chromatography and spectroscopy, and evaluation of biological activities through *in vitro* and *in vivo* assays.

By conducting such research on these understudied *Allium* species, results will aim to expand our knowledge of their potential applications in medicine. Additionally, the findings can contribute to the overall understanding of the diversity and potential benefits of *Allium* plants, further emphasizing the importance of exploring and studying different species within the genus.

The objects of the research are two plants of the genus *Allium* L growing in Georgia.: *Allium saxatile* Bieb and *Allium ponticum* Miscz, ex Grossh.

A. saxatile is a perennial herbaceous plant, mostly growing on rocks, stony and sandy ecotopes. This plant is common in Central Europe, in Georgia, mostly in Samegrelo, Imereti, Shida Kartli and Kareli floristic regions. It shows filamentous leaf, pale pink petals, elongated, flowering in VII-VIII months[1].

A. saxatile is united in Oreiprason F. Herm section [1], [2]. Plants of this section are characterized with short and indirect rhizome or bulb; round or grooved anthophore; flat, grooved, semi-cylindrical or cylindrical leaves; Scape 10-50 cm. The bulb is egg-shaped with short rhizome, covered with leathery shell. The plant has 5-7 filamentous leaves, grooved, shorter than the trunk. Petals are 1.5-3 times longer than inflorescence. Inflorescence is spherical, or semi-spherical. Anthophore is 1.5-3 times longer than perianth. Pointed egg/bell-shaped perianth with pale pink color. Filaments are 1.5-2 times longer than perianth. Style of pistil is stick out from perianth[1]–[3].

A. ponticum is common in the Caucasus and Turkey region, in Georgia this plant is spread in Abkhazia, Imereti, Guria, Javakheti, Adjara and Kartli floristic regions. The plant is flowering from May through July. It presents itself as an egg-shaped bulb, covered with almost leathery shell. Scape 30-70 cm, leaves 3-4 cm, grooved, without hollow. Inflorescence semi-spherical,

anthophore are 2-3 times longer than perianth. Spherical/bell-shaped perianth with dark red color petals. Filaments as long as perianth or shorter[1].

Figure 1. Map of Georgia.

Goals of the research

The goal of the research is phytochemical study of the secondary metabolites of some species of genus *Allium*, growing in Georgia and the determination of their biological activity and based on these results, detection of new herbal material for medicinal use.

The genus *Allium*, which includes species such as garlic, onion, and leek, is well known for its medicinal and nutritional properties. These plants are rich in secondary metabolites such as flavonoids, phenols, alkaloids, and sulfur-containing compounds, which are responsible for their various biological activities.

The aim of this study is to perform a thorough phytochemical analysis of *Allium* species present in Georgia. To achieve this objective, the morphological and anatomical characteristics of the plants will be examined initially. This study involves the extraction of crude extracts from the plants using 80% Ethanol. followed by the fractionation of the obtained crude extracts to isolate individual compounds.

Obtained fractions will be further tested to determine their biological activities, including cytotoxic, antioxidant, analgesic, gastroprotective, and anti-inflammatory activities, using *in vitro* and *in vivo* assays. The choice of these activities is influenced by the data and research already available in scientific literature. Previous studies on *Allium* species have reported promising results in terms of cytotoxic, antioxidant, analgesic, gastroprotective, or anti-inflammatory properties, motivating further investigation. Biologically active fractions will be further separated using column chromatography, to obtain biologically active individual compounds. The structure of isolated compounds will be elucidated with NMR and Mass-spectrometry.

Overall, the phytochemical study of *Allium* species in Georgia has the potential to contribute to the discovery of new herbal material for medicinal use by correlating the chemical composition of these plants with their therapeutic effects. By identifying and characterizing the active compounds present in *Allium* species, this research can contribute to the development of new drugs or therapies for various diseases.

Objective of the research

- Extraction of the sum of the secondary metabolites, and their fractionation;
- Isolation of individual compounds;
- Determination of chemical structure of isolated compounds;
- Determination of Antioxidant, Cytotoxic, Anti-protozoal, Anti-inflammatory, Analgesic and gastroprotective activities of crude extracts, fractions and individual compounds.
- Formulation of normative-technical documentation;

List of abbreviation

A.S.	*Allium saxatile*
A.P.	*Allium ponticum*
ACN	Acetonitrile
CC	Column Chromatography
COSY	Correlated Spectroscopy
DAD	Diode Array Detector
DCM	Dichloromethane
DMSO	Dimethylsulfoxide
IC_{50}	50% Inhibitory concentration
EtOAc	Ethyl acetate
EtOH	Ethanol
HMBC	Heteronuclear Multiple Bond Correlation
HPLC	High Performance Liquid Chromatography
HSQC	Heteronuclear Single Quatum Coherence
LC	Liquid Chromatography
LOD	Limit of Detection
LOQ	Limit of Quantification
m/z	masse to charge ratio
MeOH	Methanol
MS	Mass Spectroscopy
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
NMR	Nuclear Magnetic Resonance
p value	Probability value
ppm	Part per million
NMR	Nuclear Magnetic resonance
RT-qPCR	Real-time polymerase chain reaction
RSD	Relative Standard Deviation

TBARS	Thiobarbituric acid reactive substances
TLC	Thin Layer Chromatography
TOCSY	TOtal Correlated SpectroscopY
UV	Ultra-violet
δ	Chemical Shift
ε	Molar absorptivity
λ	Wavelength

CHAPTER 1 – Review of the literature

1.1. The family Alliaceae

The family Alliaceae is represented with one genus – *Allium* L. According to "Nomenclatural checklist of flora of Georgia", this genus is separated into 12 section [3]:

1) Sect. 1. *Anguinum* G. Don fil. ex Koch
2) Sect. 2. *Rhizirideum* G. Don. fil. ex Koch
3) Sect. 3. *Schoenoprasum* Koch
4) Sect. 4. *Orieprason* F. Herm.
5) Sect. 5. *Allium*
6) Sect. 6. *Scorodon* Koch
7) Sect. 7. *Codonoprasum* Koch
8) Sect. 8. *Molium* Traub
9) Sect. 9. *Briseis* (Salib.) Stearn
10) Sect. 10. *Ophioscordon* (Wallr.) Vved.
11) Sect. 11. *Porphyroprason* Ekberg
12) Sect. 12. *Melanocrommyum* Webb. & Berth.

1.2. Genus *Allium*

Genus *Allium* L. is widely represented in world flora. According to GBIF—the Global Biodiversity Information Facility, this genus includes up to 1233 species[4].

The genus *Allium* includes some of the most ancient cultivated crops, including *A. cepa* (onion), *A. sativum* (garlic), *A. schoenoprasum* (chives), *A. fistulosum*, *A. ampeloprasum*, *A. tuberosum*, *A. chinense* and etc[5].

The botany of *alliums* has been extensively studied and has been the subject of many excellent books. *Allium* species growing in particular countries or geographic areas are to be found

in *flora* of those areas, *e.g.*, the *Flora of North America* (2002), and *Wild Flowers of the United States* (Rickett, 1973), which respectively identify 96 and 79 different native species[5].

Notable among those who specifically researched the botany of the genus *Allium* was the eminent nineteenth century botanist Eduard Regel (1815-1892). He was scientific director and then Director General of the Imperial Botanical Garden in St. Petersburg, Russia. He was a founder of the Russian and Swiss Horticultural Societies and the journal *Gartenflora*. He had a particular fascination with the genus *Allium* and wrote about it in two monographs (Regel, 1875, 1887), which feature more than 250 species, including a large number not previously described, the fruits of the explorations in Asia. More than 60 of the *Allium* species he helped to identify bear his name in the full name of the plant, *e.g.*, *A. giganteum* Regel and *A. rosenbachianum* Regel[5].

While the origin of *Allium* species remains speculative, evidence suggests that garlic and onion were first domesticated in the central Asian mountainous regions of Tajikstan, Turkmenia, Uzbekistan, and northern Iran, Afghanistan and Pakistan and most likely brought to the Middle East by Marco Polo and other Silk Road/spice route travelers [6].

It has been suggested that *A. longicuspis*, genetically identical to *A. sativum*, might have been cultivated by seminomadic hunter-gatherers more than 10 000 years ago and transported along trading routes between China and the Mediterranean. From the Mediterranean region garlic was brought to sub-Saharan Africa and the Americas by explorers and colonists. It is also thought that garlic was introduced to China by traders from Central Asia, and into Japan from Korea, where it was very popular[7]. DNA analysis indicates that neither *A. longicuspis* nor *A. tuncelianum* are ancestor species of garlic, as suggested earlier. Therefore the true ancestor species of garlic still remains unknown[8].

Alliums vary in height from 5 to 150 cm, while the bulbs can range from very small (2 to 3 mm in diameter) to rather big (8 to 10 cm). The largest onion, according to the *Guinness Book of World Records*, weighed 4.9 kg, while the largest garlic weighed 1.19 kg (McCann, 2009). Some *Allium* species, such as *A. fistulosum*, develop thickened leaf-bases rather than forming bulbs.

Alliums are distributed widely through the temperate, warm temperate and boreal zones of the northern hemisphere at altitudes up to 3050 m from Mexico and North Africa and southern Asia northwards, but are limited to mountainous regions in tropical areas[5].

Many Georgian scientists have been working on *Allium* species growing in Georgia, They have studied: *A. porrum, A. rotundum, A. erubescens, A. waldsteinii, A. fuscoviolaceum* and etc [9]–[13].

In Georgia, Systematical studies about *Allium* species has started by N. Cholokashvili, she has divided *Allium* species into 16 section and 4 subgenus[14].

According to "Nomenclatural checklist of flora of Georgia" 38 species of genus *Allium* are described in Georgia. Among them 5 species are endemic to Georgia and 3 to the Caucasus region[3].

Table 1. Species of plants of the genus *Allium* described in Georgia

1. *A. affine* Ledeb.
2. *A. albidum* Fisch. Ex Bieb
3. *A. albovianum* Vved **
4. *A. atroviolaceum* Boiss
5. *A. aucheri* Boiss
6. *A. candolleanum* Albov *
7. *A. cardiostemon* Fisch. Et C.A. Mey
8. *A. chevsuricum* Tscholok **
9. *A. erubescens* K. Koch
10. *A. fuscoviolaceum* Fomin
11. *A. globosum* Bieb. Ex DC
12. *A. gracilescens* Somm. Et Levier **
13. *A. gramineum* K. Koch
14. *A. jalae* Vved
20. *A. oleraceum* L.
21. *A. oreophilum* C.A. Mey
22. *A. otschiauriae* Tscholok.
23. *A. paczoskianum* Tuzs.
24. *A. paradoxum* (Bieb.) G. Don. F (*Scilla paradoxa* Bieb.)
25. *A. ponticum* Miscz. Ex Grossh.
26. *A. pseudoflavum* Vved *
27. *A. pseudostrictum* Albov *
28. *A. rotundum* L.
29. *A. rubellum* Bieb.
30. *A. rupestre* Stev.
31. *A. ruprechtii* Boiss
32. *A. saxatile* Bieb

15. *A. karsianum* Fomin
16. *A. kunthianum* Vved.
17. *A. ledschanense* Conrath et Freyn *
18. *A. leucanthum* K. Koch *
19. *A. moschatum* L.

* - Endemic to the Caucasus

** - Endemic to Georgia

33. *A. scorodoprasum* L.
34. *A. szovitsii* Regel
35. *A. ursinum* L.
36. *A. victorialis* L.
37. *A. vineale* L.
38. *A. waldsteinii* G.Don f.

1.3. Ethnopharmacology of the plants of genus *Allium* L.

Garlic, onion and leek have always been popular foods in Egypt and elsewhere in the Mediterranean basin. Garlic, considered a strengthening food, ideal for workers, oarsmen and soldiers was such a popular plant with the Roman army that it was said that "one could follow the advance of the Roman legions and expansion of the empire by plotting range maps for garlic" [15]. The Roman legions introduced garlic to many of the peoples they conquered, especially those of Northern Europe[16]. Our knowledge of their cultivation, culinary usage and medicinal applications in ancient Middle Eastern civilizations, comes from the study of extensive archeological evidence. This includes pictograms and other ancient art appearing on papyrus scrolls, clay tablets and models, stone carvings, painted funerary objects such as coffin covers and mummy cases, and from burial relics including intact plants themselves. Sumerian cuneiform tablets from 2300 BCE describe the Sumerian diet as consisting of grains, legumes, onion, garlic, leek and other vegetables, as well as many varieties of fish. The archeological evidence suggests that there were apparently five or six varieties of *alliums*, although only onion, leek and garlic can be identified, and that the plants were generally mashed or chopped together to draw more flavor from them[17], [18].

The onion is mentioned as an Egyptian funeral offering. Ancient Egyptian wall carvings and drawings, as well as several finds of dried specimens, show that both leek and onion were part of Egyptian food production. Onion bulbs were found placed in body cavities of mummies,

perhaps to stimulate the dead to breathe again, while garlic was used in the embalming process. Excellently preserved garlic was found in Tutankhamun's tomb (1325 BCE) [19].

Ancient cultivation of garlic and onions in India can be found in Ayurvedic medical books, dating from 400 to 200 BCE, which attributes many health virtues to these plants(Jones and Mann, 1963).

In India, garlic has been used for centuries as an antiseptic lotion for washing wounds and ulcers[20]. *Allium* species, such as: *A. tuberosum, A. chinense, A. fistulosum* are cultivated in China more than 3000 years[21]. *Allium fistulosum* is used in traditional Chinese medicine as a potential remedy for the common cold, influenza, arthritis, headache, abdominal pain, constipation, dysentery, sores, ulcers, parasitic infestations, arthritis, and heart disease[22],[23]. *Allium tuberosum* seeds are commonly used in traditional Chinese medicine to treat impotence. They are also used as tonic and aphrodisiac. In contrast, the leaves of *A. tuberosum* are usually employed to cure abdominal pain, diarrhea, hematemesis, diabetes. They are used as tonic and booster of the digestive and immune systems, as well as an antidote for snake bites and poisonous bee or wasp stings [24]. However, there is also evidence from the Han Dynasty (206 BCE–220 CE) of putting onions and garlic on red cords hung on house doors to repel harmful insects. In the Han Dynasty, garlic, scallions and leek were common foods[25]. In China, garlic and onion tea have long been recommended for fever, headache, cholera and dysentery[26].

Onions, garlic and leeks were favorites in medieval monastery gardens as they were easy to grow, hardy and, above all, strong tasting. In Italian medicinal literature is described the usage of *Allium* species against many diseases, such as: abscesses, bleeding, blisters, body's detoxication, bronchitis, bruises, burns, chilblains, cold, constipation, contusions, corns, cough, cysts, diarrhea, ear pains, eczemas, female intimate inflammation, flu, gout, greasy skin, hemorrhoids, hair loss, headache, high blood pressure, high blood sugar level, high cholesterol, hoarseness, horses thrombosis (vet.), inflammation, ingrown nails, insect bites, intestinal disorders, intestinal worms, kidney stones, liver dysfunction, low milk production, mange (vet.), menstrual pains, nosebleed, oral cavity infections, overweight, pimples, pneumonia, polycythemia, poor digestion, rhagades,

rheumatism, stomachache, sty, sore throat, toothache, urinary tract diseases, warts, water retention, whitlows, wounds[27], [28]. In Spanish traditional medicine, *Allium* species were used for skin diseases, sinusitis, cold, bronchitis, pneumonia, asthma, diarrhea, wounds and cuts and etc[29].

In Iranian traditional medicine are described the following *Allium* species: *Allium akaka, Allium tripedale, Allium iranicum, Allium stipitatum, Allium atroviolaceum, Allium cepa, Allium jesdianum, Allium sativum.* Used for diabetes, hyperlipidemia, toothache, frequent colds, scorpion bites, kidney stones, pains in the digestive system, rheumatism, respiratory problems particularly cough, constipation, prevention hair to get white and etc[30].

Onions and garlic also hold an important place in traditional Turkish medicine. They are main components of almost all Turkish food. Garlic, onions, and related vegetables were very popular in Ottoman cuisine not only as a food or condiment but also due to their health benefits. In the Ottoman Navy, every ship was equipped with a chest of medicines, among the many pharmaceutical preparations and herbs, "garlic paste" was mandatory to be included in the medicinal chest[31]–[33]. *A. cepa, A. sativum, A. ampeloprasum, A. Scorodoprasum*, are most commonly used plants in traditional medicine of Ottoman empire. They were used against Asthma, Cancer, Head ache, Hypertension, Diabetes, Wound healing, Cold, Infertility, sugar management, cardiotonic, for loss of appetites and etc[34]. *A. ampeloprasum* L. was used against blurred vision and diabetes, *A. cepa* L. against Gastrointestinal diseases, renal colic, Menstrual pain and *A. sativum* L. against Diabetes, Cold and flu[35].

Medicine, pharmacy, and drug science in Georgia have ancient cultures and traditions, as evidenced by results of many archaeological studies and medieval medical literature: "Carabadin" and "Yadigar Daud", describing the use of *Allium* species in Georgian traditional medicine against nose bleeding, cancer, ulcer, stomachache, snake and dog bite, cough, flu, cold and etc. They were used also as antiseptic and antibacterial remedy(Table 2)[36], [37].

Table 2. Traditional use of *Allium* species.

Country	Plant species	Plant parts used	Traditional use
India	*Allium sativum*	Whole plant	washing wounds and ulcers
China	*Allium tuberosum, Allium chinense, Allium fistulosum*	Whole plant, bulbs, seed	common cold, influenza, arthritis, headache, abdominal pain, constipation, dysentery, sores, ulcers, parasitic infestations, arthritis, and heart disease, impotence.
Russia and central Asia	*Allium cepa* *Allium sativum*	Bulbs	Skin diseases
Italy	*Allium cepa* *Allium sativum* *Allium ampeloprasum*	Bulbs, whole plant	abscesses, bleeding, blisters, body's detoxication, bronchitis, bruises, burns, chilblains, cold, constipation, contusions, corns, cough, cysts, diarrhea, ear pains, eczemas, female intimate inflammation, flu, gout, greasy skin, hemorrhoids, hair loss, headache, high blood pressure, high blood sugar level, high cholesterol, hoarseness, horses thrombosis (vet.), inflammation, ingrown nails, insect bites, intestinal disorders, intestinal worms, kidney stones, liver dysfunction, low milk production, mange (vet.), menstrual pains, nosebleed, oral cavity infections, overweight, pimples, pneumonia, polycythemia, poor digestion, rhagades, rheumatism, stomachache, sty, sore throat, toothache, urinary tract diseases, warts, water retention, whitlows, wounds.
Spain	*Allium cepa* *Allium sativum*	Bulb, leaves	skin diseases, sinusitis, cold, bronchitis, pneumonia, asthma, diarrhea, wounds and cuts.
France	*Allium cepa*	Bulb, leaves	Flu syndrome
Iran	*Allium akaka, Allium tripedale, Allium iranicum, Allium stipitatum, Allium atroviolaceum,*	Bulbs, leaves, whole plant	diabetes, hyperlipidemia, toothache, frequent colds, scorpion bites, kidney stones, pains in the digestive system, rheumatism, respiratory problems particularly cough, constipation, prevention hair to get white and etc.

	Allium cepa, *Allium jesdianum,* *Allium sativum*		
Turkey	*Allium cepa* *Allium sativum* *Allium ampeloprasum,* *Allium Scorodoprasum*	Bulbs, whole plant	Asthma, Cancer, Head ache, Hypertension, Diabetes, Wound healing, Cold, Infertility, sugar management, cardiotonic, for loss of appetites, blurred vision, gastrointestinal diseases, renal colic, menstrual pain.
Georgia	*Allium cepa* *Allium sativum*	Bulbs, whole plant	nose bleeding, cancer, ulcer, stomachache, snake and dog bite, cough, flu, cold, antiseptic and antibacterial.

1.4. Chemical composition of species of genus *Allium*

Biological activity and health-beneficial properties of the *Allium* species are associated with their diverse biologically active compounds, such as steroidal saponins, flavonoids, sulphuric compounds, polysaccharides, micro and macroelements and etc [38], [39].

The discovery of diallyl disulfide (DADS) and a small amount of diallyl trisulfide (DATS) in garlic by Weitheim in 1844 marked the beginning of the identification of more than 90 kinds of organosulfur compounds (OSCs) from the *Allium* genus. These compounds can be classified as chain disulfide ether and trisulfide ether. The side chains of these compounds feature symmetrical or mixed methyl, propyl, propylene, and pro propyl substituents, with the main constituents of methyl allicin, DADS, ajeone, and allicin[40]. Sulfuric compounds cause characteristic smell and taste.

Content of sulfuric compounds in Garlic is higher than in other species[41]. Garlic contains 2.3% of all sulfuric compounds used in medicine. Aliicin (allicin) was isolated from *A. sativum* in 1940 by scientists Cavallito and Bailey, they have discovered that this compound had an antibacterial activity against gram-positive and gram-negative strains (Figure 1) [42]. Allicin is formed from Alliin through the action of enzyme alliinase in garlic, allicin is an odorless compound that can be found in various quantities in the bulbs. Due to its instability, different

data is given in the literature about its quantity, for example: 3.7 mg/g, 20.73-24.31 mg/g, 37 mg/g and etc[43]–[45].

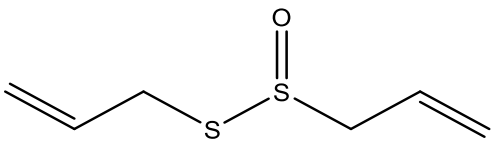


Figure 2. Structure of Aliicin

Main metabolites of Aliicin are diallyl sulfide, diallyl di-, tri- tetrasulfide and Sulfur dioxide. Aliicin exhibits hypolipidemic, antiplatelet, and procirculatory effects and includes antibacterial, anticancer and chemopreventive activities[46]. Despite undoubtedly many health benefits, garlic intake is not recommended in patients with blood clotting disorders and with certain drugs metabolized by CYP 3A4[47]. It should be noted, that all sulfuric compounds isolated from *Allium* species cannot be used in medicine, for example propyl propane thiosulfinate (PTS) present in the *Allium* species in *in vitro* assays has shown genotoxicity with high doses and induced DNA damage in Caco-2 cells [48], [49]. Taking large doses of *A. sativum* is also accompanied by other negative phenomena such as allergic reactions and an unpleasant odor [50].

Allium species also contain: γ-l-glutamyl-S-allyl-l-cysteine GSAC, γ-l-glutamyl-S-(trans-1-propenyl)-l-cysteine GSPC, γ-l-glutamyl-S-methyl-l-cysteine GSMC, γ-glutamyl phenylalanine γGPA and etc. studies have shown that these compounds possess antioxidant activity[51], [52].

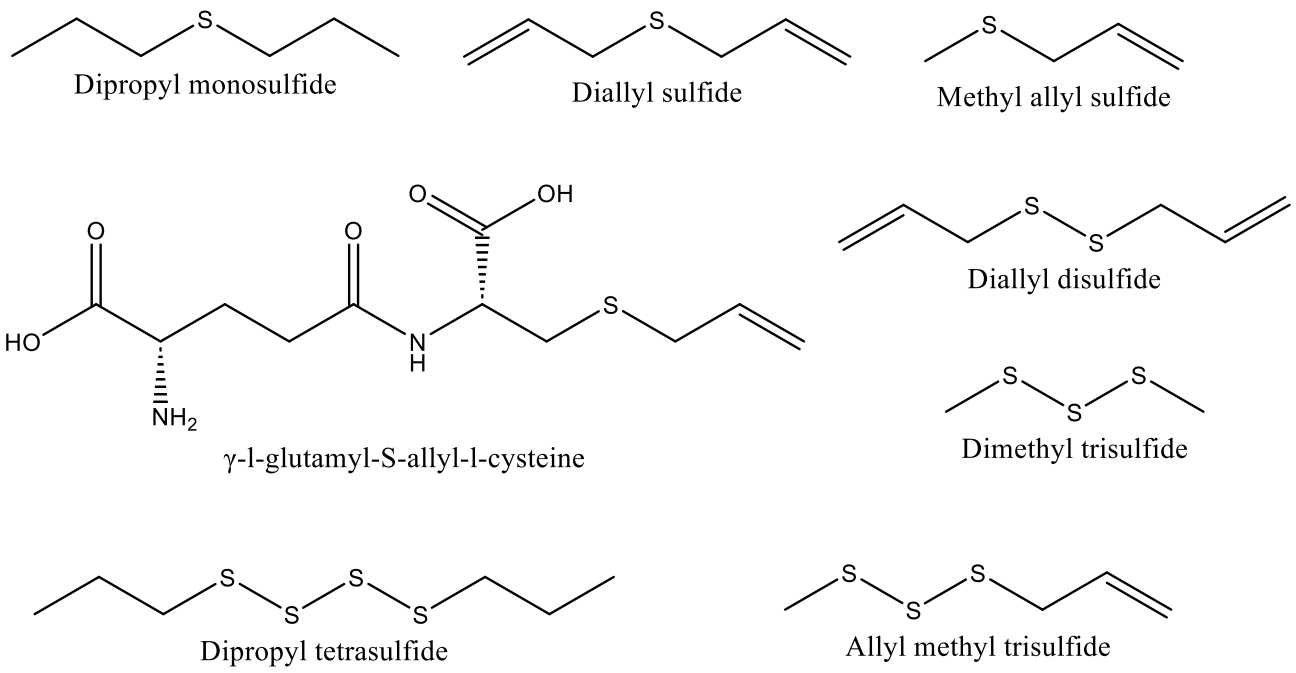


Figure 3. Structure of some major sulfuric compounds.

Table 3. Sulfuric compounds isolated from genus *Allium*[53].

1	Dipropyl monosulfide	22	Diallyl trisulfide (DATS)	43	Garlicnin B3
2	Methyl allyl sulfide	23	Di-1-propenyl trisulfide	44	Garlicnin B4
3	Diallyl sulfide	24	Dimethyl tetrasulfide	45	Garlicnin K1
4	Dimethyl disulfide	25	Dipropyl tetrasulfide	46	Garlicnin K2
5	Methyl-propyldisulphide	26	Allicin	47	Garlicnin C1
6	Pentyl hydrodisulfide	27	S-allyl cysteine	48	Garlicnin C2
7	Methyl pentyldisulfide	28	S-allyl cysteine sulfoxide	49	Garlicnin C3
8	Dipropyl disulfide	29	S-allylmercaptocysteine	50	Garlicnin A
9	Methyl-1-propenyl disulfide	30	2-vinyl-4H-1,3-dithiin	51	Garlicnin I
10	Allyl methyl disulfide/ Methyl-2-propenyl disulfide	31	1,2-vinyldithiin	52	Welsonin A1
11	1-propenyl propyl disulfide/ Cis-propenyl propyl disulfide	32	Allixin	53	Welsonin A2
12	Trans-propenyl propyl disulfide	33	Xanthiazone	54	Garlicnin J
13	Allyl propyl disulfide	34	Xanthiside	55	Garlicnin G

14	Diallyl disulfide	35	2-hydroxyxanthiside	56	Garlicnin L-1
15	Methyl-1-(methylthio) ethyl disulfide	36	Entadamide A-Î²-D-glucopyranoside	57	Garlicnin L-2
16	Methyl-1-(methylthiopropyl) disulfide	37	Glucoerucin	58	Garlicnin L-3
17	Dimethyl trisulfide	38	Onionin A1	59	Garlicnin L-4
18	Methyl propyl trisulfide	39	Onionin A2	60	E-Ajoene
19	Dipropyl trisulfide	40	Onionin A3	61	Z-Ajoene
20	Methyl-1-propenyl trisulfide	41	Garlicnin B1	62	Garlicnin E
21	Allyl methyl trisulfide	42	Garlicnin B2	63	Garlicnin F

The polyphenolic compounds in *Allium* species mainly consists of flavonoids, phenolic acids, lignans and anthocyanins[54]. Flavonoids are derivatives of a phenyl-substituted propylbenzene possessing a C15 skeleton (C6-C3-C6), their structure consists of f two phenyl rings (A and B) and a heterocyclic ring (C) [55]. Flavonoids are divided in several classes: flavones, flavonols, dihydroflavonols, flavanones, anthocyanidins, aurones, based on the degree of unsaturation and oxidation of the C-ring (Figure 4).

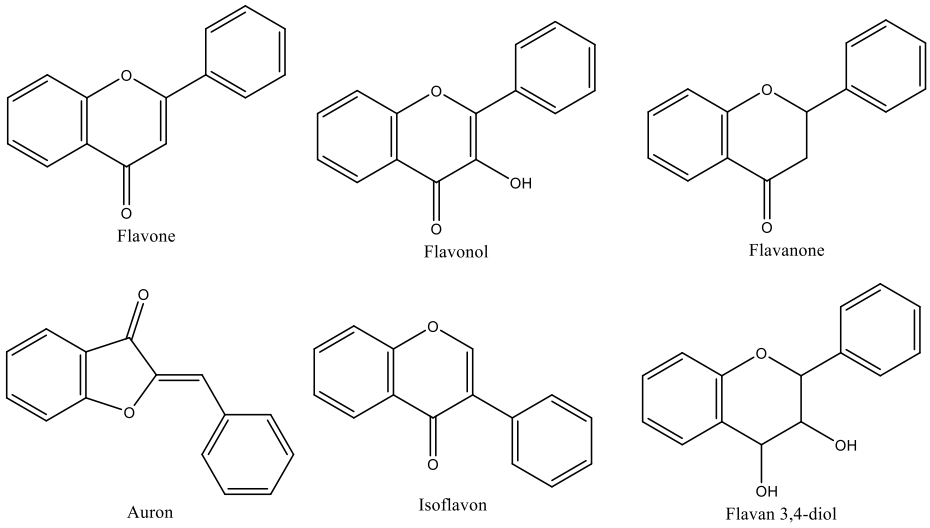


Figure 4. General structures of flavonoids.

These classes are divided into subclasses depending on characteristic and amount of substituents[55], [56]. Flavonols and anthocyanins are the main subclasses of flavonoids present in onions[57]. Total flavonoid content in *Allium* species is very different and varies from 4 mg/kg to 428 mg/kg[58]–[61]. Quercetin, kaempferol, isorhamnetin, myricetin, fisetin, and morin are the major flavonol aglycone representatives in *Alliums* (Figure 5).

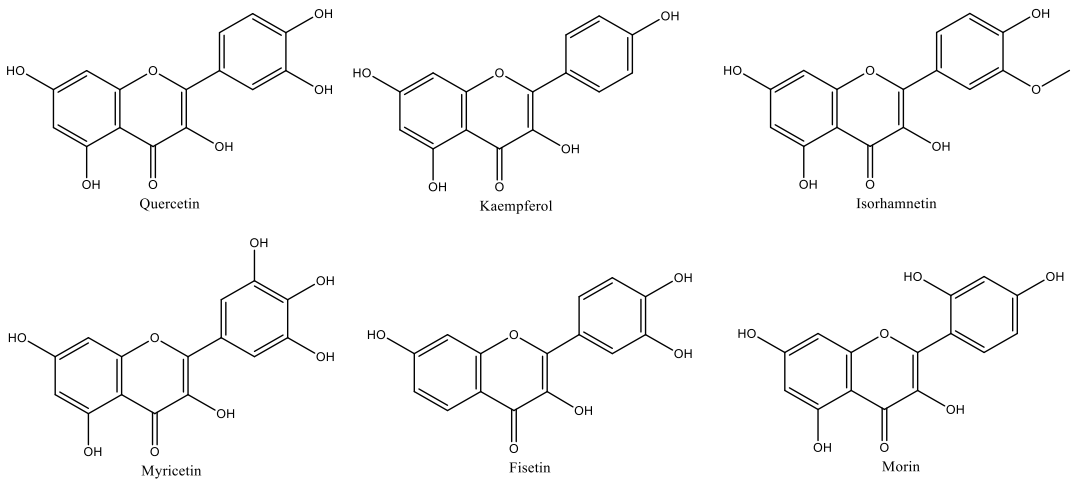


Figure 5. Structure of major flavonol aglycones in *Allium* species.

Diverse kinds of flavonoids have been isolated from *Allium* species (Table 4). Most of these flavonols exist as their glycosylated derivatives in nature, where sugars are attached through oxygen at 3, 4′, and/or 7-positions of the aglycones. Glucose is the most common sugar moiety; however, rhamnose, galactose, xylose, and glucuronic acid have also been identified[62].

Table 4. Flavonoids isolated from the species of genus *Allium*.

N	Flavonoids	Plant species	Source
1	Quercetin	*A. cepa* L.	[63]
2	Quercetin-3-O-Glucoside	*A. cepa* L., *A. sativum* L., *A. flavum* L., *A. macrostemon* Bunge	[63]–[67]
3	Quercetin-4′-O-Glucoside	*A. cepa* L.	[64]
4	Quercetin-3,4′-O-Diglucoside	*A. cepa* L., *A. tuberosum* Rottler ex Spreng	[63], [64], [68]

5	Quercetin-3-O-Rutinoside	*A. cepa* L., *A. chinense* G.Don	[63], [69]
6	Quercetin-7-O-Glucoside	*A. cepa* L.	[63]
7	Quercetin-7-O-Rhamnoside	*A. cepa* L.	[63]
8	Quercetin-7,4′-O-Diglucoside	*A. cepa* L.	[63]
9	Quercetin-3,7-O-Diglucoside	*A. cepa* L.	[63]
10	Quercetin-3,7,4′-O-Triglucoside	*A. cepa* L.	[1], [2]
11	Quercetin-3-O-Rhamnoside	*A. cepa* L., *A. fistulosum* L.	[63]
12	Quercetin	*A. cepa* L.	[63]
13	4′-glucoside of quercetin dimer	*A. cepa* L.	[63]
14	Quercetin trimer	*A. cepa* L.	[63]
15	Quercetin sophoroside glucuronide	*A. tricoccum* Aiton	[70]
16	Quercetin hexoside glucuronide	*A. tricoccum* Aiton	[70]
17	Quercetin sophoroside	*A. tuberosum* Rottler ex Spreng	[71]
18	Quercetin-3-O-β-D-xylopiranoside	*A. sativum* L.	[66]
19	Kaempferol	*A. cepa* L., *A. tuberosum* Rottler ex Spreng	[68], [72], [73]
20	Kaempferol-3-O-Glucoside	*A. cepa* L., *A. sativum* L., *A. flavum* L., *A. ursinum* L., *A. macrostemon* Bunge	[63], [65]–[67]
21	Kaempferol-4′-O-Glucoside	*A. cepa* L.	[63]
22	Kaempferol-7,4′-O-Diglucoside	*A. cepa* L.	[63]
23	Kaempferol-7-O-Glucoside	*A. triquetrum* L.	[74]
24	Kaempferol-3,4′-O-Diglucoside	*A. cepa* L., *A. tuberosum* Rottler ex Spreng, *A. macrostemon* Bunge	[63], [67], [68]
25	Kaempferol-3,7-di-O-rhamnoside	*A. roseum* L.	[75]
26	Kaempferol-3,7-di-O-Glucoside	*A. macrostemon* Bunge	[67]
27	Kaempferol-3-O-glucuronide-7-O-rhamnosyl glucoside	*A. roseum* L.	[76]
28	Kaempferol-3-O-Rutinoside	*A. roseum* L.,	[68], [74], [76]

		A. tuberosum Rottler ex Spreng, *A. triquetrum* L.	
29	Kaempferol–3-O-glucoside –7-Oglucuronide	*A. roseum* L.	[76]
30	Kaempferol-7-O-glucuronide	*A. roseum* L.	[76]
31	Kaempferol-3-O-glucuronide	*A. roseum* L.	[76]
32	Kaempferol-7-O-(6”-malonyl)-glucoside	*A. roseum* L.	[76]
33	Kaempferol-3-O-sophoroside	*A. tuberosum* Rottler ex Spreng, *A. tricoccum*	[68], [70]
34	Kaemferol-3-O-β-D-glucosyl-(1→2)-O-α-L-xylopyranoside	*A. tuberosum* Rottler ex Spreng	[68]
35	3-O-β-D-(2-O-feruloyl)-glucosyl-7,4′-di-O-β-D-glucosyl kaempferol	*A. tuberosum* Rottler ex Spreng	[68]
36	3-O-β-sophorosyl-7O-β-D-(2-O-feruloyl)-glucosyl kaempferol	*A. tuberosum* Rottler ex Spreng	[68]
37	kaempferol 3-O-β-neohesperidoside	*A. ursinum* L.	[77]
38	kaempferol 3-O-β-neohesperidoside-7-O-[2-O-(trans-p-coumaroyl)]-β-D-glucopyranoside,	*A. ursinum* L.	[77]
39	kaempferol 3-O-β-neohesperidoside-7-O-[2O-(trans-feruloyl)]-β-D-glucopyranoside,	*A. ursinum* L.	[77]
40	kaempferol 3-O-β-neohesperidoside-7-O-[2-O-(trans-p-coumaroyl)-3-O-β-D-glucopyranosyl-1-β-D-glucopyranoside	*A. ursinum* L.	[77]
41	Kaempferol 3-O[2-O-(trans-3-methoxy-4-hydroxycinnamoyl)-β-D-galactopyranosyl]-(1→4)-O-β-D-glucopyranoside	*A. porrum* L.	[78]
42	Kaempferol 3-O-[2-O-(trans3-methoxy-4-hydroxycinnamoyl)-β-D-glucopyranosyl]-(1→6)-O-β-D-glucopyranoside	*A. porrum* L., *A. triquetrum* L.	[74], [78]
43	Kaempferol-3-O-(2-O-trans-p-feruloyl) Glucoside	*A. triquetrum* L.	[74]
44	8-Hydroxy Kaempferol 8-O-Glucoside	*A. triquetrum* L.	[74]
45	kaempferol 3-O-[2-O-(trans-p-coumaroyl)-3-O-β-D-glucopyranosyl]-β-D-glucopyranoside	*A. triquetrum* L.	[74]
46	Isorhamnetin	*A. cepa* L.	[79]
47	Isorhamnetin-4′-O-Glucoside	*A. cepa* L.	[63], [64]
48	Isorhamnetin-3-O-Glucoside	*A. cepa* L., *A. vineale* L., *A. macrostemon* Bunge	[63], [67], [80]

49	Isorhamnetin-3,4′-O-Glucoside	*A. cepa* L.*;* *A. tuberosum* Rottler ex Spreng	[63], [64], [71]
50	Isorhamnetin-4′-O-galactoside	*A. cepa* L.	[64]
51	Myricetin	*A. cepa* L.	[72]
52	Fisetin	*A. cepa* L.	[72]
53	Morin	*A. cepa* L.	[81]
54	Cyanidin 3-glucoside	*A. cepa* L.	[82]
55	Cyanidin 3-laminaribioside	*A. cepa* L.	[82]
56	Cyanidin 3-(6”-malonylglucoside)	*A. cepa* L.	[82]
57	Cyanidin 3-malonyl-laminaribioside	*A. cepa* L.	[82]
58	Cyanidin 3-dimalonylaminaribioside	*A. cepa* L.	[82]
59	Delphinidin 3,5-diglycoside	*A. cepa* L.	[83]
60	Cyanidin 3,5-diglycosides	*A. cepa* L.	[83]
61	Cepaflava A,B	*A. cepa* L.	[84]
62	Cepadial A-D	*A. cepa* L.	[84]
63	Cepabifla A-C	*A. cepa* L.	[84]
64	Dasuanxinoside F, G, H	*A. sativum* L.	[85]
67	quercetin-3-O-(6-trans-feruloyl)-b-D-glucopyranosyl-(1/2)-b-D-glucopyranoside-7-O-b-D-glucopyranoside	*A. tuberosum* Rottler ex Spreng	[86]
68	kaempferol-3-O-(6-trans-feruloyl)-b-D-glucopyranosyl-(1/2)-b-D-glucopyranoside-7-O-b-D-glucopyranoside.	*A. tuberosum* Rottler ex Spreng	[86]
69	quercetin-3-O-(6-trans-p-coumaroyl)-b-D-glucopyranosyl-(1 / 2)-b-D-glucopyranoside-7-O-b-D-glucopyranoside	*A. tuberosum* Rottler ex Spreng	[86]

Steroidal saponins are an important chemical component in *Allium* plants, recently many new steroidal saponins have been isolated from these species.

Steroidal saponins from *Allium* species can be divided into three groups, according to their structure: Spirostan, Furostan and Open-chain (Figure 6). Open-chain saponins are called also Cholestan saponins[87], [88].

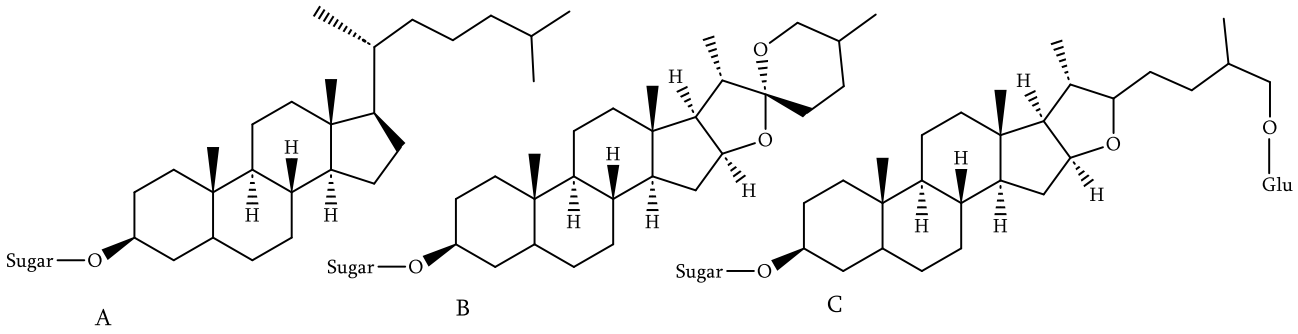


Figure 6. Classification of saponins. A-Cholestan Saponin. B-Spirostan Saponin. C-Furostan Saponin.

Steroidal saponins from *Allium* species mostly belong to mono- and bisdesmoside class saponins. Monodesmosidic saponins have a single sugar chain attached at the C-3 position, while bidesmosidic saponins have an additional sugar chain at the C-28 (for triterpenoid saponins) or C-26 (steroid saponins) position [89]. Tridesmoside saponins, having sugar chain at the C-1 position, are very rare and are only isolated from the bulbs of *A. macleanii* [90]. saponins of *Allium* spp. are mainly composed of linear or branched glucose (Glc), rhamnose, galactose (Gal), xylose, and arabinose units. The most common spirostanol sapogenins identified in *Allium* plants are: diosgenin, tigogenin, gitogenin, agigenin, alliogenin, and β-chlorogenin[91]. Steroidal saponins isolated from *Allium* species are given in Table 4.

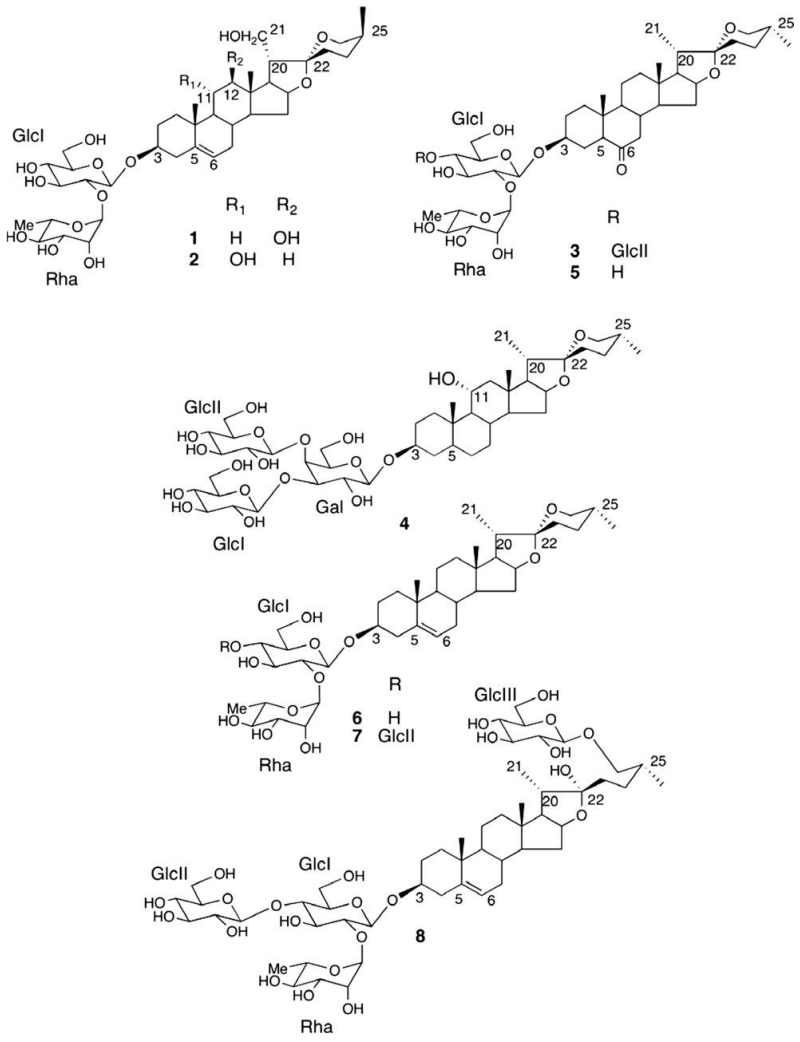


Figure 7. Structure of saponins isolated from the plants genus *Allium.*

Saponins, flavonoids, sulfuric compounds and many other classes of compounds are isolated from *Allium* species, from Georgia (Table 5).

Table 5. Steroidal saponins form *Allium* species.

Specie	Saponin	Source
A. ampeloprasum L.	Persicoside A-E; Ceposides A; Ceposides C; Tropeosides A, B; Ascalonicoside A (3β,5α,6β,25R)-3-{(O-β-d-glucopyranosyl-(1→3)-β-d-glucopyranosyl-(1→2)-O-[O-β-d-glucopyranosyl-(1→3)]-O-β-d-glucopyranosyl-(1→4)-β-d-galacto-pyranosyl)oxy}-6-hydroxyspirostan-2-one	[92] [93]
A. cepa L.	Ceposide A-C; Ceparocide E-L; Alliospiroside A, B	[94], [95] [96]
A. flavum L.	(20S,25R)-2α-hydroxyspirost-5-en-3β-yl-O-β-D-xylopyranosyl-(1→3)-[β-D-galactopyranosyl-(1→2)]-β-D-galactopyranosyl-(1→4)-β-D-galactopyranoside (20S,25R)-2α-hydroxyspirost-5-en-3β-yl-O-β-D-xylopyranosyl-(1→3)-[β-D-glucopyranosyl-(1→2)]-β-D-galactopyranosyl-(1→4)-β-D-galactopyranoside (20S,25R)-spirost-5-en-3β-yl O-α-L-rhamnopyranosyl-(1 → 4)-[β -D-glucopyranosyl-(1→2)]-β-D-glucopyranoside	[97]
A. sativum L.	Voghieroside A1/A2-E1/E2	[98]

A. schoenoprasum L.	(20S,25S)-spirost-5-en-3β,12β,21-triol_3-O-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranoside. (20S,25S)-spirost-5-en-3β,11α,21-triol_3-O-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranoside. laxogenin 3-O-α-L-rhamnopyranosyl-(1→2)-[β-D-glucopyranosyl-(1→4)]-β-D-glucopyranoside. (25R)-5α-spirostan-3β,11α-diol 3-O-β-D-glucopyranosyl-(1→3)-[β-D-glucopyranosyl-(1→4)]-β-D-galactopyranoside.	[99]
A. tuberosum Rottl. ex Spreng	(24S,25S)-5β-spirostan-2α,3β,5,24-tetraol 3-O-α-L-rhamnopyranosyl-(1→2)-O-[α-L-rhamnopyranosyl-(1→4)]-β-D-glucopyranoside; Allituberoside A-O; (25S)-5β-spirostan-2β,3β-diol 3-O-β-D-glucopyranoside (25S)-5β-spirostan-2β,3β,19-triol 3-O-β-D-Glucopyranoside (25S)-5β-spirostan-2β,3β-diol 3-O-α-L-rhamnopyranosyl-(1→4)-O-b-D-glucopyranoside	[100] [101] [102]
A. vavilovii M. Popov	Vavilosides A1/A2-B1/B2	[103]
A. ramosum L.	Ramofurosides A–C; (25R)-26-O-β-D-glucopyranosylfurost-5-ene-2α,3β,22α,26-tetraol-3-O-β-D-glucopyranosyl-(1→2)-β-D-glucopyranosyl -(1→4)-β-D-galactopyranoside	[104] [105]
A. chinense G.Don	(25R)-5α-spirostan 3-O-{O-(4-O-acetyl-α-L-arabinopyranosyl)-(1→6)-β-D-glucopyranoside; (25R)-3β-hydroxy-5β-spirostan-6-one3-O-β-D- xylopyranosyl(1→4)-[α-L-arabinopyranosyl-(1→6)]-β-D-glucopyranoside;	[106]

	(25R)-3β-hydroxy-5α-spirostan-6-one3-O-{[O-β-D-glucopyranosyl-(1→3)-O-β-D-xylopyranosyl]-(1→4)-O-[α-L-arabinopyranosyl-(1→6)]}-β-D- glucopyranoside; (25S)-3β,24β-dihydroxy-5α-spirostan-6-one3-O-[α-L-arabinopyranosyl(1→6)]-β-D-glucopyranoside (25S)-24-O-β-D-glucopyranosyl-3β,24β-dihydroxy-5α-spirostan-6-one (25R)-5α-spirostan-3β-yl-3-O-acetyl-O-β-D-Glucopyranosyl -(1→2)-O-[β-D-glucopyranosyl-(1→3)]-O-β-D-glucopyranosyl-(1→4)-β-D-galactopyranoside 5α-spirostane 25(27)-ene-2α,3β-diol-3-O-{O-β-D-glucopyranosyl-(1→2)-O-β-D-glucopyranosyl-(1→4)-β-D-galactopyranoside} 26-O-β-D-glucopyranosyl 3β,22α,26-trihydroxy-25(R)-5α-furostan-6-one 26-O-β-D-glucopyranosyl 3β,22α,26-trihydroxy-25(R)-5α-furostan-6-one 3-O-β-D-glucopyranoside 26-O-β-D-glucopyranosyl 3β,26-dihydroxy-25(R)-5α-furostan-20(22)-en-6-one	[107]

A. ascalonicum L.	3β-O-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl-19α-hydroxyolean-12-ene-28-oic acid 28-O-[α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl] ester 3-O-β-D-glucopyranosyl-(1→3)-[α-L-rhamnopyranosyl-(1→2)]-α-L-arabinopyranosyl-3β,19α-dihydroxyoleanane-12-en-28-oic acid	[108]
A. fistulosum L.	(25R)-26-[(β-Dglucopyranosyl)oxy]-3β,22β-dihydroxyfurost-5-en-1β-yl O-α-Lrhamnopyranosyl-(1→4)-O-α-L-rhamnopyranosyl-(1→4)-β-Dglucopyranoside (25R)-26-[(β-D-glucopyranosyl)oxy]-3β-hydroxyfurost-5,20-dien-1βyl O-α-L-rhamnopyranosyl-(1→4)-O-α-L-rhamnopyranosyl- (1→4)-β-D-glucopyranoside (25R)-26-[(β-D-glucopyranosyl)oxy]-3β,22β-dihydroxyfurost-5-en-1β-yl O-α-L-rhamnopyranosyl-(1 → 4)-O-β-D-glucopyranoside (25R)-26-[(β-D-glucopyranosyl)oxy]-3β-hydroxyfurost-5,20-dien-1β-yl O-α-L-rhamnopyranosyl-(1 → 4)-β-D-glucopyranoside	[109]
A. porrum L.	(25R)-5α-spirostane-3β,6β-diol-2,12-dione 3-O-{β-D-glucopyranosyl-(1→3)-β-D-glucopyranosyl-(1→2)-[β-D-xylopyranosyl-(1→3)]-β-D-glucopyranosyl-(1→4)-β-D-galactopyranoside	[9], [110]

	(25R)-5α-spirostan-3β,6β-diol-12-one 3-O-β-D-glucopyranosyl-(1→2)-[β-D-fucopyranosyl-(1→3)]-β-D-glucopyranosyl-(1→4)-β-D-galactopyranoside	

Table 6. Compounds isolated from *Allium* species, growing in Georgia.

Species	Isolated compounds	Source
A. affine Ledeb.	Diosgenin; Tigogenin; Ruscogenin	[111]
A. albidum Fisch.	Diosgenin; Ruscogenin; Hecogenine	[112], [113]
A. atroviolaceum Boiss	Atroviolaceoside; Atroviolacegenin;	[114]
A. erubescens K. Koch	Eruboside A; Eruboside B; β-chlorogenin;	[11]
A. fuscoviolaceum Fomin	Diosgenin;	[13]
A. gramineum K. Koch	isorhamnetin-3-O-β-D-glucopyranoside; diosgenin-3-O-α-rhamnopyranosyl-(1→2)-β-D-glucopyranoside (Prosapogenin A of dioscin); Deltonine; β-sitosterol 3-O-β-glucopyranoside; Quercetin 3-O-β-glucopyranoside; Isorhamnetin 3,4'-di-O-β-glucopyranoside; Isorhamnetin 3,7-di-O-β-glucopyranoside ; Eruboside B;	[112], [115], [116]

A. leucanthum K. Koch	Diosgenin; β-chlorogenin; Yucagenin; Agigenin; Yayiosaponin C; Eruboside B; Aginoside; Leucospiroside A; Leucospiroside B; (25R)-5α-spirostane-2α,3β,6β-triol-3-O-β-D-glucopyranosyl-(1--2)-O-β-D-glucopyranosyl-(1--4)-β-D-galactopyranoside; (25R)-5α-spirostane-3β,6β-diol-O-β-D-glucopyranosyl-(1->3)-O-β-D-xylopyranosyl-(1->2)-O-β-D-glucopyranosiyl-(1->4)-O-β-D-glactopyranoside; (25R)-5α-spirostane-3β,6β-diol-O-β-D-glucopyranosyl-(1->3)-O-β-D-glucopyranosyl-(1->2)-O-β-D-glucopyranosyl-(1->3)-O-β-D-glucopyranosyl-(1->4)-β-D-galactopyranoside;	[117]–[121]
A. paradoxum (M.Bieb.) G.Don	Dioscin related saponins; Astragalin; 2-Methoxy tyrosol;	[122], [123]
A. rotundum L.	Tigogenin; Diosgenin; Gitogenin; β-Chlorogenin; Yucagenin; Agigenin; Trillin; Dideglucoeruboside B; Aginoside; Eruboside B; Yayoisaponin C; Quercetin, Luteolin, Apigenin, Hyperin, Cinaroside, Apigenin-7-*O*-β-D-glucopyranoside, Scopoletin and Umbelliferone; β-Carotene, Violaxanthin, Flavoxanthin, Lutein, Rubixanthin and Zeaxanthin;	[10], [124]–[126]
A. rubellum M.Bieb.	Tigogenin;	[127]
A. ursinum L.	Allicin; Malondialdehyde (MDA); Carotenoids; Kaempferol-3,7-di-O-β-D-glucopyranoside; Kaempferol-(acetylhexoside)-hexoside; Acetyl-kaempferol-deoxyhexose propylene sulfide; Dimethyl disulfide; Dimethyl thiophene- (E)-methyl-2-propyl disulfide; (Z)-Methyl-2-propenyl disulfide; Dimethyl trisulfide; Di-2-propenyl disulfide; 2-Vinyl-1,3-dithiane; (E)-Propenyl propyl disulfide;	[128]

	(Z)-Propenyl propyl disulfide; Methyl-2-propenyl trisulfide; 3,4-Dihydro-3-vinyl-1,2-dithiine; 2-Vinyl-4H-1,3-dithiine; Dimethyl tetrasulfide; (E)-Di-2-propenyl trisulfide; (Z)-Di-2-propenyl trisulfide; Di-2-propyl trisulfide; Di-2-propenyl tetrasulfide; Inulin, Nystose and I-Ketose;	
A. victorialis L.	Allivictoside A–H; Allumine A and B; Cyclopent-1-enecarboxylate; Alliumonoate; β-Amyrin acetate; β-Sitosterol acetate, 22-Cyclohexyl-1-docosanol, β-Amyrin, β-Sitosterol, β-Sitosterol 3-O-b-D-glucopyranoside;	[129]–[131]
A. vineale L.	Diosgenin, Nuatigenin and Isonuatigenin; Ophiopogonin C; Deltonin and 7 other saponins; 2-Furaldehyde; (2E)-Hexenal; (3Z)-Hexenol; 2,4-Dimethylthiophene; Allyl methyl disulfide; Methyl (Z)-1-propenyl disulfide; methyl (E)-1-propenyl disulfide; Benzaldehyde; Dimethyl trisulfide; Diallyl disulfide; Allyl (Z)-1-propenyl disulfide; Allyl (E)-1-propenyl disulfide; 1-Propenyl propyl disulfide; Methyl (methylthio)methyl disulfide; Allyl methyl trisulfide; 4-Methyl-1,2,3-trithiolane; Methyl propyl trisulfide; Methyl (Z)-1-propenyl trisulfide; Methyl (E)-1-propenyl trisulfide; Dimethyl tetrasulfide; Allyl (methylthio)methyl disulfide; Diallyl trisulfide; Allyl (Z)-1-propenyl trisulfide; p-Vinylguaiacol; Allyl propyl trisulfide; 5-Methyl-1,2,3,4-tetrathiane; Methyl (methylthio)methyl trisulfide; Allyl methyl tetrasulfide; Allyl (methylthio)methyl trisulfide; 4-Methyl-1,2,3,5,6-pentathiepane;	[80], [132]

In addition to above-described metabolites, *Allium* species contain many other classes of biologically active compounds, such as: pigments, proteins, enzymes, amino acids, fatty acids, γ-glutamylpeptides, proteins, enzymes, mineral, and microelements and etc[133].

β-Carboline class alkaloids are also isolated from *A. sativum*: (3S)-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid, (1S,3S)-1-methyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid and (1R,3S)-1-methyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid[134]. *Allium* species contain following amino acids: tryptophan, threonine, isoleucine, leucine, lysine, methionine, phenylalanine, tyrosine, valine, histidine, aspartic acid, glutamic acid, glycine, proline and serine[135]. Plants of genus *Allium* are rich with microelements: calcium, iron, magnesium, phosphorus, sodium, zinc, copper, manganese and selenium[135]. They also contain ergosterol, brassicasterol, stigmasterol, campesterol, β-sitosterol[136].

1.5. Biological activity of species of genus *Allium* and medicinal products

Secondary metabolites of species of genus *Allium* are characterized with various biological activities, such as: cytotoxicity, antioxidant, anti-inflammatory, antimicrobial, antibacterial, antidiabetic, antihelminthic, anti-atherosclerotic, wound healing, anti-hypercholesterolemic, analgesic, neuroprotective, gastroprotective and etc.

1.5.1. Cytotoxic activity

Cytotoxic activity of secondary metabolites of *Allium* species is found in results of many *in vitro* and *in vivo* experiments. Cytotoxic and antiproliferative activity of sulphur compounds, polyphenols, flavonoids, saponins isolated from *A. cepa* has been determined against MCF-7, MDA-MB231, HL-60, HepG2, HT29, and PC3 cells [137]–[139]. Meta-analysis of 18 studies (142 921 subjects) demonstrated that high garlic consumption was associated with a reduced gastric cancer risk[140]. Also, studies have found that anticancer activity of garlic extracts may be associated with increase of both NK cell number and NK cell activity[141]. The spirostane-type glycosides isolated from the whole plant of *Allium flavum*, were evaluated for cytotoxicity against a human cancer cell line (colorectal SW480) and showed moderate cytotoxicities with IC_{50} values

of 14.3, 14.0 and 18.1 μM, respectively[142]. Spirostane-type saponines isolated from *A. schoenoprasum* are characterized with cytotoxic activity against HCT 116 and HT-29 human colon cancer cell lines, with IC_{50} values of 2.75 and 2.06 nM[99]. Vavilosides A1/A2–B1/B2 isolated from *A. vavilovii* showed cytotoxic activity on J-774, murine monocyte/macrophage, and WEHI-164, murine fibrosarcoma, cell lines[103]. Important cytotoxic activity is described in sum of saponins from *A. chinense*, they have inhibited the proliferation of B16 and 4T1 cells in a dose-dependent manner. Also, they have inhibited the growth of melanoma *in vivo* [143]. The ethanol extracts of dry and fresh *A. ascalonicum* bulb were prepared and tested for *in vitro* cytotoxic efficacy on liver cancer cell line HepG2, extracts have anticancer potential with inhibitory concentration IC_{50} of 50mg/mL[144].

Cytotoxic activity of fractions and compounds isolated from *Allium* species was discussed in many experimental articles. Khazaei et al. evaluated the cytotoxic activity of bulbs of *A. atroviolaceum* in MCF7 and MDA-MB-231, HeLa and HepG2 cell lines. The MTT cytotoxicity assay exhibited different growth responses in MCF7, MDA-MB-231, HeLa, and HepG2 cells. The IC_{50} values after 24, 48, and 72 h of treatment were 91.5, 88, and 75.7 μg/ml for MCF-7 cells, 149, 114, and 101 μg/ml for MDAMB-231, 154, 89.7, and 74.7 μg/ml for HeLa cells and 97, 70, and 58.7 μg/ml for HepG2, respectively[145]. In the study, the anticancer effect of 4′,5,7-Trihydroxy-3′,5′-dimethoxyflavone (Tricin) was investigated with docetaxel on PC3 cell line. Tricin was initially isolated from the *A. atroviolaceum.* IC_{50} of tricin and docetaxel were assessed at 117.5 ± 4.4 μM and 0.1 ± 0.02 nM by MTT assay, respectively[146].

The 80% EtOH extract of the flowers of *Allium gramineum* has been shown to strongly inhibit the growth of breast adenocarcinoma cell lines, with an IC_{50} of 4.5 ± 0.7μg/mL for MDA-MB-231 and 4.8 ± 0.9μg/mL for MCF-7 cells. The cytotoxic activity was related to the saponins which exhibited a potent cytotoxicity, with an IC_{50} around 3μM[116]. The cytotoxic activities of 7 glycosides extracted from *Allium leucanthum* were evaluated against A549, DLD-1. According to the results, compounds 1-3 and 5 possess a relatively similar cytotoxicity against both tumor cell lines, with IC_{50} values ranging from 3.7 to 5.8 μM for A549 and 5.6 to 8.2 μM for DLD-1[118].

Demir et al. (2022) have also determined that the extract of *A. scorodoprasum* has cytotoxic activity. The amounts of the *A. scorodoprasum* L. extract inhibiting the 50% growth of MCF-7 and MG-63 cells were recorded as 82.78 and 76.53 μg/mL, respectively[147].

Cytotoxicity of the extract of *A. ursinum* was evaluated in the research by Korga et al. (2019) on MKN28 and MKN74 cell lines. The extract of *A. ursinum* was toxic for both cell lines with a 66.77 ± 3.00% viability and a 31.55 ± 2.04% viability respectively[148].

The apoptosis-inducing capabilities of the extracts of *A. victorialis* was evaluated by the 3-(4,5-dimethyl-2-thiazolyl)- 2,5-diphenyl-2H-tetrazolium bromide assay, 4',6-diamidino-2-phenylindole staining, and the DNA fragmentation assay in human colon cancer HT-29 cells, extracts have reduced cell viability in a dose-related manner and induced apoptotic morphological changes and internucleosomal DNA fragmentation in HT-29 cells[149].

1.5.2. Antioxidant activity

Among the biological activities, described in *Allium* species, antioxidant activity is most important. Oxidative stress is characterized by an excess production of reactive oxygen species and reactive nitrogen species. These free radicals: NO, Superoxide anion, hydroxyl radical and hydrogen peroxide damage lipids, proteins and nucleic acids may trigger various chronic diseases[150]–[152].

Natural antioxidants are mainly derived from food and medicinal plants. These natural antioxidants from plant materials are mainly polyphenols (phenolic acids, flavonoids, anthocyanins, lignans and stilbenes), carotenoids (xanthophylls and carotenes) and vitamins (vitamin E and C) compounds which are inhibiting the initiation or propagation of oxidative chain reaction, acting as free radical scavengers, quenchers of singlet oxygen and reducing agents[153].

Allium species contain phenolic compounds, mostly flavonoids which are characterized with antioxidant activity[154]. The antioxidant activity of methanolic extract of *A. cepa,* determined using DPPH and ORAC tests shows that their activity directly depends on total flavonoid and total phenolic content[155]. Antioxidant activity is described also in *A. sativum*,

studies have shown that DPPH, ABTS, FRAP, and H2O2 scavenging activities of distilled water extract of aged garlic were higher than those of fresh garlic[156]. Alliin, isolated from *A. sativum*, is characterized with broad spectrum of antioxidant activity, which acts as an important inhibitor via controlling the generation of ROS and inhibiting the MAP kinase[157]. Studies have determined that, flavonoids from *A. sativum* increase activity of antioxidant enzymes[158]. Antioxidant activity of species *A. cepa, A. sativum, A. schoenoprasum, A. ursinum* corelates with their total polyphenol content[159].

Antioxidant activity is determined also in following species *A. schoenoprasum*[135], *A. hookeri*[51], *A. roseum*[160], *A. saralicum*[161], *A. ampeloprasum*[162], *A. mongolicum*[163] using DPPH, which can be explained with high total polyphenol content. DPPH test was used for evaluation of free radical scavenging activity of *A. affine* hydroalcoholic extract. RC_{50} for Vit C as a standard antioxidant was 43 μg/mL. The scavenging effect of the plant extract RC_{50} was found to be 201 μg/mL[164].

Antioxidant activity of isolated compound isorhamnetin-3-O-β-D-glucopyranoside from *Allium gramineum* was evaluated, with IC_{50} values of 20.1 ± 0.8μM[116].

Antioxidant activity of the aerial part and bulbs of *A. paradoxum* was investigated using *in vitro* assay systems by Ebrahimzadeh et al. The total phenolic content of aerial parts and bulbs was 62.7±3.5 and 7.4±0.2 mg gallic acid equivalent/g of extract, respectively and the total flavonoid contents of aerial parts and bulbs was 47.9±2.6 and 23.61±1.1 mg quercetin equivalent/g of extract powder, respectively. The aerial and bulb extracts of *A. paradoxum* exhibited good but different levels of antioxidant activity in all the models studied, both cell-free and in cell systems[165].

Assadpour et al. have investigated *in vitro* antioxidant and antihemolytic effects of the essential oil and methanolic extract of *A. rotundum*. IC_{50} for DPPH radical-scavenging activity were 284 ± 11.64 for methanol extract and 1264 ± 45.60 μg/ml for essential oil, respectively. The extract has shown better reducing effects versus essential oil. The extract also demonstrated better activity in nitric oxide-scavenging activity. IC_{50} were 464 ± 19.68 for extract and 1093 ± 38.25 μg/ml for essential oil. The extract shows better activity than essential oil in Fe^{2+} chelating system.

IC_{50} were 100 ± 3.75 for extract and 1223 ± 36.25 μg/ml for essential oil. The *A. rotundum* extract and essential oil showed significant H_2O_2 scavenging effects at dose-dependent manners in H_2O_2 induced hemolysis[166].

The research of Motamed et al. has showed that among studied 10 plants (*Allium paradoxum*, *Allium rubellum*, *Foeniculum vulgare*, *Mentha longifolia*, *Origanum vulgare*, *Prunus divaricata*, *Rubus sanctus*, *Rumex tuberosus*, *Satureja mutica* ϙ *Spinacia turkestanica*) all the extracts had moderate inhibitory activity against deoxyribose (DR) damage. but *A. rubellum* showed the highest DR degradation inhibitory activity (56.45 ± 1.56%)[167].

As a result of the analyses conducted, in the bulb and leaf parts of the *A. scorodoprasum*, total phenolic content values were 254.51-927.81 and 1929.05-19645.24 mg/kg, FRAP was 0.80-5.20 and 14.31-47.83-mM TE/g, DPPH free radical scavenger effect was 0.99-9.02 and 36.61-241.06 μmol TE/g and ascorbic acid content was 29.14-314.01 mg/kg and 200.64-1383.16 mg/kg, respectively. These data reveal that the leaf's of *A. scorodoprasum* is rich with antioxidants[168]. Antioxidant enzyme activity of *A. scorodoprasum* has showed following results: An increase in catalase activity, compared with A. *sativum*, was observed in *A. scorodorpasum* (36.8%)[169].

The antioxidant and anti-tyrosinase activity of different *A. ursinum* extracts and their metal complexes were evaluated using DPPH radical scavenging assay. The results showed that the polarity of extracting solvents and the solubility of the phenolic compounds in the solvents had a noticeable influence on the yield, the phenolic content, the antioxidant and anti-tyrosinase activity[170].

Total antioxidant activity of crude extract and isolated compounds of *A. vineale* and Trolox and α-tocopherol were determined by the ferric thiocyanate method in the linoleic acid system. The activities of 80 μg/ml concentration over the incubation period (20 h) of crude extract, four flavonoids, Trolox and α-tocopherol were found to be 64.8%, 79%, 75.6%, 82.2%, 75.7% and 31.4%, respectively[80].

1.5.3. Antibacterial activity

Secondary metabolites of genus *Allium* are indicated to have antibacterial activity against Gram-positive and Gram-negative bacteria such as *Staphylococcus, Streptococcus, Micrococcus, Enterobacter, Escherichia, Klebsiella, Lactobacillus, Pseudomonas, Shigella, Salmonella, Proteus, Helicobacter pylori* [171]. Studies have shown that, the extract of *A. sativum* fulfilled the criteria for its therapeutic use, against standard strain of *Pseudomonas aeruginosa*, with MIC of 600 µg/ml[172]. Antibacterial activities of four different concentrations of garlic extract (5%, 10%, 20%, and 100%) were evaluated against *Streptococcus mutans*, *Streptococcus sanguis*, *Streptococcus salivarius*, *Pseudomonas aeruginosa*, and *lactobacillus* spp. using the disk diffusion method, among them 5% extract proved to be useful[173]. *A. sativum* also possess effective anti-bacterial activity against multi-drug clinical pathogens, such as: *E. coli, P. aeruginosa, Proteus sp., S. aureus,* and *Bacillus*[174]. Studies have determined that combination of honey and garlic extract is effective against the strains of *Salmonella strain, S. aureus, Lyesria moncytogenes,* and *Streptococcus pneumoniae* [175]. Garlic extract has also antifungal activity against *Candida, Torulopsis, Trichophyton, Cryptococcus, Aspergillus, Trichosporon* and *Rhodotorula*[176]. Antifungal activity, against *B. cinerea* and *T. harzianum* strains, is described in furostanolic saponins, isolated from *A. sativum*[177]. Saponins with antifungal activity is isolated from *A. cepa* (Ceposide C and A) also, they have inhibitory effect against *B. cenerea, A. alternata, Phomopsis spp, Mucor spp, R. solani, T. harzianum* and *T. atroviride* strains[95]. *In vitro* antibacterial activity of the methanolic extracts of *A. cepa* was investigated on Gram-negative (*Escherichia coli* and *Pseudomonas aeruginosa*) and Gram-positive (*Staphylococcus aureus* and *Bacillus cereus*) respectively, by using a modified disc diffusion method. The highest zone of inhibition for gram positive bacteria was observed to be 13.5 ± 0.9 mm, for gram negative bacteria, the inhibition zone was found to be relatively smaller[178]. Studies have determined antibacterial and antifungal activity of *A. subhirsutum* L. Aqueous Extract, against *E. coli, P. aeruginosa, P. mirabilis, K. pneumoniae, P. mirabilis, S. sciuri, S. pyogens, P. aeruginosa, S. aureus, E. cloacae, S. paucimobilis, A. baumannii, C. albicans, C. neoformans, C. vaginalis, C. albicans, A. fumigatus,* and *A. niger* strains, the mean diameter of the growth inhibition zone (mm ± SD) varied from 6.00

± 0.00 to 15.66 ± 0.57[179]. Saponins, Persicosides A and B, isolated from *A. ampeloprasum* have also antifungal activity against *P. italicum, A. niger* and *T. harzianum* strains[180]. Antifungal activity has also the extract of *A. hirtifolium*[181]. The hydroalcoholic extract of A. tripedale is characterized with antifungal activity against *Candida* species, especially the extract exhibited the highest antimicrobial properties against *C. albicans* strains[182]. Flavonoids, Phenolic acids, amino acids and aother compounds isolated from *A. mongolicum* exhibits antifungal activity against *Fusarium solani, Valsa mali, Fusarium oxysporum, Fusarium sulphureum, Botrytis cinerea, Alternaria solani, Fusarium graminearum, Exserohilum turcicum, Corynespora cassiicola* strains[183].

Antibacterial effect of aqueous and alcoholic extracts of *A. atroviolaceum* was investigated, results has shown that After 48 hours of incubation, the minimum inhibitory concentration of aqueous and alcoholic extractions against *S. aureus* was 3.125 mg/ml and 6.25 mg/ml respectively. Also, the minimum inhibitory concentration results of aqueous and alcoholic extractions against *Escherichia coli* was 3.125 mg/ml and 12.50 mg/ml respectively[184]. The antimicrobial activities of the concentrated extract, obtained from *A. scorodoprasum*, was tested against different microorganisms that are important in terms of food technology (*S. aureus*; 20.00 mm, *E. faecalis*; 17.50 mm, *E. coli*; 14.00 mm, *A. niger*; 18.50 mm, *A. flavus*; 14.5 mm). MIC results showed that only *E. coli* presented a high resistance (7.5 mg/mL) against *A. scorodoprasum* L. extract. Among the selected bacteria and mold, *A. scorodoprasum* L. extract indicated the highest antibacterial activity against *S. aureus* and the highest antifungal activity against *A. niger*[147].

Results of the research of *Mskhiladze et al.* has demonstrated that the sum of steroidal saponins from *A. leucanthum* has moderate activity towards bacteria, but spirostanolic fraction has strong activity. Bacterial strains were resistant towards furostanol fraction. Glycosids of β-chlorogenine are characterized by stronger activity, than agigenin glycosids[185].

Phytochemical study of *A. paradoxum*, specially the saponin constituents of the plant, resulted in isolation and identification of a dioscin related steroidal saponin from bulbs of the plant by Rezaee et al. The leishmanicidal effects of the isolated compound was evaluated, which

has exhibited significant activity on promastigotes of *L. major* with both 10 and 50 μg/mL concentrations[123].

1.5.4. Antileishmanial and antiplasmodial activity

Antileishmanial activity was also studied in *A. leucanthum* by Mskhiladze et al. obtained results suggested that the spirostanolic and furostanolic fractions, extracted from the plant, have activity against *L. infantum* with 0.7 μg/ml and 0.9 μg/ml concentrations respectively[186].

The results of *in vitro* antiplasmodial activity demonstrated that the highest efficiency of *A. paradoxum* extract was at 80 μg/mL dose which led to a 60.43%-growth inhibition of parasites in culture, compared to the control groups. In case of 40 μg/mL concentration, growth inhibition was 52.48% in comparison with the control groups[187].

1.5.5. Anti-inflammatory activity

Inflammation is the immune system's response to harmful stimuli, such as pathogens, damaged cells, toxic compounds, to restore homeostasis. The inflammation is characterized by redness, swelling, heat, pain, and loss of tissue function[188]. These changes are caused by proinflammatory cytokines such as IL-1, IL-6, IL-8, TNF-α, G-CSF, GM-CSF, which play an important role in development of inflammation[189]. Many studies report anti-inflammatory activity of *Allium* species and compounds isolated from them[38].

Results of the research indicates that anti-inflammatory activity of *Allium* species are mostly related to tannins, anthocyanins, flavonoids, saponins and etc[190]. Studies have shown that extract of *A. cepa* decreases the expression of COX-2 gene in J774A.1 mouse macrophage cells, induced by LPS[191]. Also, hot-water extract of onion, decreases the production of proinflammatory cytokines IL-6, TNF-α, and IL-1β in in mice[192]. *in vivo* studies have demonstrated that onion extract declines oedema of mice paw, induced by carrageenan, this effect can be related to the inhibition of COX and LOX enzymes by the secondary metabolites of *Allium*

species[193]. Red onion extract inhibits also the activity of NF-κB, MARK and STAT-1, proinflammatory mediators[194]. Compounds obtained from *A. sativum*, express anti-inflammatory activity with inhibition of TNF-α, IL-1β, IL-6, MCP-1, and IL-12 cytokines[195]. Ethyl linoleate from garlic reduces lipopolysaccharide-induced pro-inflammatory cytokines nitric oxide (NO) and prostaglandin E2 production in RAW264.7 cells[196]. Results of *in vivo* and *in vitro* assays shows, that extracts from *A. schoenoprasum* leaves exert anti-inflammatory activities by inhibiting phagocytosis[197]. An *in vivo* test of the inhibition of carrageenan induced edema shows that the edema volume was reduced by 29.6% after treatment with the extract of *A. fistulosum*[198]. Same assay have shown the anti-inflammatory activity of *A. subhirsutum*[199]. Anti-inflammatory activity is described in secondary metabolites isolated from *A. hookeri,* they decrease the expression of pro-inflammatory cytokines IL-1β, IL-6, IL-13, and TNF-α, as well as they decrease the oedema induced by carrageenan[200]. *A. stipitatum* expresses anti-inflammatory activity via inhibition of COX-1 and COX-2 enzymes[201].

Woo et al. have investigated the inhibitory activities of isolated compounds (Allivictoside A–H (1–8)) from *A. victorialis* on neuroinflammation by measurement of produced NO levels in LPS-activated BV-2 cells, Allivictoside B and Allivictoside F from the leaves of *A. victorialis* significantly inhibited NO production in LPS-activated BV-2 cells. These results indicate that flavonoid derivatives from *A. victorialis* have anti-neuroinflammatory effects[129]. Anti-inflammatory activity of *A. scorodoprasum* extract is also studied. The extract concentration inhibiting the 50% activity LOX and XO were found as IC_{50} 9.75 and 9.71 mg extract/mL, respectively, while the quercetin and allopurinol IC_{50} values for LOX and XO were 1.22 and 2.69 mg/mL, respectively[147].

1.5.6. Effect on cardio-vascular diseases

Allium species are important for treatment of cardio-vascular diseases, flavonoids and other compounds isolated from these species, are widely used in cardio-vascular diseases[202]. studies have shown that, the extract of *A. sativum* have an antihypertensive effect[203].

Secondary metabolites from *A. sativum* stimulate NO production, which causes vasodilatation and therefore antihypertensive effect [204], also it decreases the level of cholesterol (TC), low-density lipoprotein cholesterol, and triglyceride (TG) in plasma [205]. The secondary metabolites from *Allium* species are characterized with cardioprotective activity, they can prevent Isopriterenol-induced hypertrophic growth in rat heart, though partially increasing Na+/K+-ATPase level[206]. *A. sativum* expresses also other cardioprotective activity such as inhibition of platelet aggregation[207]. Research data suggests that extract of *A. sativum* suppresses lipid deposition in the vessels during the early stage of atherosclerotic development[208]. Furostanesaponins from the Seeds of *A. ramosum,* Ramofurosides A and C, showed a significant reduction in total lipid content by 27.93±3.05 and 27.54±1.68 %, respectively, in HepG2 cell lines[104]. Anti-atherosclerotic activity is described in saponins from *A. macrostemon*, which blocks platelet activation through the CD40 signaling pathway[209].

Some steroidal saponins and sapogenins with thrombolytic activity including diosgenin, tigogenin and ruscogenin have been isolated from *A. affine*[164]. Study has found that *A. atroviolaceum* extract has excellent antiplatelet activity and is able to inhibit platelet aggregation in vitro induced by ARA and ADP with each IC_{50} value of 0.4881 (0.4826–0.4937) and 0.4945 (0.4137–0.5911) mg/mL[210]. Antiplatelet activity is also described in the leaf extract of *A. ursinum*. Testing of antiplatelet activity in vitro was conducted using light transmission aggregometry which has been induced with adenosine diphosphate (ADP), collagen, A23187, epinephrine and arachidonic acid (ARA)[211].

1.5.7. Other biological activities

Apart above-mentioned biological activities, *Allium* species are characterized with many other biological activities.

in vivo experiments have determined gastroprotective effect of the extracts of *A. ampeloprasum* and *A. sativum,* in ethanol and indometiacine induced gastric ulcer model[212], [213].

Species of *Allium* are characterized with wound healing properties. Studies show that onion extract inhibit the proliferation of fibroblasts by 50.8% and can be used in the treatment of patients with keloids and hypertrophic scars[214]. *in vivo* tests determined also wound healing effect of the extract of *A. sativum*[215]. Same effect is described in *A. ascalonicum*[216]. Extract of *A. stipitatum* significantly increases epithelialization and collagenation of thermal injuries[217].

Species of *Allium* have important antidiabetic activity, for example, the flavonoids from A. cepa showed substantial inhibitory activities against both α-glucosidase and protein tyrosine phosphatase 1B (PTP1B), with IC_{50} values of 0.89–6.80 μM[84]. The crude extract of *A. tuberosum* expresses antidiabetic and hepatoprotective activities, in the CCl_4 treated mice groups[68]. Silver nanoparticles synthetized using *A. cepa* revealed that the particles have high level of α-amylase and α-glucosidase inhibitory activities[218]. Administration of different doses of *A. saralicum* extract significantly reduces blood glucose level. Also, it normalizes the levels of catalase and superoxide dismutase enzymes[219].

Allium species have also analgesic activity. Studies on *A. paradoxum* extract showed significant analgesic activity, evaluated by hot plate and acetic acid induced writhing test on male Balb/C mice[220]. Tail-flick and hot plate tests has also demonstrated that extract of *A. fistulosum* has analgesic effect [221]. Studies have shown that the analgesic action of *A. ampeloprasum* is mediated by interaction with β-adrenergic receptor[222]. The hotplate test revealed that the pain reaction time was significantly increased in group treated with the extract of *A. cepa*[223]. Results have revealed that sodium channel Nav1.7 is the potential target of *A. macrostemon* for its analgesic activity[224].

The extracts of *A. paradoxum* obtained from aerial parts and bulbs, shows significant hepatoprotective effect by reducing the serum marker enzymes, serum aspartate aminotransferase (AST) and alkaline phosphatase (ALP)[225]. The extract of *A. paradoxum* expresses also nephroprotective effect by change in the blood urea nitrogen and creatinine [226].

In silico analysis of *A. sativum* and *A. cepa* determined their potential therapeutic efficacy against COVID-19 infection. Among studied compounds, alliin showed the best binding efficacy against COVID-19 main proteases[227].

First medicine maid from *Allium* species was "Alilchep", in Russia, which has antiatheromatic, hypoglycemic and antimicrobial activity (against *Bacillus subtilis, Salmonella typhi, Pseudomonas aeruginosa, Escherichia coli*). *A. cepa* is main component of "Contractubex" Gel, with Allantoin and Heparin, this gel supports the healing process inside the skin, regulating the production of scar tissue and working to positively influence scar formation. Onion and garlic extracts are active components of nasal spray "Aviruspray", for the prevention of viral infections. These extracts are active components of other medicinal products, such as: syrup "Wormitel" with anthelmintic activity, cream "Cepan" for local treatment of scars and acestomas (keloids) after burns and surgical procedures, also for treating scars after furuncles, ulcerations and acne, syrup "Alcep" used in mild upper respiratory tract infections. Oral paste "Fitolizyn" is containing an extract of *A. cepa* in combination with other herbs. Onion is component of homeopathic medicine "Rhinallergy", for treatment of allergic rhinitis (hay fever) and allergic conjunctivitis. Garlic is active ingredient of "Allochol", which improves the secretory function of hepatocytes (primary liver cells); it has a mild choleretic action and stimulates the synthesis of bile acids. Onion and Garlic bulbs, as food additive, are used for lowering cholesterol and triglycerides. The "Vit'Allium", contains a unique garlic extract, obtained by an innovative ripening process which reduces unpleasant odors and preserves the beneficial compounds present in fresh garlic. Aliicin is active ingredient of drugs which are used for the prevention of cardiovascular diseases, colds, and flu symptoms. medicinal products based on dried powder of Garlic are marketed in the EU/EEA, as herbal medicinal product in the prevention and treatment of mild hypercholesterolemia and hypertriglyceridemia as a supplement to a diet and where no other medical treatment is required, also For oral use, in adults and adolescents: prophylaxis of generalized arteriosclerosis[228].

To ensure quality of the products, European pharmacopoeia includes monograph about quality control of "Garlic powder - Allii sativi bulbi pulvis". This monograph involves identification of the product with microscopic examination and Thin-layer chromatography. Also involves tests for determination of Starch, Loss on drying and Total ash. Monograph also describes content of allicin in powdered plant material and the assay for its quantification[229].

European Medicines Agency (EMA) has registered *Allium sativum* as traditional remedy for following therapeutic indications[230]:

- Traditional herbal medicinal product used as an adjuvant for the prevention of atherosclerosis.
- Traditional herbal medicinal product used for the relief of the symptoms of common cold.

With following forms are recognised:

- Powdered herbal substance. Single dose: 300 mg to 750 mg. Daily dose: 900-1380 mg divided into 3 to 5;
- Liquid extract. Single dose: 110-220 mg 4 times daily. Daily dose: 440-880 mg. extraction solvent rapeseed oil, refined;
- Dry extract. Single dose: 100-200 mg 1-2 times daily. Daily dose: 100-400 mg. extraction solvent ethanol 34% V/V;

Medicines containing *Allium* species as active components are indicated in table 6.

Table 7. Medicines from *Allium* species

N	Name	Therapeutic use	Dosage Forms	Manufacturer/Country	Source
1	Allilchep	Antiatheromatic and hypoglycemic	Extract	Russia	[231]

2	Contractubex	keratolytic	Gel	„Merz Pharma GmbH & Co. KGaA“, Germany	[232]
3	Aviruspray	For the prevention of viral infections	Spray	„Georg BioSystems“, Ukraine	[233]
4	Cepan	For local treatment of scars and keloids	Cream	Unia, Poland	[234]
5	Alcep	For mild upper respiratory tract infections	Syrup	„Hasco-Lek“, Poland	[235]
6	Fitolizyn	Anti-inflammatory	Paste	„Herbapol“, Poland	[236]
7	Allochol	For chronic cholecystitis, uncomplicated cholelithiasis	Tablet	“Borshchahivskiy CPP” Ukraine	[237]

Table 8. Dietary supplements from *Allium* species

N	Name	Therapeutic use	Dosage Forms	Manufacturer/Country	Source
1	Wormitel	Antihelminthic	Syrup	„Vorwarts Pharma“, Ukraine	[238]
2	Rhinallergy	For treatment of allergic rhinitis and conjunctivitis	Tablet	„Boiron“ , France	[239]
3	Vit’allium	For cardiovascular diseases	Capsule	„Yalacta“, France	[240]

4	Allicin Complex	For cardiovascular diseases	Capsule	„Deba Pharma“, Belgium	[241]
5	AllicinMax	Food additive, antisaptic	Capsule, Cream	„Allicin International“ UK	[242]
6	HeartFast	For normal function of the heart	Capsule	„Allicin International“ UK	[243]
7	Prepro	Probiotic for the gastrointestinal tract	Capsule	„Allicin International“ UK	[244]
8	Lesterol	For the maintenance of normal blood cholesterol levels	Capsule	„Allicin International“ UK	[245]
9	Allimed	anti-bacterial, Imunostimulator	Capsule, Spray, Gel	„Allicin International“, UK	[246]
10	Garlicin Cardio	For heart and cardiovascular health	Tablet	„Nature’s Way“, USA	[247]
11	Garlic Oil	Dietary supplement	Capsule	„Solgar“, USA	[248]
12	Garlic extract	For heart and cardiovascular health	Softgels	„Nature’s Bounty“, USA	[249]
13	Kyolic	For heart and cardiovascular health	Capsule	„Wakunaga“, USA	[250]

2. CHAPTER 2 – Objects of the research, Materials and Methods

2.1. Objects of the research

The objects of the research are two plants of the genus *Allium* L growing in Georgia.: *Allium saxatile* Bieb and *Allium ponticum* Miscz, ex Grossh.

Plants were collected and identified by Dr. Tsiala Gviniashvili, Botanical Institute of Ilia State University(Voucher specimen: *A. saxatile*_ TBI1034088; *A. ponticum*_ TBI1060252).

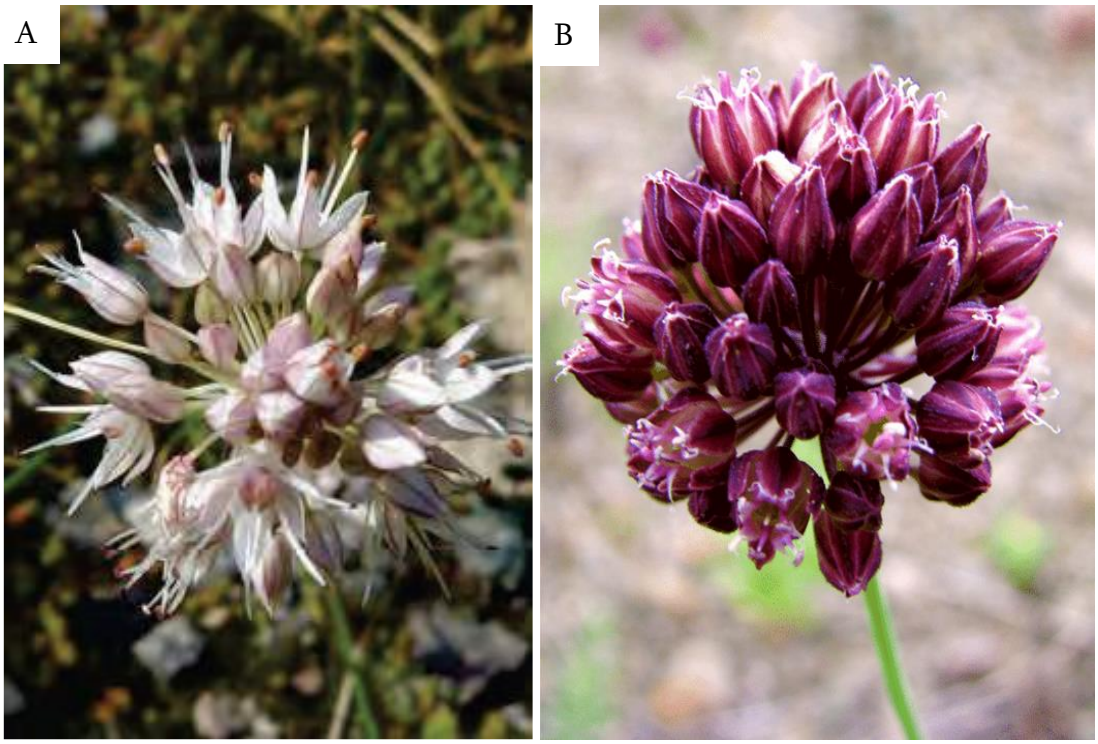


Figure 8. A – *Allium saxatiel;* B – *Allium ponticum*

2.2. Materials and Reagents

During the research following reagents were used:

N	Name	Manufacturer	Country
1	Methanol (22C154003)	VWR	France
2	Chloroform (MFCD00000826)	VWR	Belgium
3	Dichloromethane (21L1440015)	VWR	France
4	Ethyl Acetate (21K024009)	VWR	France

5	Butanol (MFCD00002964)	Sigma Aldrich	USA
6	Glacial Acetic Acid (1000631000)	Merck	Germany
7	Sulfuric acid (1007311000)	Merck	Germany
8	Hydrochloric acid (1003172500)	Merck	Germany
9	Acetone (21I234009)	VWR	France
10	Formic acid (BDH4554)	VWR	France
11	Dimethyl sulfoxide (1029521000)	Merck	Germany
12	Acetonitrile (1000302500)	Merck	Germany
13	Polyethylene glycol (AAB21992-0B)	VWR	France
14	Vanillin (8187180500)	Merck	Germany
15	2-Aminoethyl diphenylborinate (10207480)	Alfa Aesar	UK
16	4-(Dimethylamino) benzaldehyde (156477)	Merck	Germany
17	Dragendorff Reagent (44578)	Merck	Germany
18	3,5-Dinitrobenzoic acid (121258)	Merck	Germany
19	Sodium hydroxide (S5881)	Merck KGaA	Germany
21	Carrageenan (C1013)	Merck KGaA	Germany
22	Astragalin (04500585)	Merck KGaA	Germany
23	Ferulic acid (1270311)	Merck KGaA	Germany
24	Protodioscin (G0299)	Merck KGaA	Germany
25	Artemisinin (69532)	Merck KGaA	Germany
26	Deuterated Methanon (CD_3OD) (611646)	VWR	Belgium

Cell strains used in *in vitro* assays:

N	Name	Strain	Supplier
1	Human Melanoma Cell Line	A2058	ATCC®; USA
2	Breast Adenocarcinoma	MDA-MB-231	ATCC®; USA
3	Macrophage	RAW 264.7	ATCC®; USA
4	*Plasmodium falciparum* strain	3D7	ATCC®; USA

Reactive used in *in vitro* assays:

N	Name	Manufacturer	Country
1	RPMI medium	Gibco™	USA
2	RPMI-1640 medium	Gibco™	USA
3	Prestoblue	Invitrogen™	USA
4	Dulbecco's modified Eagle's medium (DMEM)	Gibco™	USA
5	Glucose	Gibco™	USA
6	Glutamine	Gibco™	USA
7	Penicillin	Gibco™	USA
8	Streptomycin	Gibco™	USA
9	Sodium pyruvate	Gibco™	USA
10	10% Fetal bovine serum (FBS)	Sigma-Aldrich®	USA

Apparatus used during the research:

N	Name	Model
1	Microscope	1. Carl Zeiss, Jeneval 2. Omax
2	Ultrasound water bath	Runyes clean-02

3	Spectrophotometre	Nanolytik NanoSpec 2
4	High-performance liquid chromatography (HPLC)	Agilent Technologies 1260 infinity
5	Nuclear Magnetic Resonance (NMR)	Bruker Avance Neo 500 MHz with cryoprobe
6	Microplate reader	1. Tecan Spark® Multimode Microplate Reader 2. Flexstation 3
7	Melting Point Apparatus	Mel-Temp™
8	Freeze dryer	Kambic LIO-5PLT
9	Mass-Spectrometer	Synapt XS HDMS Q-TOF mass spectrometer
10	Polarimeter ($[\alpha]_D^{25}$)	Anton Paar GmbH MCP 500

2.3. Methods

2.3.1. Microscopy

Transverse, longitudinal and superficial section slides of the plants, for microscopy, were prepared from dry plant material, which was thermally processed and cut with sharp knife. Sections were colored with safranin solution during 24 hours and were paced on microscopic slides. Microscopic examination was performed using Carl Zeiss, Jeneval and Omax microscopes. Pictures were processed in Adobe Photoshop CS5 software.

2.3.2. Extraction of secondary metabolites

Aerial part of each plant was dried and milled to 1 mm particles. Powdered plants were extracted with 80% EtOH, using an ultrasonic water bath at 50 °C. Extracts were concentrated with a rotary evaporator (Speed – 100 RPM; Temperature – 55 °C; Frequency – 35000 Hz) and dried using freeze dryer. Apparatus: Kambic LIO-5PLT; Condenser temperature: -90 °C; Drying chamber temperature – 35 °C; Pressure – 0.012 mbar; Duration of drying – 32 hr.

2.3.3. Column chromatography (CC)

Open Column chromatography method was employed for fractionation of the crude extracts and isolation of individual compounds.

A) For fractionation

Stationary phase: Diaion HP-20 (Mitsubishi, Japan)

mobile phase: Water-Methanol (100:0; 50:50; 0:100), Ethyl acetate (100%)

B) For isolation of individual compounds

Stationary phase: Silica Gel 60 F (0,04-0,063) (Merck)

Mobile phase: Chloroform-Methanol-Water (60:10:1; 50:10:1; 45:12:1; 40:12:1; 30:12:2; 40:10:1; 26:12:1; 30:12:1;).

2.3.4. Thin-layer chromatography (TLC)

For thin layer chromatography, we have used TLC Silica gel 60 F_{254} plates as a stationary phase, with 20 cm x 20 cm dimensions. Manufacturer: Sigma-Aldrich Canada Co.

Mobile phase:

1) Chloroform-Methanol-Water (30:12:1; 40:11:1; 40:10:1; 26:14:3; 65:35:10)

2) Chloroform-Methanol (15:1; 23:2)

3) Dichloromethane-Methanol-Water (30:12:2; 26:12:2)

4) Butanol-Acetic Acid-Water (4:1:5)

5) Ethyl Acetate-Formic Acid-Acetic Acid-Water (100:11:11:26)

6) Chloroform-Methanol-Ammonia (85:14:1)

Revelation:

A) For spirostanolic saponins: 1% Vanillin in methanol and conc. sulfuric acid (98:2);

B) For furostanolic saponins: 4-Dimethylamino benzaldehyde methanolic solution with conc. hydrochloric acid (50:50);

C) For flavonoids: 2% 2-aminoethyl diphenylborinate in methanol;

D) For alkaloids: Dragendorff reagent;

E) For cardiac glycosides: 2% 3,3-Dinitrobenzoic acid in ethanol;

F) For anthraquinones: 10% Sodium hydroxide solution;

G) For tannins: Ammonium iron(III) sulfate dodecahydrate;

2.3.5. Acid hydrolysis

Acid hydrolysis was used to determine structure of isolated compounds and qualitative analysis of aglycones[251]. Objects were heated on water bath for 4 hours with 2N HCl, at 100-120 °C. After 4 hours, solution was cooled down to room temperature and neutralized with 5% $NaHCO_3$ solution and extracted with Dichloromethane (1:1) in separatory funnel. TLC was utilized for the qualitative analysis of aglycons.

2.3.6. Mass-spectrometry

To perform mass spectrometry, objects were prepared with a concentration of 100 μg/ml in acetonitrile and then subjected to mass spectrometry. The experiments were performed on an Acquity ultra-performance liquid chromatography (Waters) system equipped with an ACQUITY UPLC BEH C18 column (2.1 mm x 50 mm, 1.7 μm particle size; Waters). The UPLC is coupled to a Synapt XS HDMS Q-TOF mass spectrometer with an ESI source (Waters). The mobile phase consisted of were water and acetonitrile, each containing 0.1% formic acid. The LC was performed at a flow rate of 0.4 mL/min and the gradient conditions were 5% of acetonitrile from 0 to 1 minute, 5–100% of acetonitrile from 1 to 28 minutes, 100% of acetonitrile from 28 to 30 minutes.

The samples were analysed using a UPLC-HDMSe workflow with a mass range from 50 to 2000 Da and a collision energy ramp from 20 to 70 V. Data analysis was performed using MassLynx V4.2.

2.3.7. NMR Spectroscopy

Chemical structure of isolated compounds was determined with NMR spectroscopy. Bruker AVANCE NEO equipped with cryoprobe. Frequency of NMR was 500 MHz. Objects were dissolved in Deuterated methanol.

Following number of scans were used:

Proton spectrum (^{1}H): 128 scans;

Carbon spectrum (^{13}C): 5120 scans;

COSY: 4 scans;

HMBC: 16 scans;

HSQC: 8 scans;

TOCSY: 8 scans;

2.3.8. Determination of moisture and total ash content

Total ash and moisture content in plant materials was determined according to World Health Organization guideline for "Quality control methods for herbal materials" [252].

2.3.9. Quantification of furostanolic saponins

Quantification of furostanolic saponins in plant material and crude extract was performed spectrophotometrically. Protodioscin was used as chemical marker.

To prepare sample of plant material, 2.5 g of plant powder were placed in 100 ml volumetric flask, 50 ml methanol was added and heated for 1 hour on a magnetic stirrer. After 1 hour, the solution was cooled to room temperature and filtered.

To prepare sample of crude extracts, 250 mg of were placed in a 100 ml volumetric flask, 50 ml methanol was added and heated for 1 hour on magnetic stirrer. After 1 hour, was cooled to room temperature and filtered.

10 ml of filtered solution was transferred in a 50 ml volumetric flask and volume was adjusted with methanol (Solution "A").

5 ml of solution "A" was transferred to a 25 ml volumetric flask and 5 ml 4-Dimethylamino benzaldehyde in 4N hydrochloric acid in methanol was added (Solution "B"), volume was adjusted with methanol. Prepared mixture was heated for 2 hours in water bath at 57.5-58.5 °C. Absorbance of mixture was measured at 518 nm wavelength, in 10 mm cuvettes. Same procedure was repeated for both plants, for crude extracts and plant materials.

Content of furostanolic saponins, in percents (X), was calculated with formula:

$$X = \frac{C * 50 * 25 * 100 * 100}{a * (100 - b)}$$

Where:

C – concentration of furostanolic saponins from calibration curve;

50 – Initial volume of studying solution;

10 – Dilution factor;

a – Weight of the object;

b – Moisture content;

Preparation of 4N Hydrochloric acid in methanol

34 ml of concentrated Hydrochloric acid were transferred into 100 ml volumetric flask, and volume was adjusted with methanol.

1% 4-Dimethylamino benzaldehyde in 4N hydrochloric acid in methanol preparation

2.5 g of 4-Dimethylamino benzaldehyde was dissolved in 250 ml 4N hydrochloric acid in methanol.

Preparation of standard solution

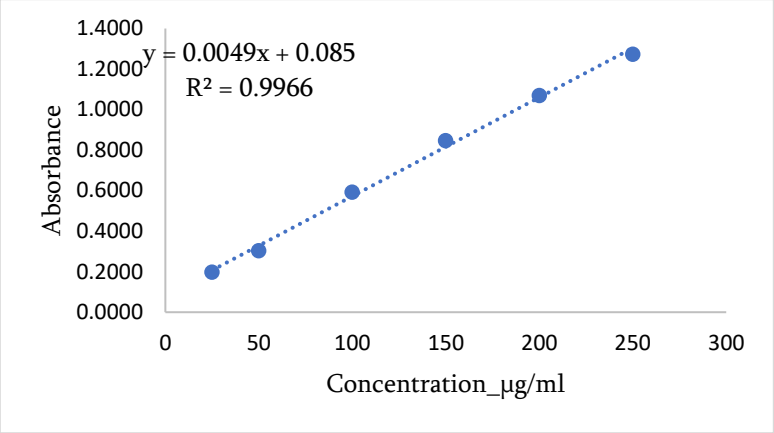
For calibration curve of protodioscin, a standard solution was prepared with 25 µg/ml, 50 µg/ml, 100 µg/ml, 150 µg/ml, 200 µg/ml and 250 µg/ml concentrations in a mixture of water and 1% 4-Dimethylamino benzaldehyde in methanol (1:1).

Prepared mixture was heated for 2 hours in water bath at 57.5-58.5 °C. Absorbance of mixture was measured at 518 nm wavelength, in 10 mm cuvette. mixture of water and 1% 4-dimethylamino benzaldehyde in methanol (1:1) was used for comparison.

Table 9. Absorbance of standard solutions.

Conc.	Absorbance			
µg/ml	n=1	n=2	n=3	Avg.
25	0.2001	0.1973	0.1971	0.1982
50	0.3047	0.3050	0.3049	0.3049
100	0.5900	0.5990	0.5900	0.5930
150	0.8472	0.8491	0.8485	0.8483
200	1.1000	0.9993	1.1100	1.0698
250	1.2765	1.2737	1.2727	1.2743

Graph 1. Calibration curve of Protodioscin.



2.3.10. Quantification of phenolic compounds

HPLC method was used for quantification of phenolic compounds. Chemical marker for *A. saxatile* was – astragalin, for *A. ponticum* – ferulic acid.

The HPLC system consisted of an Agilent Technologies Model 1260 infinity liquid chromatography, equipped with a vacuum degasser, a binary pump, an auto-sampler, and a photodiode array detector (DAD).

Mobile phase: Water (0.1% Acetic acid) and Acetonitrile (77-23, v/v);

Column: Eclipse plus C-18 (4.6 x 250 mm; 5 µm); Temp : 25 °C ;

Detector: For *A. saxatile* crude extr.– 320 nm, for *A. ponticum* crude extr.– 280 nm;

Flow rate: For *A. saxatile* crude extr.– 0.8 ml/min, for *A. ponticum* crude extr.– 0.7 ml/min;

Preparation of solutions:

1 g of powdered plant material of *A. saxatile* was dissolved in 100 ml of 50% methanol, 1 ml of this solution was filtered through 0.45 µm membrane filter (Millipore, ref HVPL04700), for HPLC analysis.

10.0 mg of *A. saxatile* crude extr. was dissolverd in 1 ml 50% methanol and filtered through 0.45 µm membrane filter (Millipore, ref HVPL04700).

2 g of powdered plant material of *A. ponticum* was dissolved in 100 ml of 50% methanol, 1 ml of this solution was filtered through a 0.45 μm membrane filter (Millipore, ref HVPL04700), for HPLC analysis.

20.0 mg of *A. ponticum* crude extract was dissolverd in 1 ml of 50% methanol and filtered through 0.45 μm membrane filter (Millipore, ref HVPL04700).

Standard solution of astragalin and ferulic acid were prepared in 50% methanol with 1.0 mg/ml concentration separately. A series of dilutions of astragalin and ferulic acid (n=5) was prepared to obtain samples with various concentration. All prepared standard solutions were filtered through 0.45 μm membrane filter (Millipore, ref HVPL04700) before HPLC analysis.

Dilutions were prepared with 1 mg/ml; 0.5 mg/ml; 0.25 mg/ml; 0.125 mg/ml; 0.0625 mg/ml; 0.03125 mg/ml and 0.015625 mg/ml concentrations and the peak areas with HPLC.

2.3.11. in vitro research methods

2.3.11.1. *Cell Cultures*

The following cell cultures were utilized for in vitro studies.: Macrophage cell line_RAW 264.7 (RAW264.7 ATCC® TIB-71™), Breast adenocarcinoma cell line_(MDA-MB-231 ATCC® HTB-26™) and human melanoma cell line_A2058 (A2058 ATCC® CRL-11147™). All these cell cultures were obtained from ATCC (American Type Culture Collection, USA). Cells were incubated in medium containing 10% FBS, in incubator at 37 °C, with 5% CO_2 in the air, in a humid atmosphere. Antiplasmodial activity was studied on *P. falciparum* 3D7 strain.

2.3.11.2. Cytotoxicity

Assays were performed on A2058 cells (melanoma) and MDA-MB-231 cells (breast cancer) to evaluate the cytotoxicity of the tested compounds. Cells were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with glucose (4.5 g/L), glutamine (2 mM), penicillin (50 IU/mL), streptomycin (0.5 mg/ml), sodium pyruvate (0.5 mM) and 10 % FBS (Foetal Bovine Serum) at 37°C with 5% CO2 in air, in a humid atmosphere. Compounds were tested in 96-well

microplates using the Prestoblue® colorimetric assay for cell viability based on the reduction of resazurin in resorufin dependant to the metabolic activity of cells. When added to cells, the reducing power of living cells will reduce the resazurin and a fluorescent red colour will be produced. This colour change can be detected using fluorescence measurements (SpetraMax i3, Molecular Devices™) at 560 and 590 nm.

MDA-MB-231 cells (2.5 x103 cells/well) and A2058 cells (2 x103 cells/well) were incubated with serial dilutions of the tested compound (1 μg/ml; 2 μg/ml; 5 μg/ml; 10 μg/ml; 25 μg/ml; 50 μg/ml) for 72 hours and then 75 μL of a 10x dilute solution of Prestoblue reagent were added to each well. Each dilution of the tested compounds were tested in triplicate. After 2 hours at 37 °C, the fluorescence values were recorded at 560 and 590 nm. IC_{50} values were calculated as those concentrations of the tested compound yielding a 50% of cell growth, based on a regression line in which the fluorescence values at 560 and 590 nm were plotted against the logarithm of drug concentration. Doxorubicin was used for positive control and 1.5% DMSO solution for negative control[253].

2.3.11.3. MTT Test (Cell Viability Assay)

The MTT assay is used to measure cellular metabolic activity as an indicator of cell viability and proliferation. This colorimetric assay is based on the reduction of a yellow tetrazolium salt (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide or MTT) to purple formazan crystals by metabolically active cells. Decreasing the viability of cells in cell culture, proportionally decreases intensity of Formazan. Cells were incubated with studied objects, after incubation, 10% MTT solution in RPMI medium is added. After 30 minutes, MTT solution is removed and 0.5 ml DMSO is added. Resulting coloured solution is quantified by measuring absorbance at 560 nm using a multi-well spectrophotometer[254].

2.3.11.4. TBARS Test (For detection of lipid peroxidation)

Thiobarbituric acid reactive substance (TBARS) assay is a method to detect lipid oxidation. Because reactive oxygen species (ROS) have extremely short half-lives, they are difficult to measure directly. Instead, what can be measured are several products of the damage produced by

oxidative stress, such as TBARS. ROS are highly reactive and attack various classes of biomolecules including proteins, DNA and lipids. As a result of lipid oxidation, malondialdehyde (MDA), 4-hydroxy-2-nonenal (HNE) and other reaction products are formed. These are biomarkers of oxidation and this assay measures malondialdehyde (MDA) [255]. The MDA reacts with Thio barbituric acid (TBA) forming a pink chromogen (TBARS), which is measured fluorometrically, at 515-548 nm wavelength.

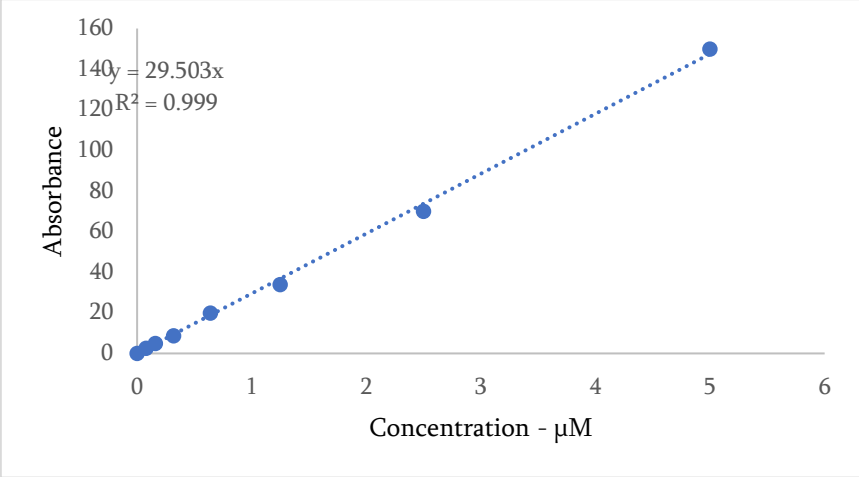
Test procedures

RAW 264.7 cells were incubated with studied objects with different concentrations and Low-density lipoprotein and $CuSO_4$ (100 μM). Crude extracts of the plants were prepared with 0.5 μg/ml, 5 μg/ml, 50 μg/ml and 100 μg/ml concentrations. Fractions were prepared with 0.1 μg/ml, 1 μg/ml, 10 μg/ml and 20 μg/ml concentrations. $CuSO_4$ solution was used to induce lipid oxidation in cells. For control only cells were used in appropriate medium and for second control – cells with $CuSO_4$ solution and LDL. After incubation, mediums were collected and transferred into microtube for TBARS assay.

For TBARS quantification, we prepared standard solution of MDA with 5 μM; 2,5 μM; 1,25 μM; 0,64 μM; 0,32 μM; 0,16 μM; 0,08 μM concentrations.

100 μl of standard solutions and studied objects were transferred into microtubes, 10 μl of 0.5N HCl and 100 μl Thio barbituric acid were added. The solution was mixed and heated for 10 mins at 95 °C, after cooling down, 200 μl of Butanol was added. After adding BuOH, each tube was vortexed for 10 sec and centrifuged at 2000 RPM for 10 min. After centrifugation, 100 μl of the supernatant (butanolic phase), is transferred into 96-well plate and measured using fluorometric spectroscopy (Excitation wavelength - 515 nm; Emission wavelength - 548 nm).

Graph 2. Callibration curve of Malondialdehyde (MDA).



2.3.11.5. Antiprotozoal activity

In vitro antiplasmodial assay was conducted according to the article[256]. Cultures of *P. falciparum* in the asexual erythrocyte stage were maintained. The 3D7 chloroquine sensitive strain of parasites was obtained from the Malaria Research and Reference Reagent Resource Center, MR4. The culture medium was composed of RPMI 1640 (Gibco, Fisher Scientific, Merelbeke, Belgium) containing $NaHCO_3$ (32 mM), HEPES (25 mM), and L-glutamine. The host cells were human red blood cells (A+ or O+). The medium was supplemented with 1.76 g/L of glucose (Sigma-Aldrich, Overijse, Belgium), 44 mg/ml of hypoxanthin (Sigma-Aldrich, Overijse, Belgium), 100 mg/L of gentamicin (Gibco, Fisher Scientific, Merelbeke, Belgium), and 10% human pooled serum (A+ or O+), as previously described. Each crude extract was dissolved in DMSO at a concentration of 10 mg/ml. The solutions of crude extracts were then diluted in the culture medium: for each solution to test, two-fold dilutions were performed eight times on a 96-well plate. With this method, the highest concentration tested is 100 µg/ml. Artemisinin (Sigma-Aldrich, Machelen, Belgium) was used as a positive control for all assays, at an initial concentration of 100 ng/ml. After leaving the parasites in incubation with the diluted solutions of the crude extracts for 48 h, the impact on parasite growth was revealed using SYBR Green, a DNA intercalating compound. The procedure was adapted from the method described in the article of Dery et al. (2015). The SYBR Green solution was diluted in a lysis buffer composed of TRIS buffer (Sigma-Aldrich, Overijse, Belgium), EDTA (Merck, Darmstadt, Germany), saponin (Alfa Aesar, Karlsruhe, Allemagne), and triton (Merck, Darmstadt, Germany). Thus, 500 ml of lysis buffer

contains 1.20 g TRIS buffer, 0.73 g EDTA, 40 mg saponin, and 0.4 ml triton. To reveal a plate, 2 µl of SYBR Green solution is diluted in 10 ml of lysis buffer. In new 96-well plates, 100 µl of solutions from assays are placed and 75 µL of SYBR Green is added. After 2 h of incubation, the plates are read with the FlexStation® (Molecular Devices, Winnersh, United Kingdom) at 490 nm excitation wavelength and 530 nm emission wavelength. The half maximal inhibitory concentration (IC_{50}) values were calculated from graphs. Averages of three IC_{50} values from three independent experiments (n = 3) performed on different days were calculated.

2.3.12. in vivo research methods

2.3.12.1. Animals

Outbred white mice weighing 24-28 g were obtained from the animal house of Tbilisi State Medical University I. Kutateladze Institute of Pharmacochemistry and quarantined for 1 week in the Department of Preclinical Pharmacological Research of above Institute. Animals were kept under standard conditions (temperature 20 ± 2°C, humidity 55-65%, 12/12-hour light/darkness cycle, granulated food - 4 g/animal/day, water *ad libitum*). All experiments were carried out in accordance with the requirements of the EU Directive 2010/63[257].

Research protocol was authorized by the Tbilisi State Medical University Ethics Committee on Animal Research (approval #AP-56-2022).

2.3.12.2. *Hot Plate assay*

The animals were individually placed in an open cylindrical space consisting of a metal floor heated to a temperature of 52 ± 1°C and transparent vertical walls. The time between placing the animal on the floor and the first nociceptive reaction (hind paw licking or jumping) was recorded as the hot-plate latency. The measurements were taken before and after the intraperitoneal administration of 50 mg/kg A.s.tot and A.p.tot extracts and fractions: A.s.F2, A.s.F3, A.p.F2, A.p.F3 with 25 mg/kg and 50 mg/kg concentrations (baseline latency), as well as 30 minutes and one hour.

The analgesic effect was calculated by the formula: E% = ((T_0-T_n) /T_0) × 100, where T_0 is the reaction time prior to the A.s.tot and A.p.tot extracts and fractions injection, and T_n - after the corresponding period (30 or 60 min) after injection, respectively[258].

2.3.12.3. Ethanol-induced gastric ulcer assay

18 outbred mice were randomly distributed in three groups of animals, each consisting of six mice. 24 hours prior to the experiment, the access to food was restricted, and animals were relocated in cages with raised floors of wide wire mesh to prevent coprophagy. During the fasting period, all mice received a nutritive solution of 8% sucrose in 0.2% NaCl to avoid excessive dehydration. On day 2, absolute ethanol was given orally (1 ml/100 g) to all animals. A.s.tot extract in a dose of 50 mg/kg, i.p. (Group III) and A.p.tot extract _ 50 mg/kg, i.p. (Group II) was given 1 hour prior the ethanol administration. Mice of control group (Group I) got 0.2 ml of saline. Animals were euthanized by CO_2 inhalation 1 hour after the ethanol administration. The stomachs were immediately removed, opened along the great curvature, rinsed consequently with water and 10% formalin solution which contains about 4% formaldehyde w/v, fixed on white EPS foam board, and digitally photographed. Ulcer index (UI) was calculated for each stomach according to the following scale, by three independent observers: 1 - no lesions; 2 - single petechial lesions; 2.5 - multiple petechial or short linear haemorrhagic lesions; 3 - long linear haemorrhagic lesions; 4 - continuous linear haemorrhagic lesions along the entire length of the glandular part of stomach. The efficacy of A.s.tot and A.p.tot extracts expressed as percentage of ulcer inhibition (% I) was estimated based on the UI and calculated using the formula:

$$\% I = \frac{UI_C - UI_T}{UI_T} \times 100$$

Where UI_C and UI_T are macroscopic ulcer indexes in control and test groups, respectively[259].

2.3.12.4. Carrageenan-induced Paw edema assay

50 µl of 1% carrageenan solution in saline was injected in the aponeurosis of the right hind paw of the animal. One hour prior the onset of oedema, 0.5 ml of saline and 0.5 ml of A.s.tot and A.p.tot extracts at a dose of 50 mg/kg were administered intraperitoneally to control and

experimental animals, respectively. The thickness of the paw was measured with a digital micrometre before the carrageenan injection (baseline) and after 2 hours.

Anti-inflammatory efficacy was calculated by the following formula:

$E\% = (1-(\Delta T_{exp} / \Delta T_{con})) \times 100$

where ΔT_{con} and ΔT_{exp} are the mean differences in paw thickness before and 2 hours after carrageenan administration in control and experimental group animals, respectively[260].

2.3.13. Statistical analysis

Each experiment performed independently 3 to 6 times before statistical analysis. All data were processed statistically using one-way ANOVA Tukey test. Results were considered significant at $p<0.05$.

(*-P<0.05; **-P<0.01; ***-P<0.001; ****-P<0.0001)

3. CHAPTER 3 – Results

3.1. Microstructural and Macrostructural characterization of research objects and determination of moisture and total ash

3.1.1. *Allium saxatile* Bieb microstructural and macrostructural characterization

Studying object was collected in Racha, floristic region of Georgia, during flowering period. Voucher specimen of the plant is deposited at Botanical institute of Ilia state University (# TBI1034088) (Figure 2). During the research, microstructural characteristics of vegetative and generative organs were studied (Method 2.3.1).

Leaf. The basal cells of the epidermal tissue of the leaf of *A. saxatile*, are lock-stitched in a row, the cell membrane straight linear, longitudinally elongated, narrowly spindle-shaped in configuration; The disposition of the ventilation system of the leaf, taking into account the direction of the inter-petals hole, is orderly both with respect to each other and parallel differentiated veins. Stomatas of the leaves are mostly large, their number is significantly abundant. On the narrowed edge of the basal cells of the leaf epidermis, the stomata, concentrated between two narrow spindle-shaped cells, is of the paracytic type, the guard cells are elongated, lenticular shaped, their stomal pores are large, spindle-shaped (Figure 9).

The mesophyl of the leaf is characterized by an isolateral structure, a tendency to obliteration of the spongy parenchyma is observed during the research. In terms of arrangement of the ventilation system, the leaf is amphistomatic type.

Figure 9. Voucher specimen of *A. saxatile*

The covering tissue of the leaf is sharply cutinized, the cell membrane of the single-row epidermal tissue is strongly thickened. The apparatus of stomata is equal to the epidermal tissue. On the cross section of the leaf between the covering tissue, both in the ventral and in the dorsal area, cells with an atypical palisade habitus with a pronounced square outline and a more or less close mutual arrangement of thin-walled cells can

be observed. In the central area of the mesophyll of the leaf, the coil-walled, thin-walled, fibrously interconnected tissue of the spongy parenchyma is differentiated. In the mesophyll of the leaf, large and small conductive vessels are arranged alternatively; Between two large conducting vessels, 2 or 3 smaller conducting vessels are differentiated. In the mesophyll of the leaf, there is a conducting vessel of a collateral type, in the xylem a group arrangement of slightly angular-membraned and rarely oval-shaped lumens is recorded; There are 3 to 5 conducting vessels of small volume, and 9 to 11 conducting vessels of xylem in the large one(Figure 10).

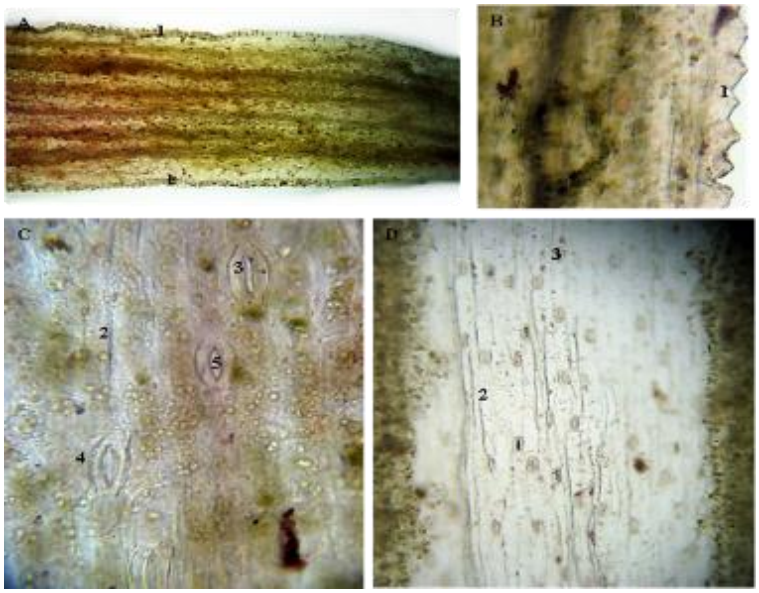


Figure 10. Microstructure of the epidermis of the leaf of *A. saxatile*

A., B. fragments of leaf macrostructure; C., D. Structure of the leaf epidermis;

1. Leaf with dentated margin; 2. Epidermal cells; 3. Paracytic stomata; 4. Guard cells; 5. Stomatal aperture

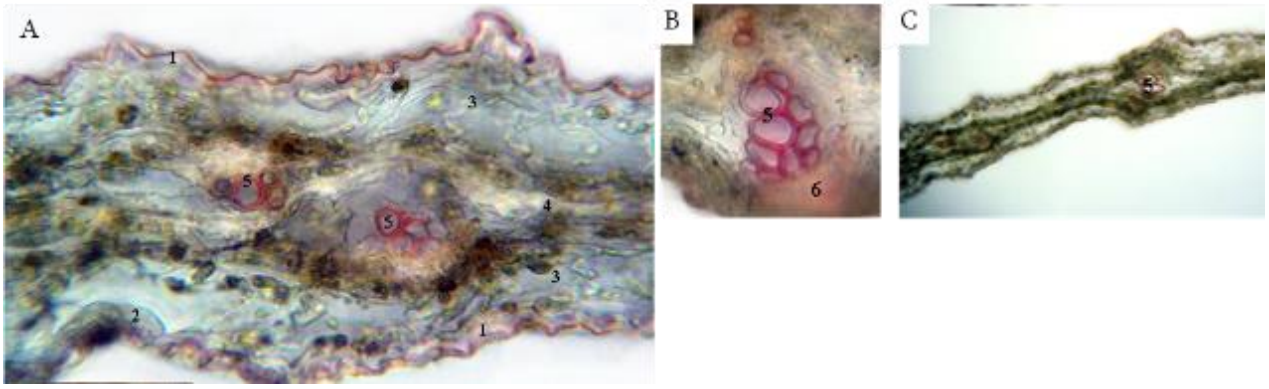


Figure 11. Microstructure of the leaf of *A. saxatile*

A. Panorama of the texture of the leaf; B. Conductive vessels; C. Collateral conductive vessel;

1. Cuticle; 2. Epidermis; 3. Pallisade and 4. Spongy parenchima; 5. Tracheary elements of the xylem; 6. Phloem;

Stem. The tangential section of the stem of *A. saxatile* is spherical, unevenly hilly; The panorama of its structure shows a small cortex area and a central cylinder surrounded by a belt of sclerenchymal tissue, the medulla of which is perforated. The conductive system differentiated in the stem is conical, symmetrically arranged in the radial direction; Conductive vessels are located in the central cylinder in two rows - in the outer zone of the sclerenchymal tissue and in the inner zone of the perimedullary tissue (Figure 13).

As a result of the exogenous study of the epidermis of the stem, the arrangement/stitching of the basal cells was recorded, elongated, straight, slightly narrowed at the ends, periclinal walls of the basal cells of the epidermis were identified. An paracytic type of stomata is located between two basal cells in the covering tissue of the stem. The guarding cells of the stomata are lenticular, they are mostly aligned correctly with each other, although the facts of a slight overlapping of the closing cells with respect to each other are also noticeable(Figure 14).

The stem is bare, the covering tissue is actively cutinized, the epidermis is arranged in a single layer, its cells are characterized by a square shape and have strong thickening of the outer periclinal walls. Stomata are fixed at the height of the covering tissue. The central cylinder of the stem begins with a girdle of sclerenchyma tissue, at the edge of which the conductive vessels of the collateral structure are differentiated. The degree of thickening of the membrane of the differentiated mechanical cells in the stem, the intensity of the thickening and the caliber are non-uniform; In the xylem of conducting vessels, there is a group arrangement of large and small-caliber conducting vessels of oval and slightly angular outline; The thickening of the sheath of conducting vessels is predominantly spiral (Figure 13, 14).

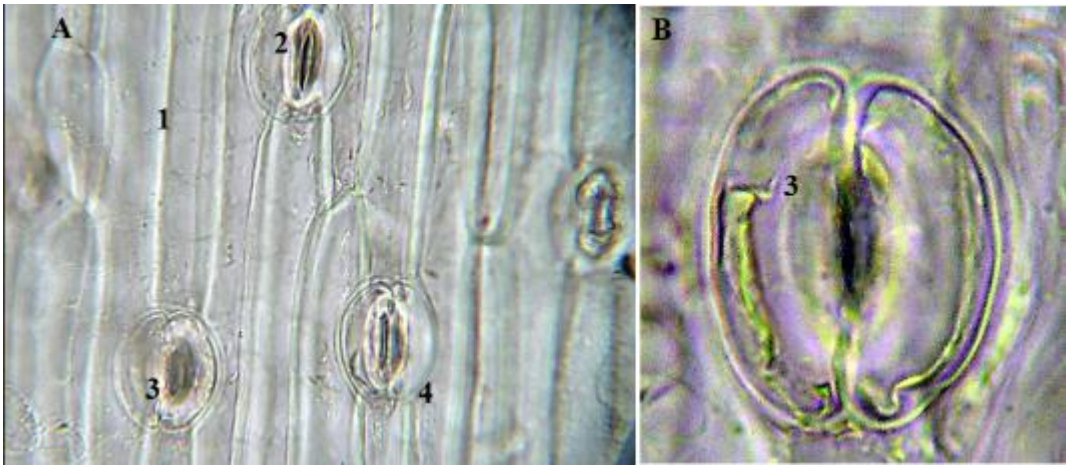


Figure 12. Microstructure of the epidermis of the stem of *A. saxatile*

A. Panorama of epidermal tissue of stem and B. Paracytic type stomata;

1. Basal cell; 2. Paracytic type stomata; 3. Guard cells; 4. Stomatal aperture;

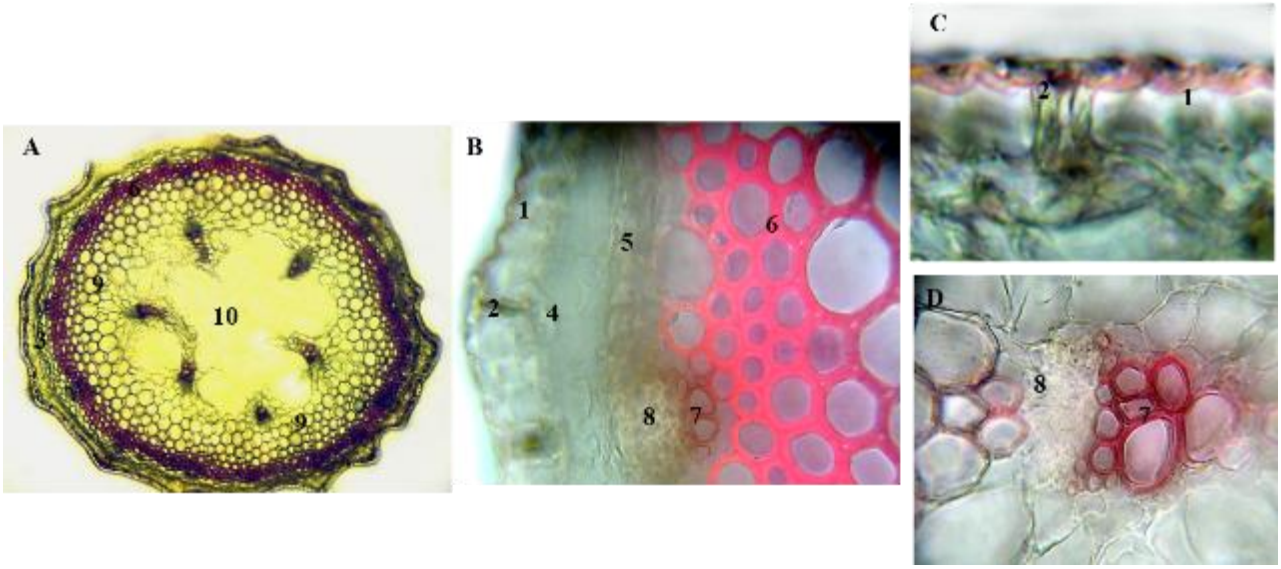


Figure 13. Cross section of Microstructure of the stem of *A. saxatile*

A. Structure of the stem; B. Structural elements of the stem, including sclerenchymal tissue; C. Stomata differentiated in covering tissue; D. Collateral conducting vessel;

1. Cuticle with epidermis; 2. Stomata; 3. Chlorenchyma; 4. Collenchyma; 5. Parenchyma; 6. Sclerenchymal cells; 7. Tracheal elements of the xylem; 8. Phloem; 9. Peridermal tissue; 10. Area of disrupted medullary tissue;

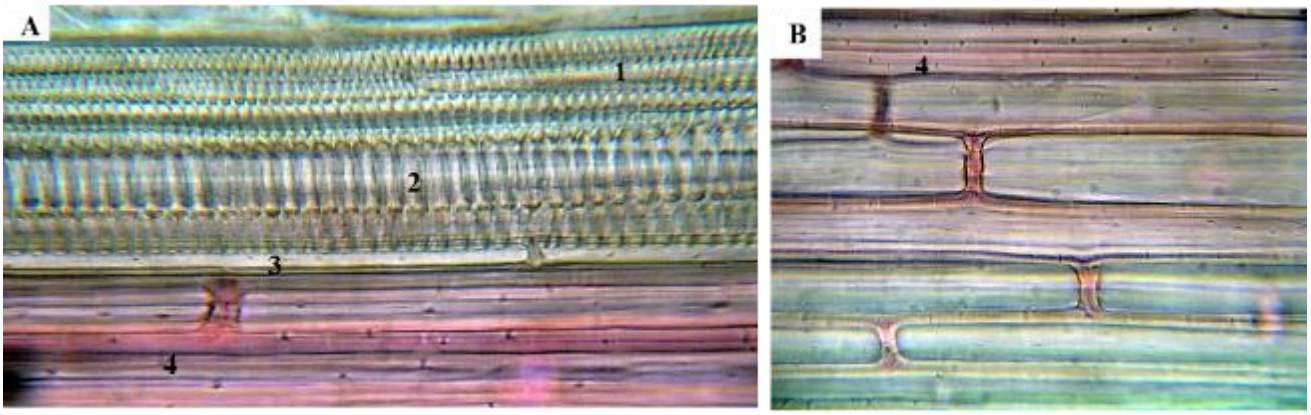


Figure 14. Longitudinal section of the stem of *A. saxatile*

A. Vascular bundle of Phloem and Xylem; B. Part of sclerenchimal tissue;

1. Annular and 2. Spiral vascular bundle of xylem; 3. Cells of the phloem; 4. Sclerenchyma cells;

Flower. The inflorescence is round, multi-flowered umbrella; Pedicels are mostly subequal to each other, equal to or longer than the length of the perianth. The perianth is ovoid-bell-shaped, its cover is free, elongated, pointed, pale pink in color. Stamen filaments are longer than the petals; Pistil's style is cut from the perianth. The pistil's ovary is triple socket like, the seed bud is syncarpous, with an angular placenta (Figure 15).

The basal cells of the epidermis of the petals are arranged in a row, elongated, straight epidermal cells are present. On the cross-section of the leaf of the perianth, significantly thin, single-rowed, pitted epidermal cells and monochromatic, thin-walled, densely interconnected structure of the mesophyll, can be seen, in the medial zone of which the tracheal elements of the xylem are differentiated (Figure 15).

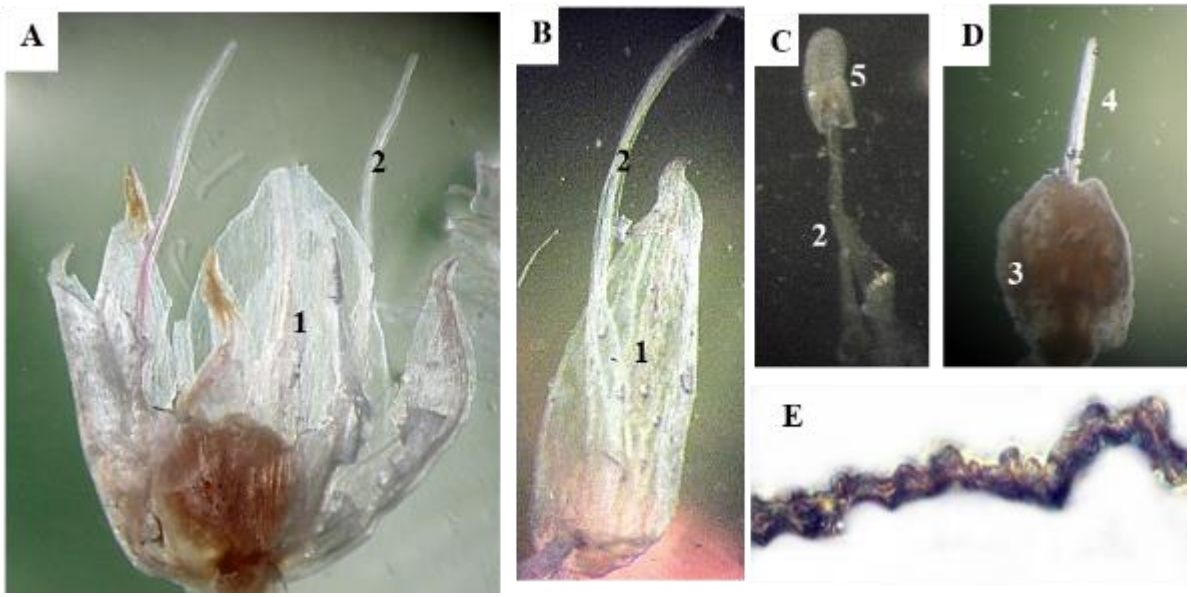


Figure 15. Macrostructural characteristics of the flower of *A. saxatile*

A. Flower; B. Disposition of petals and filamens; C. Stamen; D. Pistil; E. Cross section of petal

1. Petal; 2. Filament; 3. Ovary; 4. Style; 5. Anther;

Bulb. The bulb of *A. saxatile* is free, elongated shape, dry, with leathery shell. On the cross section of the tunic of the bulb, single layered monochromatic, thin-walled parenchyma is visible. Conducting vessels are mostly spiral shaped (Figure 9).

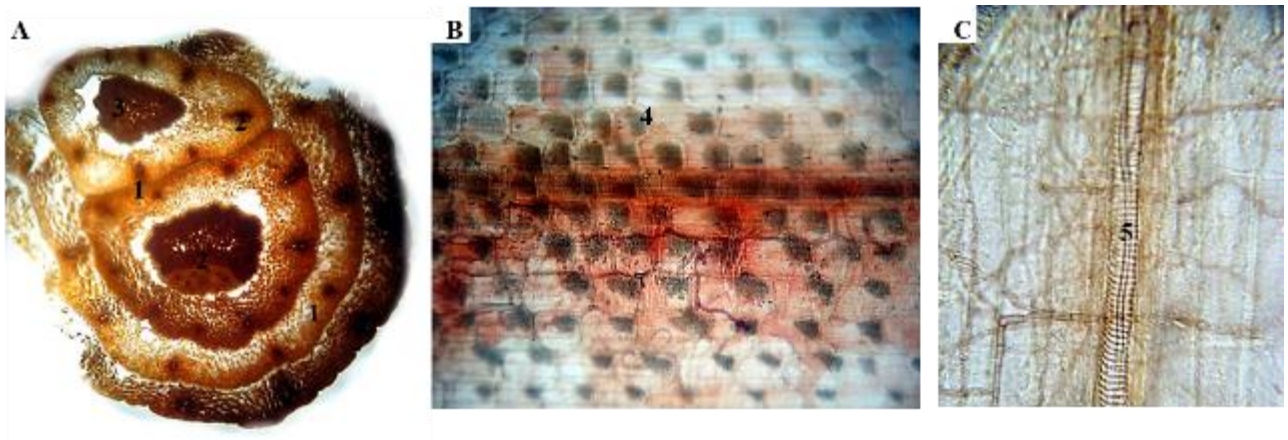


Figure 16. Microstructure of the bulb of *A. saxatile*

A. Structure of the bulb; B. Epidermal tissue of shell; C. Conductive vessel of the bulb;

1. Main tissue of the shell of the bulb; 2. Conductive vessel; 3. Tracheal elements of xylem; 4. Basal cells of the epidermis of bulb shell; 5. Spiral shaped Conducting vessel;

Conclusions:

Microstructural characteristics of *A. saxatile* Bieb is established. The following set of diagnostic features is provided:

- Stomatas of the leaves are mostly large, their number is significantly abundant
- The mesophyl of the leaf is characterized by an isolateral structure;
- The tangential section of the stem is spherical, unevenly hilly; The panorama of its structure shows a small cortex area and a central cylinder surrounded by a belt of sclerenchymal tissue, the medulla of which is perforated;
- The thickening of the sheath of conducting vessels is predominantly spiral;
- An paracytic type of stomata is located between two basal cells in the covering tissue of the stem;
- The degree of thickening of the membrane of the differentiated mechanical cells in the stem, the intensity of the thickening and the caliber are non-uniform;
- The perianth is ovoid-bell-shaped, its cover is free, elongated, pointed, pale pink in color;
- Stamen filaments are longer than the petals; Pistil's style is cut from the perianth;
- On the cross section of the tunic of the bulb, single layered monochromatic, thin-walled parenchyma is visible;

3.1.2. *Allium ponticum* Miscz. Ex Grossh microstructural and macrostructural characterization

Studying object was collected in Javakheti, floristic region of Georgia, during flowering period. Voucher specimen of the plant is deposited at Botanical institute of Ilia state University (# TBI1060252) (Figure 17). During the research, microstructural characteristics of vegetative and generative organs were studied (Method 2.3.1).

Leaf. The leaf of *A. ponticum* is flat, grooved, bifacial. Covered with unicellular, cone shaped trichomes (Figure 18). Mesophyll of leaf has isolateral structure. Amphistomatic type of stomata. The mesophyll of the leaf is characterized by an isolateral structure, a tendency to obliteration of the spongy parenchyma is observed.

The covering tissue of the leaf is sharply cutinized, the cell membrane of the single-row epidermal tissue is strongly thickened. Stomata is immersed in the epidermal tissue. In the pulp of the leaf, palisade parenchyma is sorted in one row, big, square-shaped, thin-membraned, tightly packed tissue is outlined. The leaves are characterized with collateral type of conducting vessel. Spongy mesophyll is only represented around the conductive vessels.

On the cross section of the pulp of the leaf, conductive vessel is covered with massive sclerenchymal tissue, in dorsal plane. On ventral plane, above conductive vessels, lumen of one or two mechanical cells is outlined. Conductive vessel of the leaf is mostly characterized with group of large, oval-shaped 6-8 cell lumens(Figure 19).

Structure of covering tissue on both sides of the leaf is identical. The outer wall of the cells of the leaf protective tissue is thickened. On the basal cells of leaf

Figure 17. Voucher specimen of *A. ponticum*

epidermis, paracytic type of stomata is differentiated. Guard cells of stomata are elongated (Figure 12).

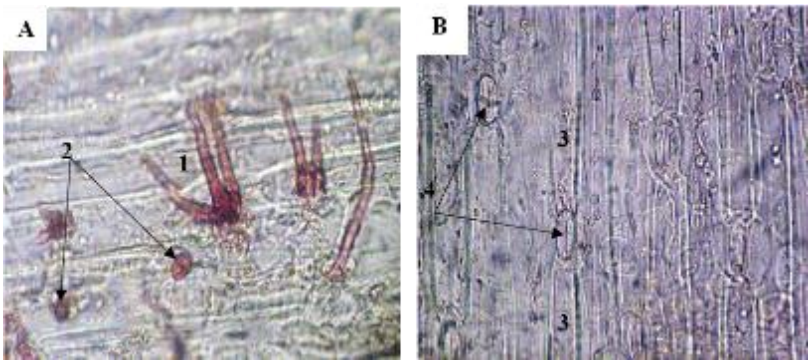


Figure 18. Microstructure of the epidermis of the leaf of *A. ponticum*

A. Trichomes differentiated on covering tissue; B. Epidermal tissue of the leaf;

1. Trichomes and 2. Its trace; 3. Epidermal cells; 4. Paracytic stomata

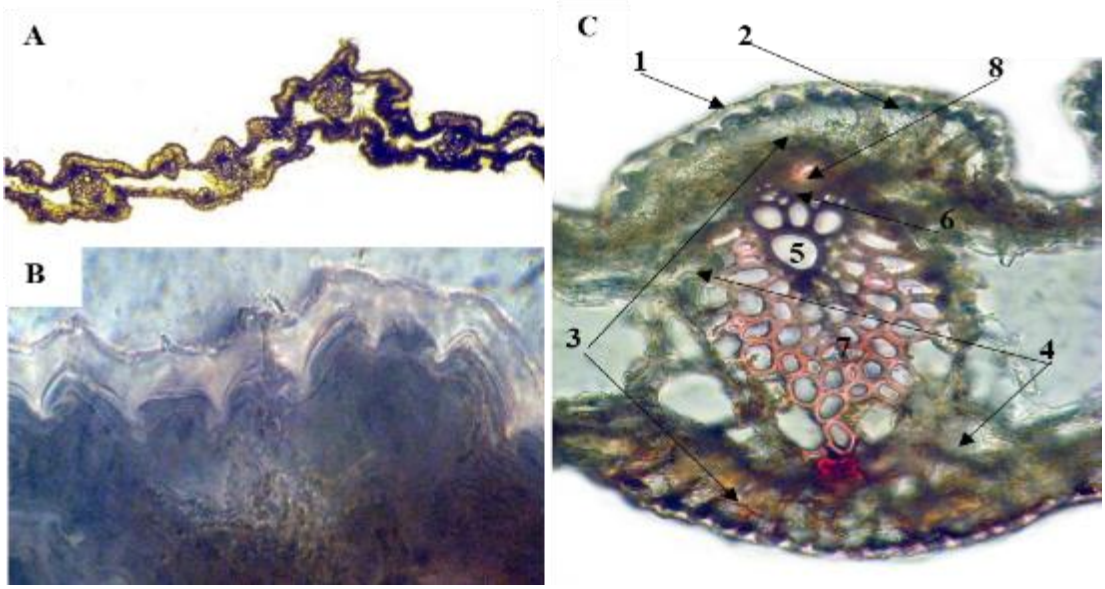


Figure 19. Microstructure of the leaf of *A. ponticum*

A. Panorama of the texture of the leaf; B. Fragment of the covering tissue; C. Main structural elements of the leaf;

1. Cuticle; 2. Epidermis; 3. Pallisade and 4. Spongy parenchima; 5. Xylem; 6. Phloem; 7. Sclerenchymal tissue; 8. Machanical cell.

Stem. The conductive system of the stem of *A. ponticum* is conical, symmetrically arranged in the radial direction. The covering tissue is actively cutinized, the epidermis is arranged in a single layer, its cells are characterized by a square shape and have strong thickening of the outer periclinal walls. Stomatas are slightly immersed in covering tissue. As a result of the exogenous study of the epidermis of the stem, the arrangement/stitching of the basal cells was recorded, configuration of elongated, straight, slightly narrowed at the ends, periclinal walls of the basal cells of the epidermis were identified. Paracytic type of stomata is located between two basal cells in the covering tissue of the stem. The guarding cells of the stomata are lenticular, they are mostly aligned correctly with each other (Figure 20).

The epidermis of the stem is separated by the continuous belt of chlorenchyma. In the central cylinder of the stem, a massive sclereenchymal tissue is presented. The degree of thickening of the membrane of mechanical cells, the intensity and the caliber decreases from the periphery in the direction of the center. The lumen of sclerenchymal cells are square shaped. In the outer peripheral area of mechanical cells, colateral type of conducting vessel, with large caliber, is differenciated. The thickening of the sheath of conducting vessels is predominantly spiral (Figure 20, 21).

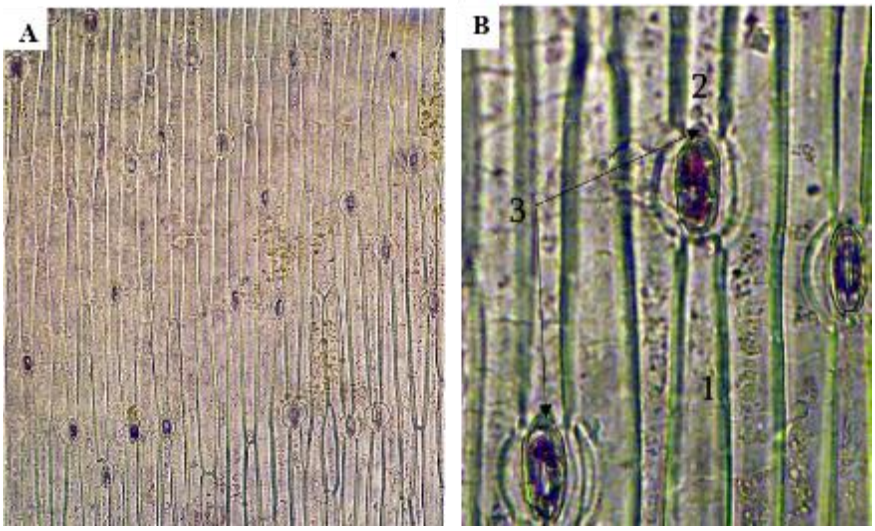


Figure 20. Microstructure of the epidermis of the stem of *A. Ponticum*

A. Panorama of epidermal tissue of stem and B. Structure

1. Basal cell; 2. Paracytic type stomata; 3. Guard cells;

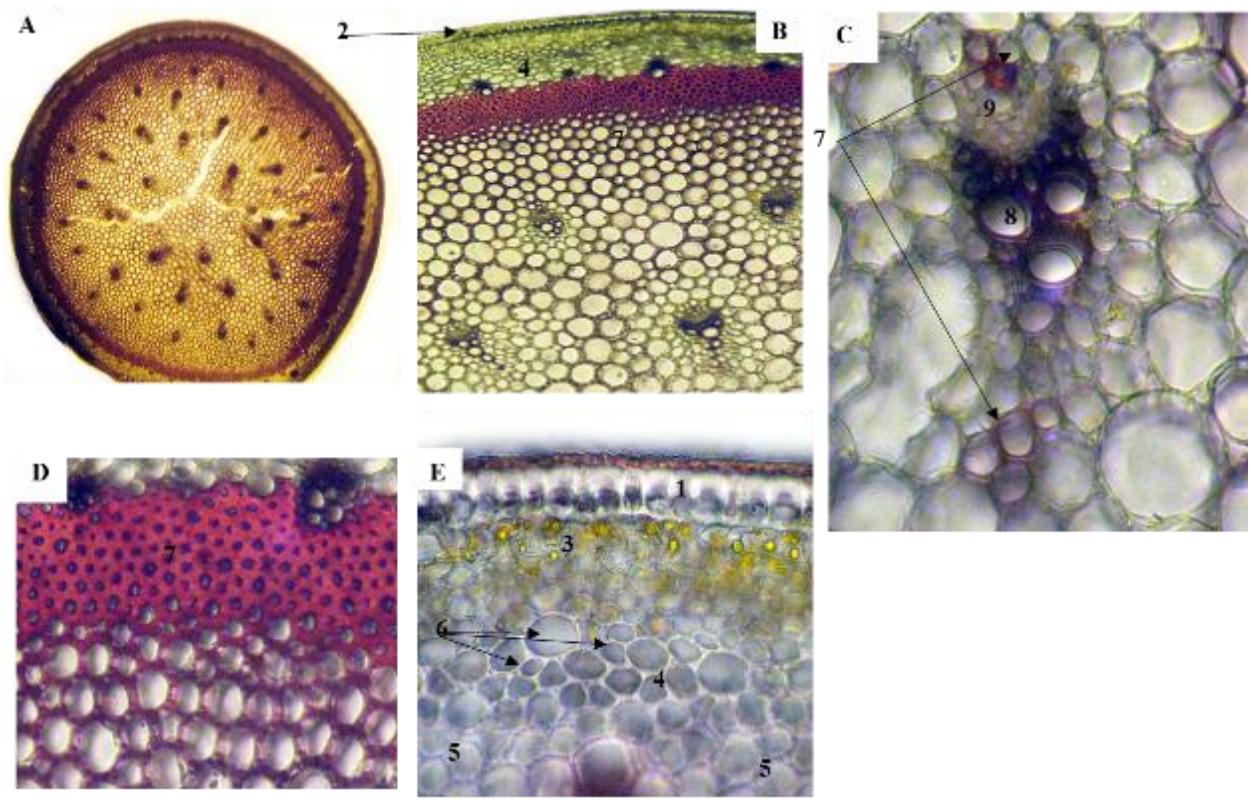


Figure 21. Cross section of Microstructure of the stem of *A. Ponticum*

A. Structure of the stem; B. Structural elements of the stem; C. Collateral conductive vessel; D. Sclerenchyma tissue; E. Covering tissue;

1. Cuticle with epidermis; 2. Stomata; 3. Chlorenchyma; 4. Collenchyma; 5. Parenchymal cells of the stem; 6. Secretarial cells with surrounding tissue; 7. Sclerencymal cells; 8. Xylem; 9. Phloem;

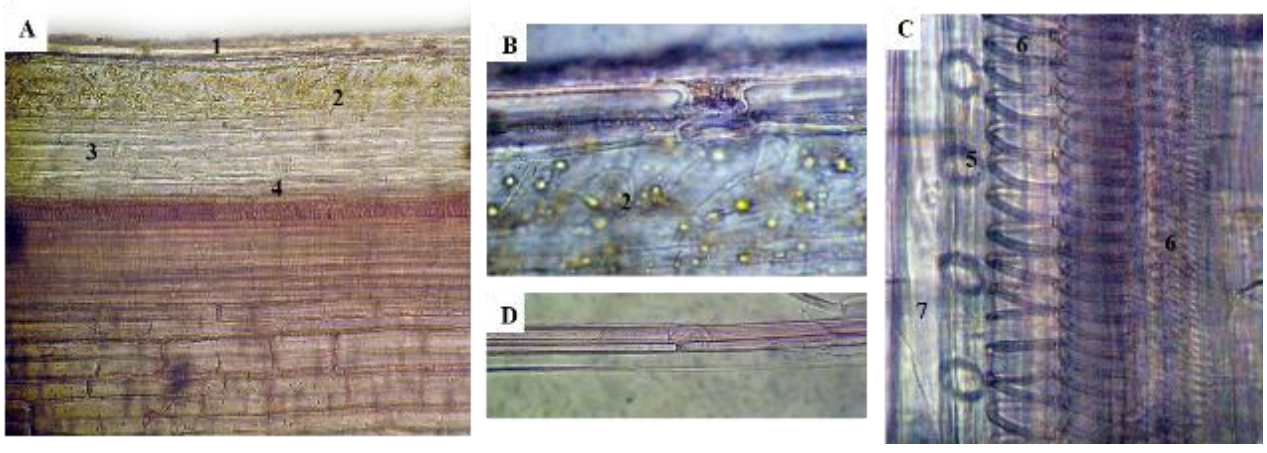


Figure 22. Longitudinal section of the stem of *A. Ponticum*

A. Structure of the stem; B. Stomata differentiated in covering tissue; C. Vascular bundle of Phloem and Xylem; D. Sclerenchimal tissue;

1. Cuticle with epidermis; 2. Chlorenchyma; 3. Collenchyma; 4. Parenchyma; 5. Tracheal elements of the xylem; 6. Spiral vascular bundle; 7. Sclerenchymal cells;

Flower. The inflorescence of *A. ponticum*, is round, multi-flowered umbrella. Pedicels are mostly subequal to each other, equal to or longer than the length of the perianth. Stamens are 6, ordered in two rows. The length of the outer filaments is slightly shorter or equal to the length of petal. Inner filaments are split into three parts, lateral parts are higher than middle. Style of carpel is long, higher than petals (Figure 16).

On the cross section of the petals, single layered, papilliform cells of epidermis and densely interconnected structure of mesophyll is presented. In the centre of main tissue, tracheal elements of xylem are differentiated (Figure 23).

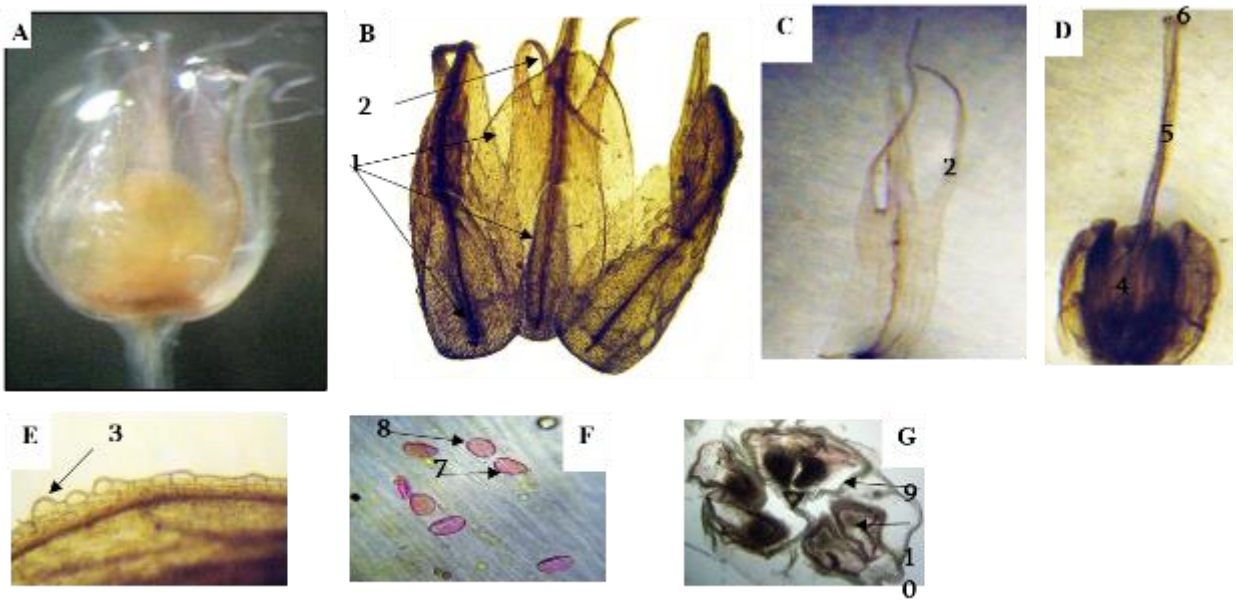


Figure 23. Macrostructural characteristics of the flower of *A. Ponticum*

A. Flower; B. Disposition of petals and filaments; C. Stamen; D. Pistil; E. Fragment of the edge of filament; F. Pollen grain; G. Seed bud;

1. Perianth; 2. Filament split into three parts; 3. Hills on the edge; 4. Ovary; 5. Style 6. Stigma 7. Layer of exine and intine of pollen grain; 8. Pollen grain; 9. Carpel; 10. Syncarpous seed bud

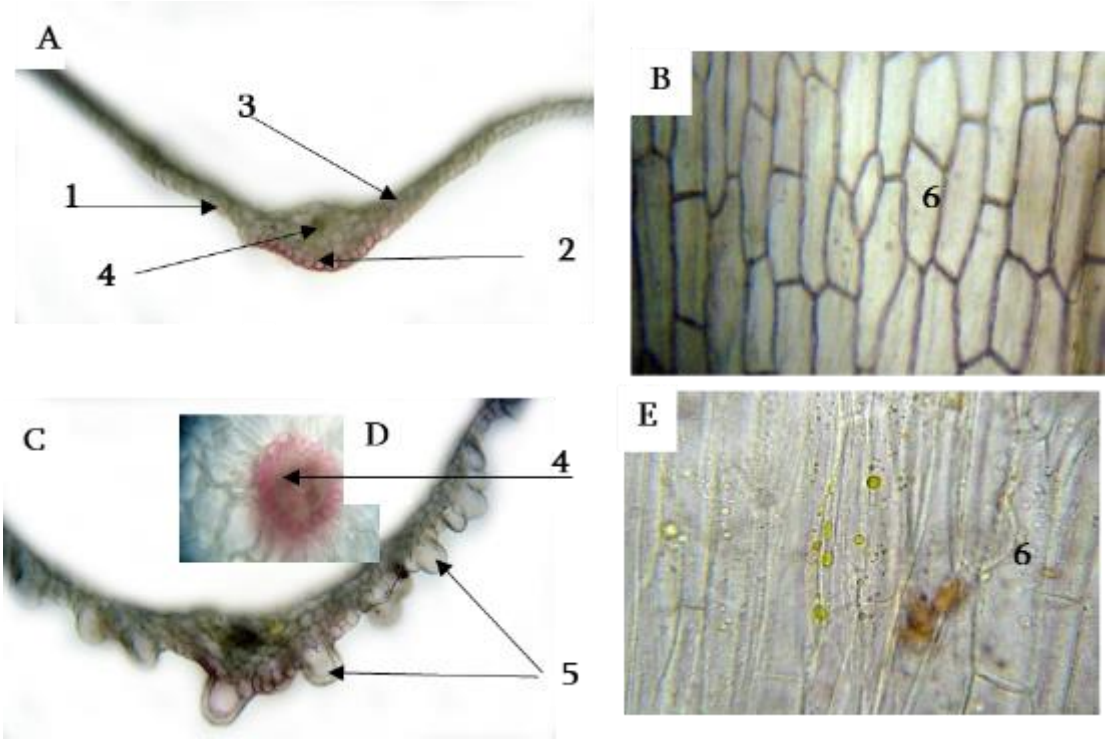


Figure 24. Microstructure of the flower of *A. Ponticum*

A. Structure of the perianth; B. Epidermal tissue; C. Structure of the filament; D. Conductive vessel; E. Epidermal tissue;

1. Epidermis; 2. Midrib; 3. Parenchyma; 4. Tracheal elements of xylem; 5. Appendiculate; 6. Elongated basal cells of the epidermis;

Bulb. The bulb of A. ponticum is free, well developed. In the texture of the shell of the bulb different size of crystals are concentrated. Covering tissue of the bulb has multiple layer. Parenchyma of the tunic in represented with thin-layered, elongated cells. Conducting vessels are mostly spiral shaped (Figure 25).

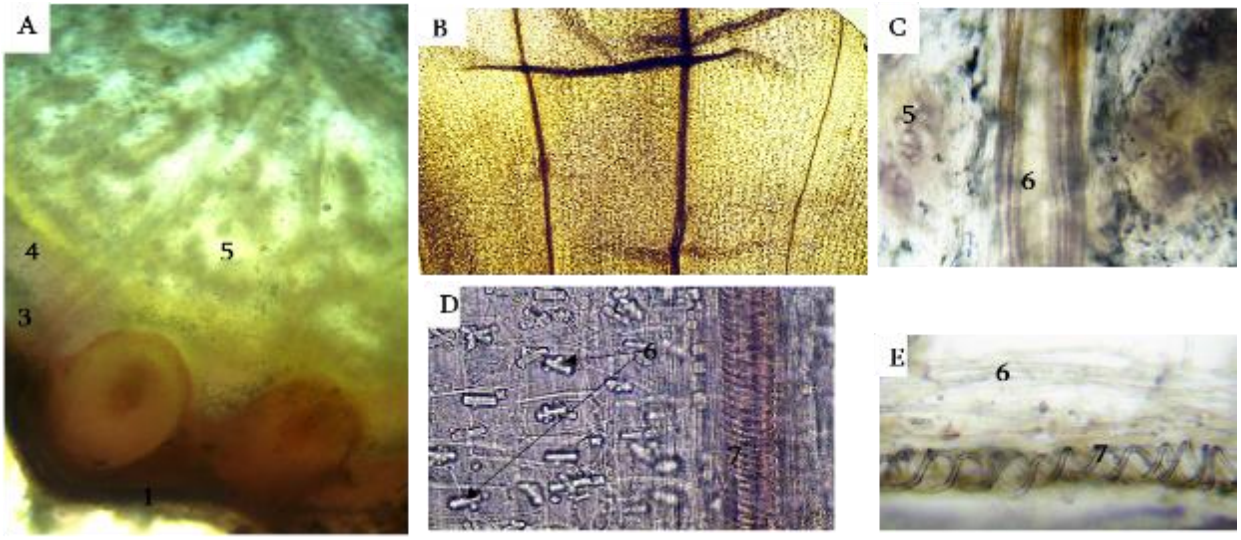


Figure 25. Microstructure of the bulb of *A. Ponticum*

A. Structure of the bulb; B., D. Fragments of the structure of the shell of the bulb; C., E. Fragments of the conductive vessels of the bulb;

1. Covering tissue; 2. Contour of the Root; 3. Shell; 4. Cambium; 5. Main tissue; 5. Xylem; 6. Phloem; 7. Spiral shaped Conducting vessel of xylem; 8. Crystals

Conclusions:

Microstructural characteristics of *A. ponticum* are established. The following set of diagnostic features is provided:

- The leaf of *A. ponticum* is covered with unicellular, cone shaped trichomes;
- Epidermis of the leaf of A. ponticum is single layered. Stomatas are immersed in the cells of epidermis;
- Palisade parenchyma is sorted in one row, big, square-shaped, thin-membraned, tightly packed tissue is outlined;
- Collateral type conductive vessel is represented in the leaf;
- Paracytic type of stomata is placed on the epidermis of the leaf;

- The covering tissue is actively cutinized, the epidermis is arranged in a single layer, its cells are characterized by a square shape and have strong thickening of the outer periclinal walls;
- Paracytic type of stomata is located between two basal cells in the covering tissue of the stem. The guarding cells of the stomata are lenticular, they are mostly aligned correctly with each other;
- In the central cylinder of the stem, a massive sclereenchymal tissue is presented. The degree of thickening of the membrane of mechanical cells, the intensity and the caliber decreases from the periphery in the direction of the center;
- In the outer peripheral area of mechanical cells, colateral type of conducting vessel, with large caliber, is differenciated;
- The thickening of the sheath of conducting vessels is predominantly spiral;
- Stamens are 6, ordered in two rows. The length of the outer filaments is slightly shorter or equal to the length of petal.
- Inner filaments are split into three parts, lateral parts are higher than middle. Style of carpel is long, higher than petals;
- Covering tissue of the bulb has multiple layer. Parenchyma of the tunic in represented with thin-layered, elongated cells. Conducting vessels are mostly spiral shaped;

Conclusion for both plants

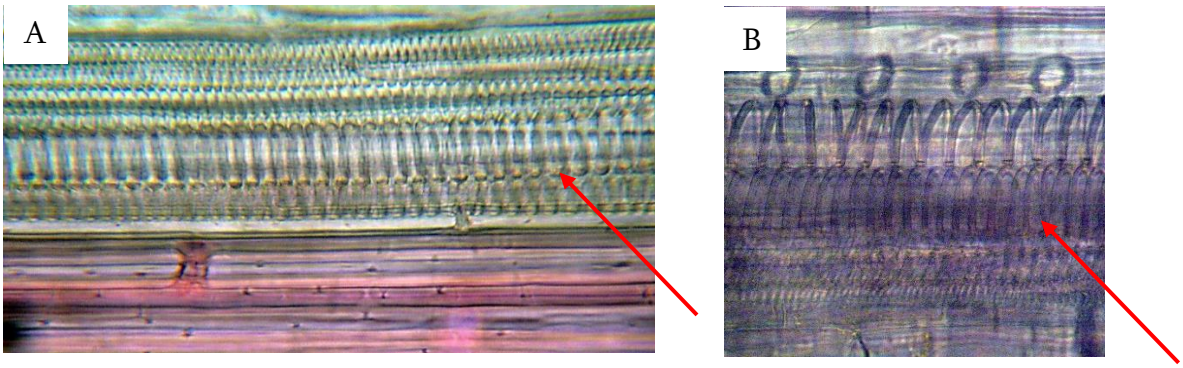


Figure 26. Spiral structure of vascular bundle. A- *Allium saxatile;* B-*Allium ponticum*

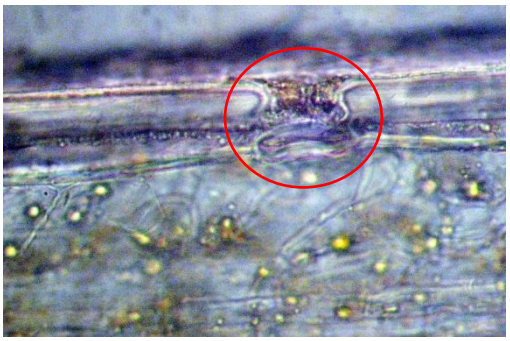


Figure 27. Stomatas of the leaves of *A. ponticum* immersed in eppidermis

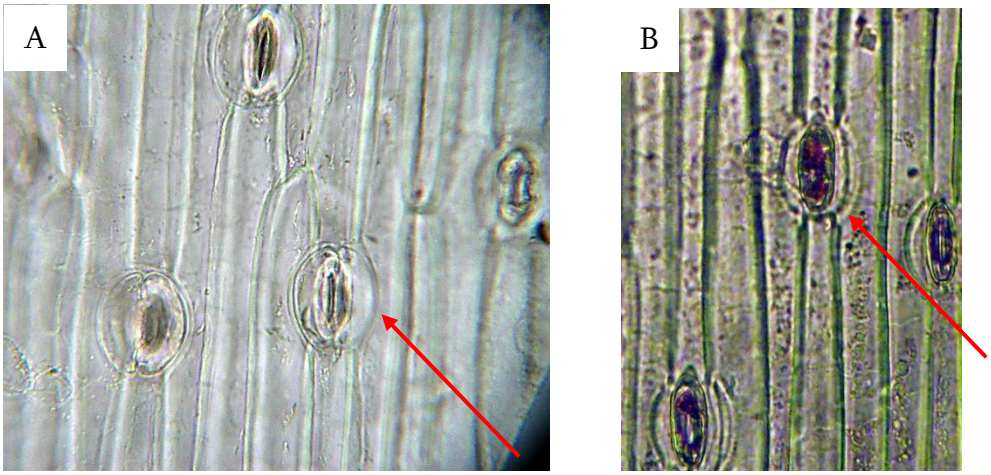


Figure 28. Paracytic type stomata of A – *A. saxatile* and B – *A. ponticum*

Figure 29. Trichomes differentiated on covering tissue of the leaf of *A. ponticum*

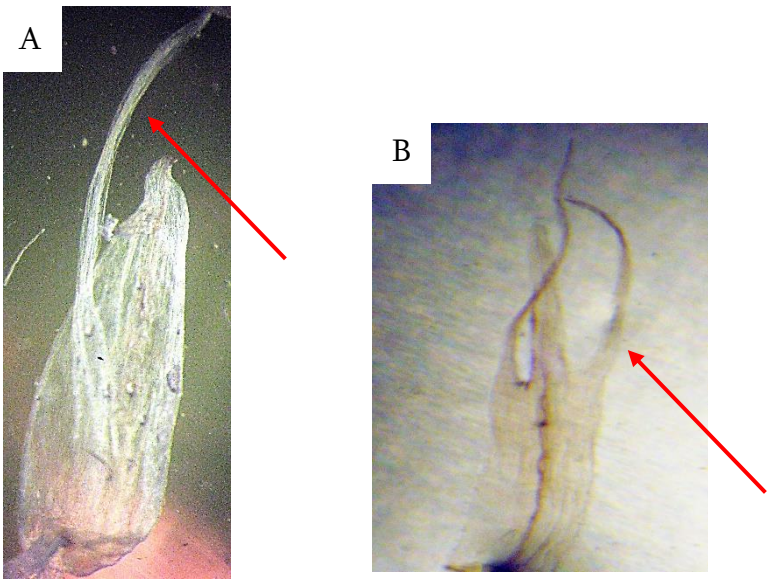


Figure 30. Stamen filaments of A – *Allium saxatile* and B – *Allium ponticum*

Microscopic characterisation of both plant material revealed the elements that could distinguish these species frome one another. Spiral structure of vascular bundle is common for the plants genus *Allium*. Both plants have paracytic type stomatas. Stomatas on the leaf of *A. saxatile* are displaced on the level of epidermis, in case of *A. ponticum* stomatas are immersed in eppidermis. The leaf of *A. ponticum* is covered with unicellular, cone shaped trichomes. Stamen filaments of *A. saxatile* are longer than the petals, Pistil's style is cut from the perianth. In case of *A. ponticum* filaments are split into three parts and lateral parts are longer than middle, their length is shorter or equal of the length of perianth.

3.1.3. Numerical indicators of studied objects

During the research several numerical indicators were determined, such as: moisture and total ash content, total furostanolic saponins in plant material and crude extract of both plants, total phenolic compounds. Considering the results of phytochemical analysis, we decided to quantify furostanolic saponins and flavonoids in studied objects, as they were major compounds for both plants. To determine total ash and Moisture we used World Health Organization guideline for "Quality control methods for herbal materials". To determine total furostanolic saponin content

we used spectrophotometric reference method and to determine content of Astragalin and Ferulic acid, we developed HPLC method (Methods: 2.3.8.; 2.3.9. and 2.3.10.). All the calculations were performed on dried plant material of research objects. Plants were dried in a air oven.

Table 10. Numerical indicators of studied objects

	Plant material	
Indicator	*A. saxatile*	*A. ponticum*
Moisture	7.21 %	7.77 %
Total ash cont.	8.09 %	4.9 %
Furostanolic saponins in plant material	1.69%	0.37%
Furostanolic saponins in crude extract	37.15%	11.54%
Astragalin	0.87%	-
Ferulic acid	-	0.076%

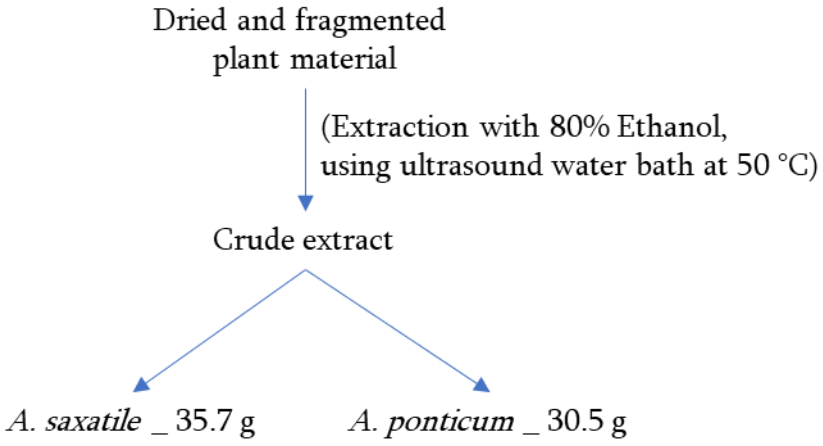
Conclusion

Several numerical indicators of studied objects are determined. Moisture - *A. saxatile* 7.21%, *A. ponticum* 7.77%; Total ash cont. *A. saxatile* 8.09%, *A. ponticum* 4.9%; furostanolic saponins in plant material *A. saxatile* 1.69%, *A. ponticum* 0.37%; furostanolic saponins in crude extract *A. saxatile* 37.15%, *A. ponticum* 11.54%; Astragalin: *A. saxatile* 0.87%; Ferulic acid: *A. ponticum* 0.076%.

3.2. Phytochemical study of secondary metabolites

3.2.1. Extraction of secondary metabolites from studied objects.

500 g of aerial part of each plant, fragmented to 1 mm size particles were extracted with 80% ethanol (Method 2.3.2)(Graph 3).



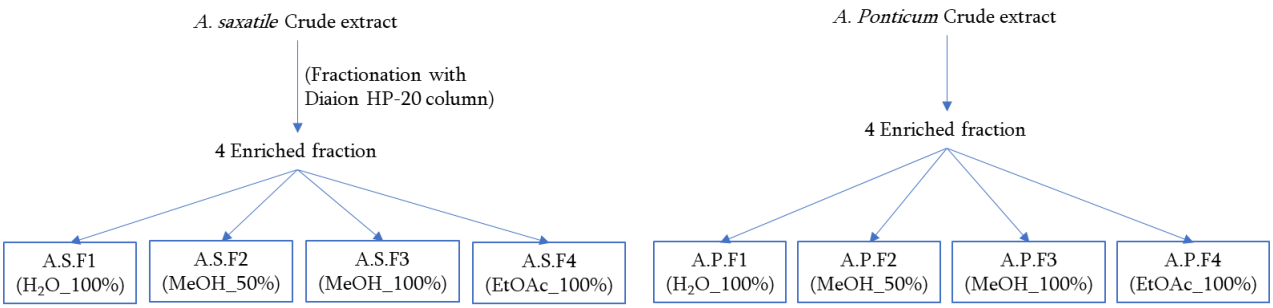
Graph 3. Extraction of the sum of secondary metabolites.

Conclusion

The yield of crude extract was 35.7 g for – *A. saxatile* resulting 7.5% of plant material, and 30.5 g for *A. ponticum,* resulting 6.1% yield from plant raw material.

3.2.2. Fractionation of the sum of secondary metabolites

15 g of crude extracts of each plant were subjected to fractionation, with column chromatography, and Diaion HP-20 as a stationary phase (Method 2.3.3.), to obtain 4 enriched fractions from each plant. Yield of each of the fractions is indicated in Table 12.



Graph 4. Fractionation of the crude extracts of the plants

Table 11. Yield of the fractions.

Fraction	Mobile Phase	Yield
A. saxatile F1(A.S.F1)	100% water	22.6% (8.1 g)
A. saxatile F2 (A.S.F2)	50% water : 50% MeOH	4.7% (1.7 g)
A. saxatile F3 (A.S.F3)	100% MeOH	9.8% (3.5 g)
A. saxatile F4 (A.S.F4)	100% Ethyl acetate	0.05% (0.02 g)
A. ponticum F1(A.P.F1)	100% water	21.9% (6.7 g)
A. ponticum F2 (A.P.F2)	50% water : 50% MeOH	12.7% (3.9 g)
A. ponticum F3 (A.P.F3)	100% MeOH	7.5% (2.3 g)
A. ponticum F4 (A.P.F4)	100% Ethyl acetate	0.6% (0.2 g)

3.2.3. Qualitative characterization of secondary metabolites of research objects.

Qualitative characterisation of secondary metabolites was studied using TLC (Method 2.3.4.).

At the first stage of the research, we studied the qualitative content of secondary metabolites in the total extracts obtained from the studied objects, in particular the presence of steroidal saponins, generally present in the *Allium* genus (Figure 31).

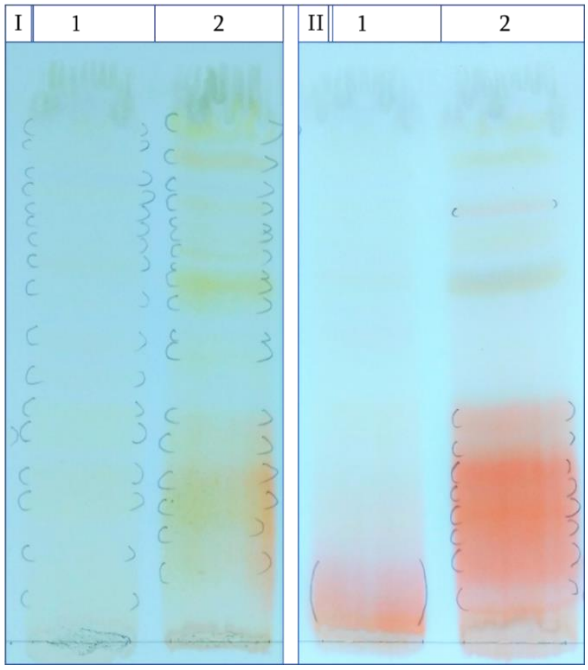


Figure 31. TLC of saponins in studied objects.

1. *A. ponticum* crude extract. 2. *A. saxatile* crude extract

I. Mobile phase _ 1; Revelation _ Reagent A;

II. Mobile phase _ 1; Revelation _ Reagent B;

Results of the TLC shows that, both plants are rich with steroidal saponins, because they give specific yellow coloration with “Reactive A” (1% Vanilline solution). Revelation of TLC with “Reactive B” (4-Dimethylamino benzaldehyde solution), gives specific purple-red coloration which is characteristic for furostanolic saponins (Figure 19).

We studied then the qualitative content of secondary metabolites in the total extracts and fractions obtained from the studied objects in order to better understand the distribution of steroidal saponins in fractions, obtained with the fractionation described above (Figure 32).

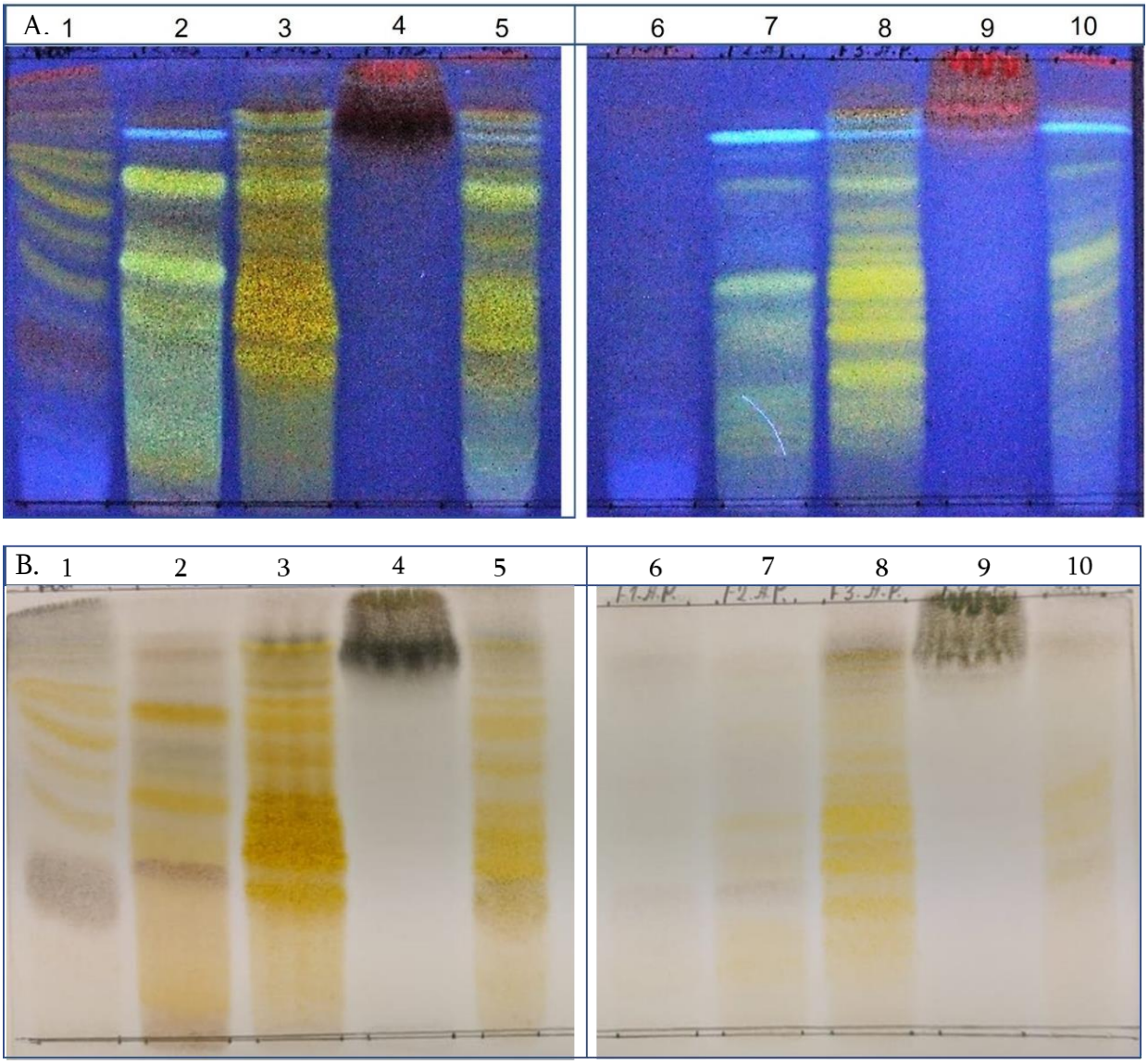


Figure 32. TLC of saponins in studied objects. A- 366 nm. B-visible light.

1- A.S.F1; 2- A.S.F2; 3- A.S.F3; 4- A.S.F4; 5- *A. saxatile* crude extract; 6- A.P.F1; 7- A.P.F2; 8- A.P.F3; 9- A.P.F4; 10- *A. ponticum* crude extract;

Mobile phase: _ 1; Revelation _ "Reagent A";

Saponins give yellow coloration, after revelation with 1% Vanillin solution in methanol. Results of TLC shows that 100% methanolic fraction of both plants is rich with steroidal saponins

(Furostanolic and spirostanolic), but saponin content in 50% methanolic fraction is lower (Figure 20). Saponins were not found in fractions obtained with 100% Ethyl acetate.

To determine which aglycones are present in the observed saponins, we used acid hydrolysis method to obtain the aglycones from both plants. Qualitative analysis was then performed with TLC method and reference compounds (Figure 33).

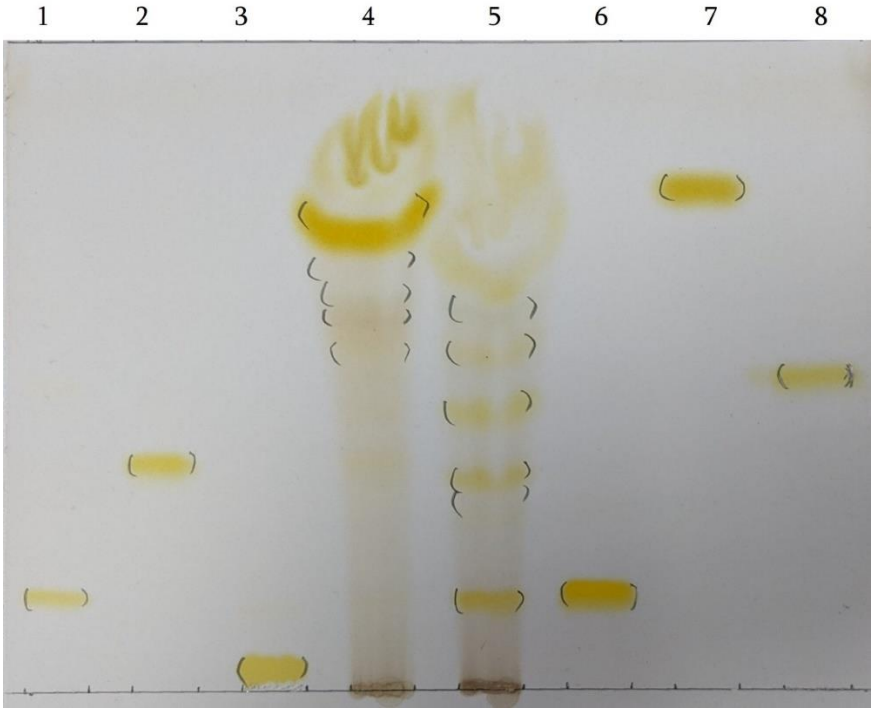


Figure 33. TLC of sapogenins.

1 – Agigenin; 2- β-Chlorogenin; 3 - Alliogenin; 4 - *A. saxatile* Sum of the sapogenins; 5 - *A. ponticum* Sum of the sapogenins; 6 - Neoagigenin; 7 - Diosgenin; 8 - Yucagenin;

Mobile phase: _ 2; Revelation _ "Reactive A"

TLC analysis of the sum of the aglycones showed possible content of Diosgenin in *A. saxatile* and Agigenin, Yucagenin and β-Chlorogenin in *A. ponticum.*

During the research we also studied content of flavonoids in crude extract and fractions of studied objects, to determine distribution of flavonoids among the fractions (Figure 34).

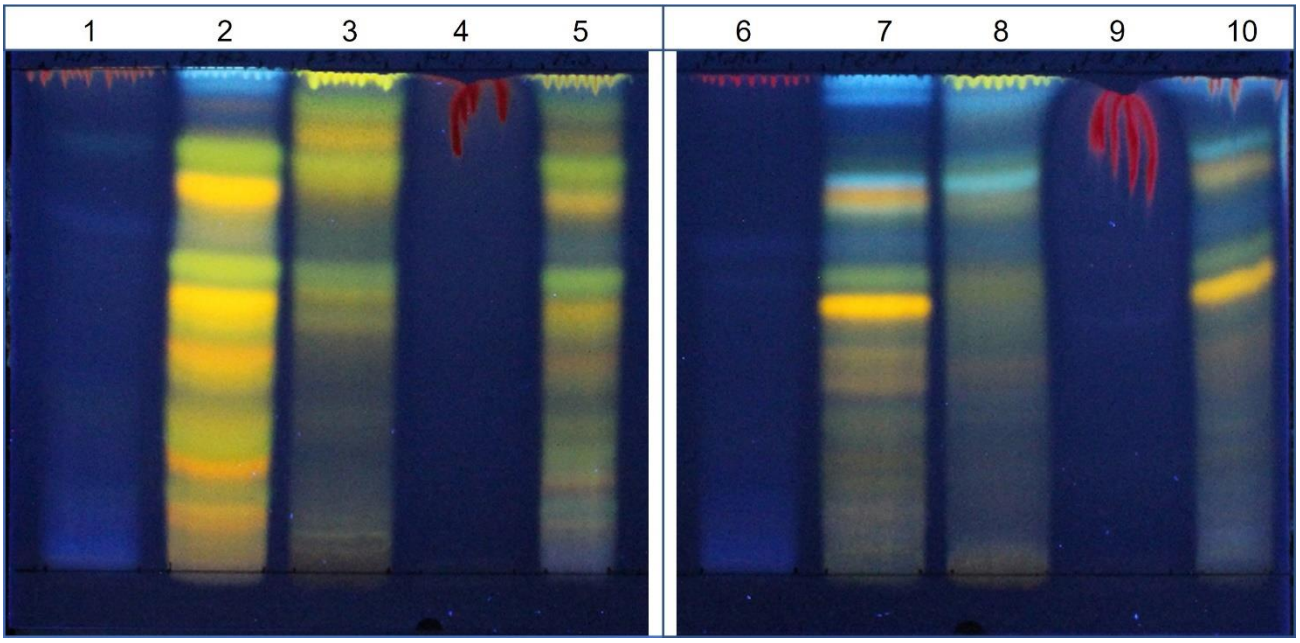


Figure 34. TLC of flavonoids.

1- A.S.F1; 2- A.S.F2; 3- A.S.F3; 4- A.S.F4; 5- *A. saxatile* crude extr.; 6- A.P.F1; 7- A.P.F2; 8- A.P.F3; 9- A.P.F4; 10- *A. ponticum* crude extr.

Mobile phase: _ 5; Revelation _ "Reagent C";

After spraying with "Reagent C" (DPBAE), phenolic compounds give blue fluorescence. Flavonoids give mostly yellow, orange, and green fluorescence. Precisely: flavanons give dark green fluorescence; flavones and flavonols give orange fluorescence, under 366 nm wavelength.

Results of TLC analysis has determined that 50% methanolic fractions of both plants contain mostly flavonoids and phenolic compounds, especially A.S.F2 fraction is rich. 100% methanolic fraction of each plant contains also phenolic compounds and flavonoids, but with less concentrations. According to the fluorescence of TLC spots, we can conclude that fractions of *A. saxatile* are rich with flavonoids, while fractions of *A. ponticum* contain mostly phenolic compounds.

Apart from the above-mentioned secondary metabolites, the presence of additional compounds, namely: Alkaloids, Cardiac glycosides, Antraglycosides and Tannins was analyzed through TLC analysis (Table 12).

Table 12. Phytochemical screening of crude extract and fractions of studied objects.

	A.S. Crd. extr	A.S.F1	A.S.F2	A.S.F3	A.P. Crd. extr	A.P.F1	A.P.F2	A.P.F3
Alkaloids	-	-	-	-	-	-	-	-
Cardiac glycosides	-	-	-	-	-	-	-	-
Flavonoids	+	-	+	-	+	-	+	-
Saponins	+	-	-	+	+	-	-	+
Anthraquinones	-	-	-	-	-	-	-	-
Tannins	-	-	-	-	-	-	-	-

Conclusion:

TLC analysis has determined that crude extracts of both plant is rich with saponins. Among the fractions, saponin content in 100% methanolic fraction of both plant is high.

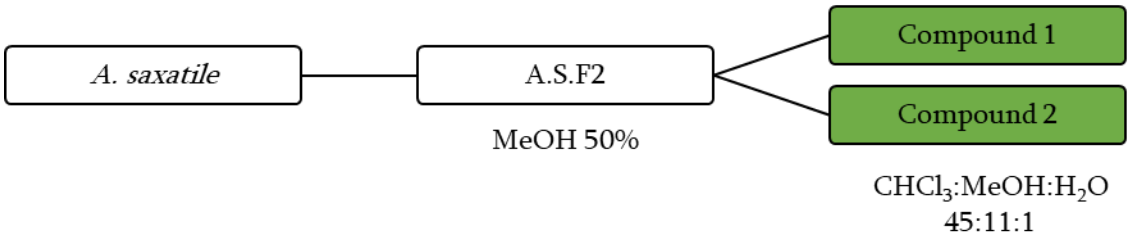
Studies have determined that *A. saxatile* probably contains saponins with Diosgenin aglycone and *A. ponticum* with Agigenin, Yucagenin and β-Chlorogenin aglycones.

TLC analysis determined that 50% methanolic fraction of both plant is rich with flavonoids, especially A.S.F2

Alkaloids, Cardiac glycosides, Antraglycosides and Tannins were not found in crude extract and fractions of both plants with TLC analysis.

3.2.4. Isolation of individual compounds from biologically active fractions

Two distinct compounds, Compound 1 (16 mg) and Compound 2 (11 mg), were obtained by separating 1000 mg of the 50% methanolic fraction of *A. saxatile* using chromatography. The mobile phase used was a mixture of chloroform, methanol, and water in the ratio of 45:11:1.



Graph 5. Isolation of individual compound from 50% methanolic fraction of *A. saxatile.*

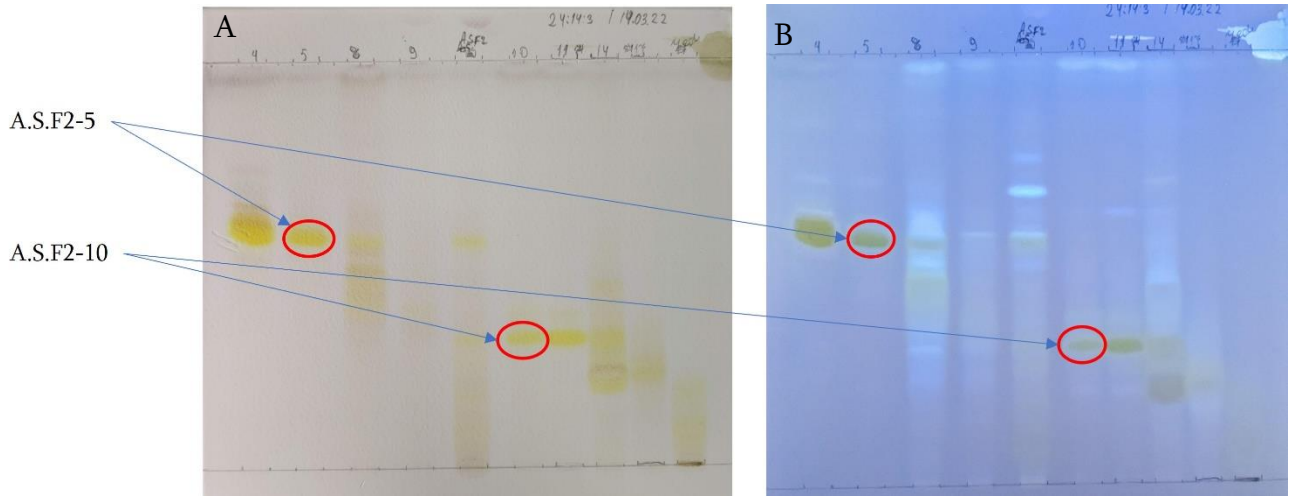
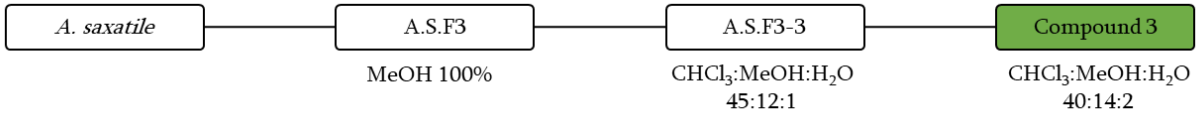


Figure 35. TLC of individual compounds obtained from 50% methanolic fraction of *A. saxatile.*

Mobile phase: _ 1; Revelation _ "Reagent A"

A- visible light; B-366 nm

Finally, one individual compound (**Compound 3** 14 mg), was obtained by chromatographically separating 1500 mg of the 100% methanolic fraction of *A. saxatile*.



Graph 6. Isolation of individual compound from 100% methanolic fraction of *A. saxatile*

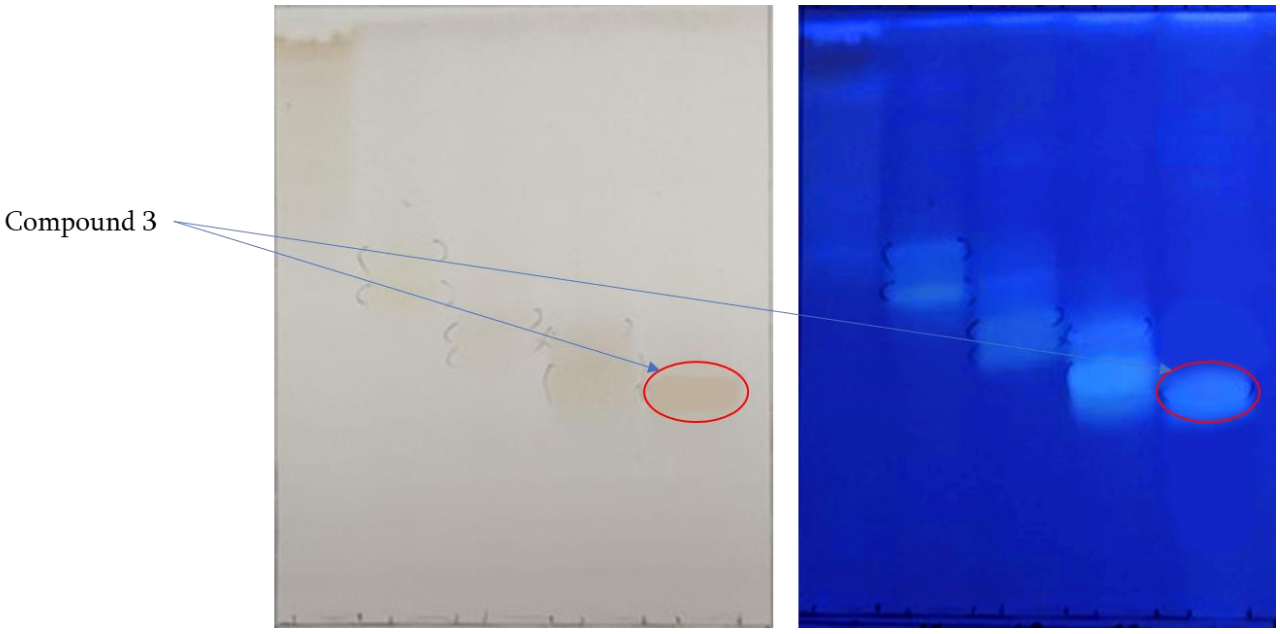


Figure 36. TLC of individual compounds obtained from 100% methanolic fraction of *A. saxatile.*

Mobile phase: _ 1; Revelation _ "Reactive B";

Conclusion

Separation of 50% and 100% methanolic fraction of *A. saxatile* with column chromatography gave three individual compounds, among them two flavonoids and one steroidal saponin **(Compounds 1, 2 and 3).**

3.2.5. Determination of chemical structure of individual compounds

NMR (Method 2.3.6) and Mass spectrometry (Method 2.3.7) were employed to determine chemical structure of individual compounds.

Compound 1 – This compound was isolated from the 50% methanolic fraction of *A. saxatile* as a yellow amorphous powder, with a melting temperature of 178 °C, a specific rotation of Compound 1 $[\alpha]_D^{25}$ = -36.3°, a molecular weight of 447.0992 m/z [M-H] corresponding to the molecular formula $C_{21}H_{20}O_{11}$ (theoretical isotopic mass of 448.1006). Chemical shift of carbon and hydrogen atoms are given in table 13.

Table 13. Chemical shift of ^{1}H and ^{13}C atoms of **Compound 1**.

Aglycone			Sugar		
C-position	δC	δH	C-position	δC	δH
2	157.7		Glucose		
3	134.4		1''	102.63	5.25
4	178.15		2''	74.33	3.44
5	161.7		3''	76.63	3.43
6	98.48	6.21	4''	69.95	3.31
7	164.6		5''	77.03	3.2
8	93.34	6.41	6''	61.21	3.79
9	157.12				
10	104.35				
1'	121.39				
2',6'	130.88	8.06			
3',5'	114.68	6.89			
4'	160.18				

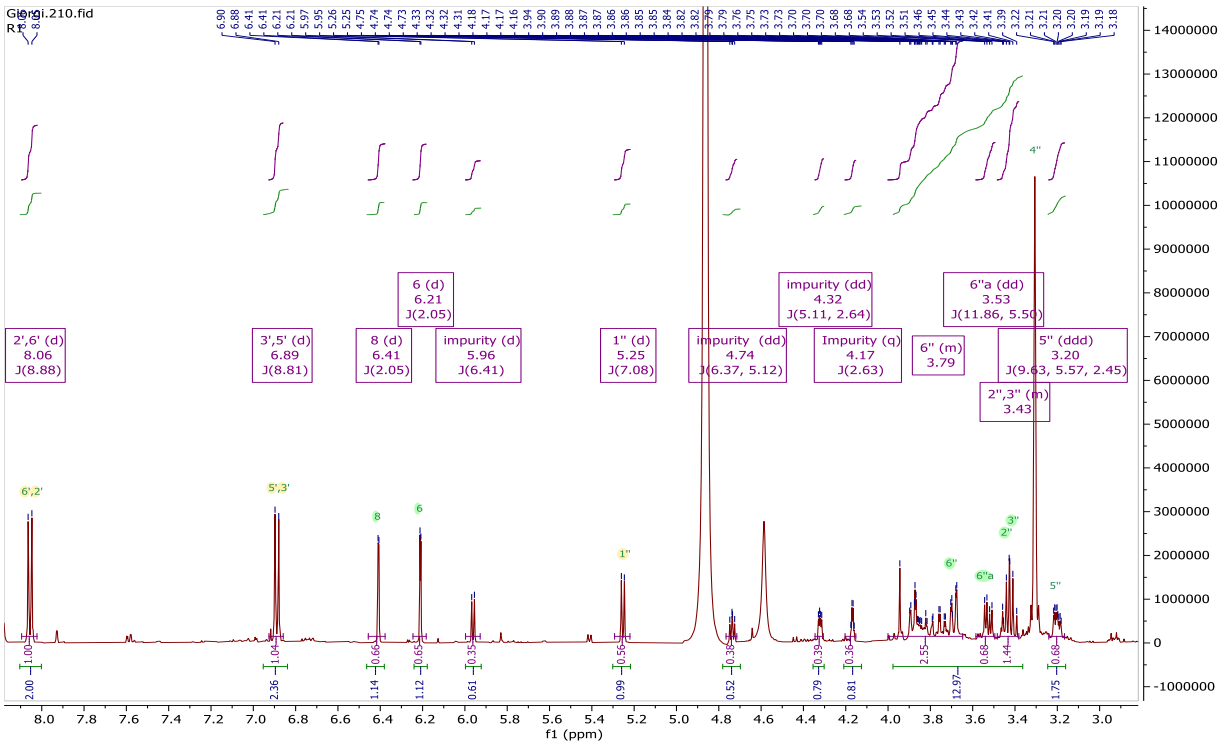


Figure 37. ^{1}H spectrum of Kaempferol 3-O-glucoside.

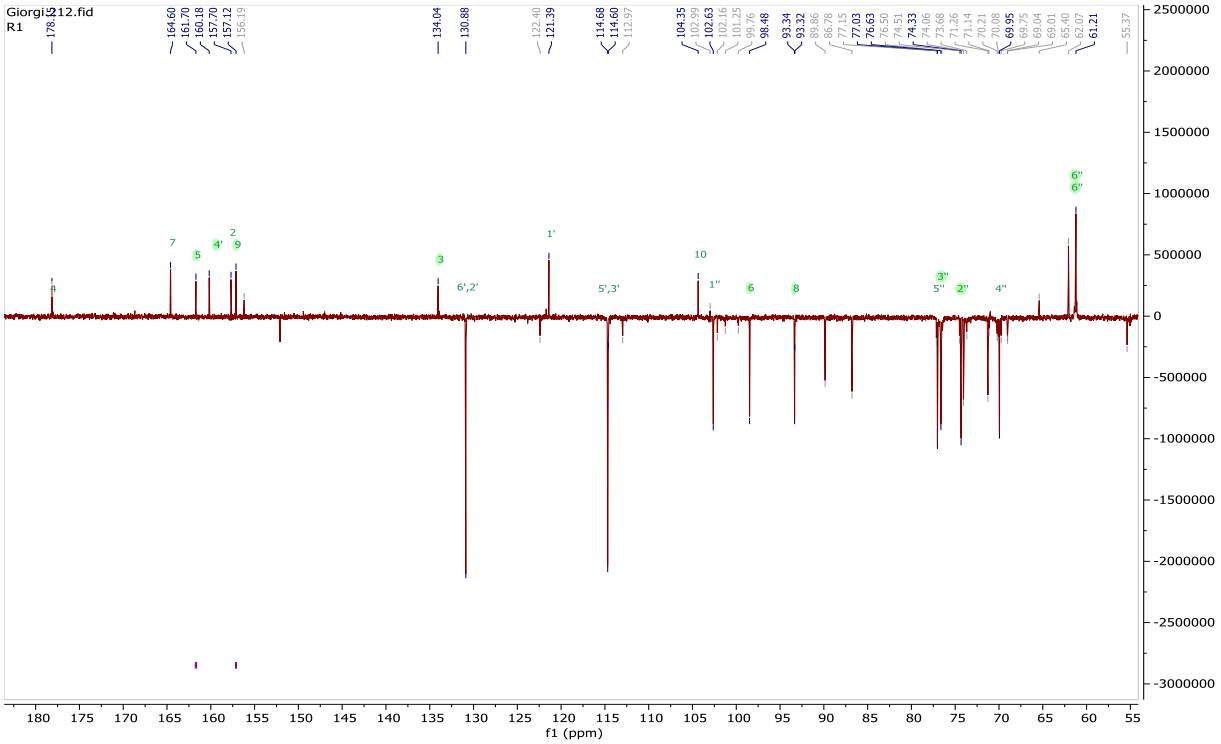


Figure 38. ^{13}C Spectrum of kaempferol 3-O-glucoside.

Figure 39. COSY Spectrum of kaempferol 3-O-glucoside.

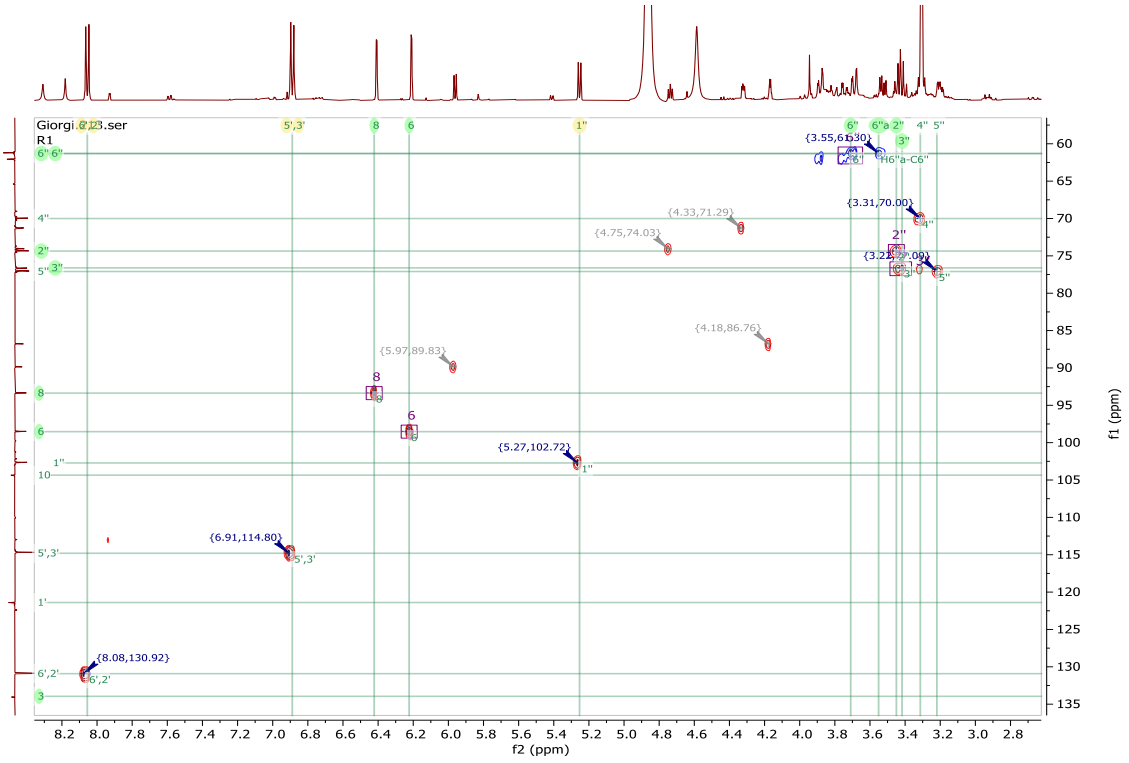


Figure 40. HSQC Spectrum of kaempferol 3-O-glucoside.

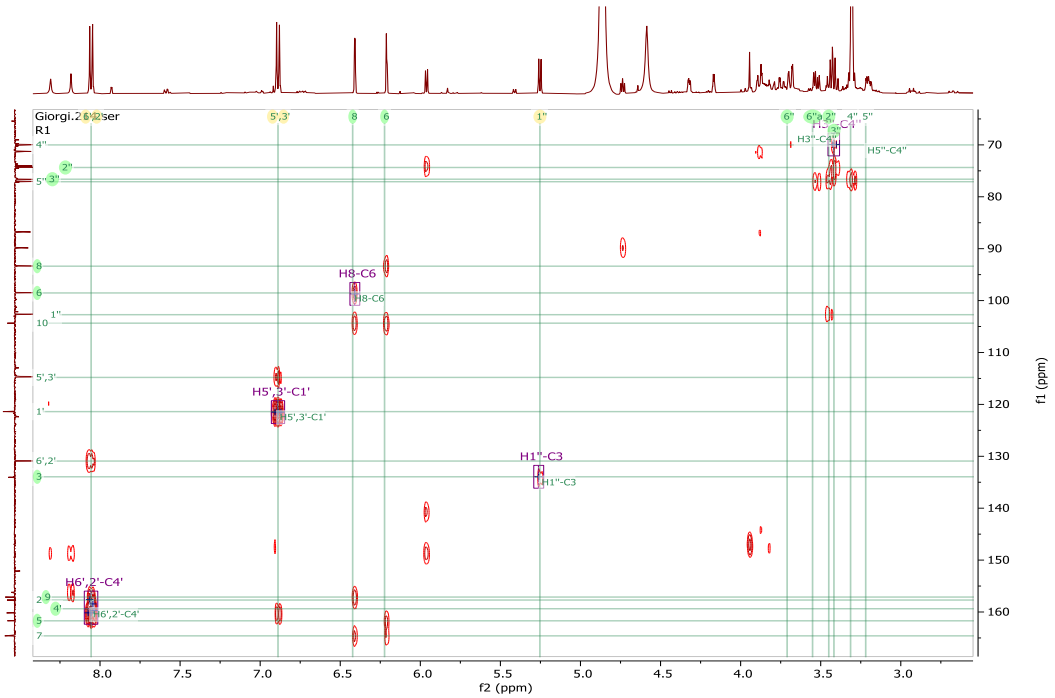


Figure 41. HMBC Spectrum of kaempferol 3-O-glucoside.

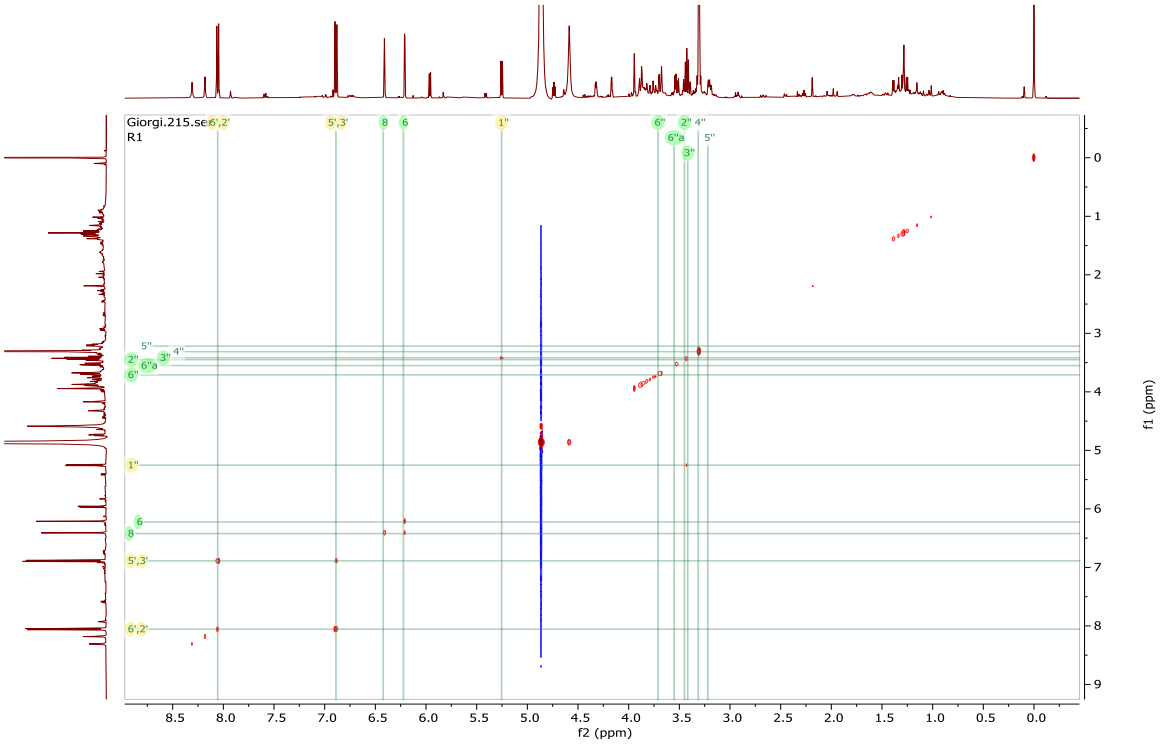


Figure 42. TOCSY Spectrum of kaempferol 3-O-glucoside.

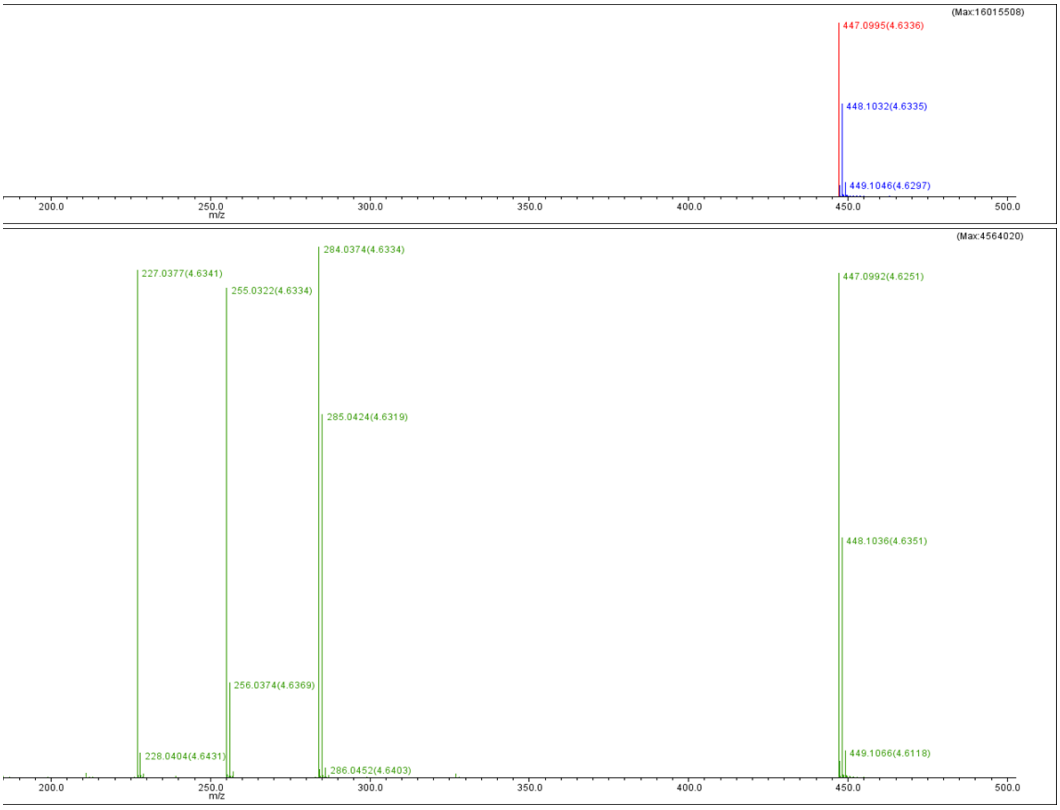


Figure 43. MS fragmentation of Compound 1 kaempferol 3-O-glucoside in negative mode. Fragment with 284-285 m/z corresponds to Kaempferol, after removing the glucose moiety.

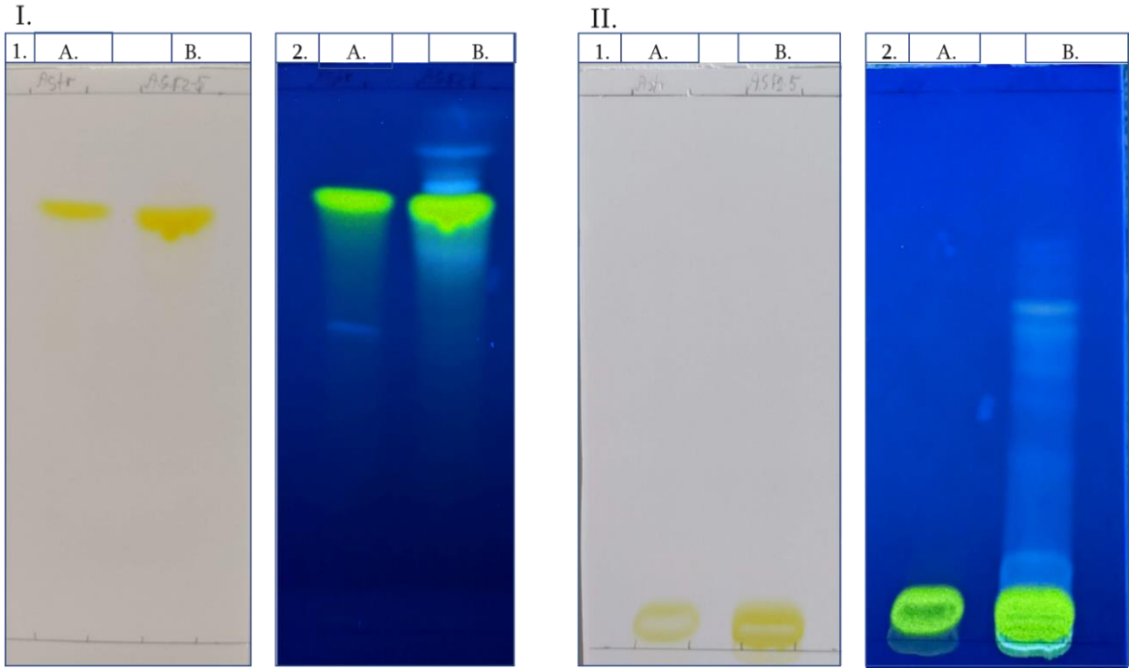


Figure 44. TLC of Astragalin and Compound 1

A. Astragalin; B. Compound 1.

Mobile phase : I. Dichloromethane : MeOH : H_2O _ 24:14:3; II. Formic acid : Acetone : Dichloromethane _ 8.5:25:85

Revelation_"Reagent C" 1. Visible light; 2. 366 nm;

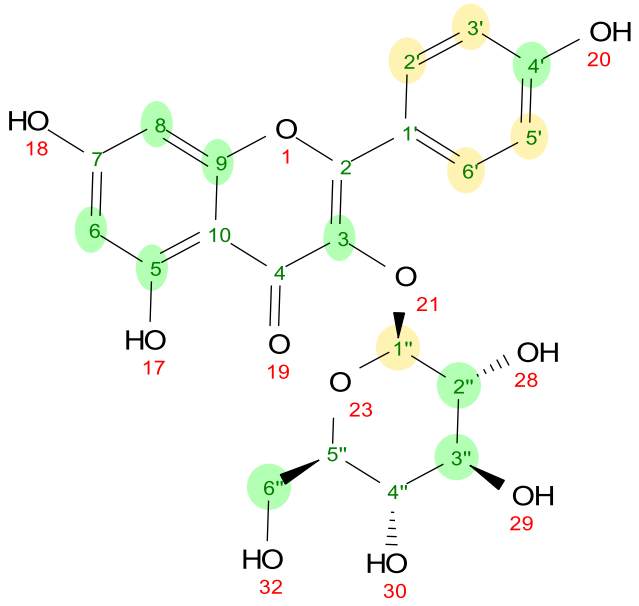


Figure 45. Structure of Kaempferol 3-O-glucoside.

Conclusion

Compound 1 – ^{1}H NMR spectrum has revealed an aromatic A_2B_2 spin system for B ring at δH 8.06 (2H, H-2′,6′) and 6.89 (2H, H-3′, 5′) and aromatic proton signals for A ring at δH 6.41 (1H, s, H-8) and 6.21 (1H, s, H-6). These data suggests that this compound represents a Kaempferol derivative, and effectively, proton and NMR data are coherent with those of a kaempferol aglycon, that could be found in the literature[261]. Proton signals of four CH in the δH 3.2-3.44 range (4H, m, H-2″, -3″, -4″, -5″), CH_2 at δH 3.71 and anomeric CH at δH 5.25 indicates the existence of a glucose moiety, which can be also confirmed by the ^{13}C spectrum with values at δC 102.63 (C-1″), 74.33 (C-2″), 76.63 (C-3″), 69.95 (C-4″), 77.03 (C-5″), and 61.21 (C-6″)[122]. The position of the glucose moiety at C-3 is confirmed by the existence of an HMBC correlation between H-1" and C-3.

According to the results of TLC, nuclear-magnetic resonance and mass spectrometry, the structure of compound 1 was determined as kaempferol 3-O-glucoside (Astragalin).

Obtained NMR data of sample was compared to NMR data in literature, comparison of the data showed that, NMR data is coherent to 13C-NMR data of astragalin in literature [262]. Identity of astragalin was also confirmed with TLC, with different mobile phases, using reference standard of Astragalin. Results have determined identity of Compound 1 as Astragalin (Figure 32). This compound was also isolated from other *Allium* species, such as *A. ursinum, A. victorialis, A. ampeloprasum, A. paradoxum* [122], [263]–[265]. Astragalin is also isolated from the plants of other families, such as: Convolvulaceae Ebenaceae, Rosaceae, Eucommiaceae and etc [266]. *Cuscuta chinensis* has extremely high content of kaempferol 3-O-glucoside (29–34% of the total phenolics) among all species[267].

Compound 2 – was isolated from 50% methanolic fraction of *A. saxatile* – as yellow amorphous powder, with a melting temperature 197-200 °C; a specific rotation of $[\alpha]_D^{25}$ = -58.8°; a molecular weight of 594.5181 m/z [M-H], 617.1475 m/z [M+Na], corresponding to a Molecular formula of $C_{27}H_{30}O_{15}$ (corresponding to a theoretical isotopic mass of 594.1584). Chemical shift of carbon and hydrogen atoms are given in table 14.

Table 14. Chemical shift of ^{1}H and ^{13}C of **Compound 2**.

Aglycon			Sugar		
C-position	δC	δH	C-position	δC	δH
2	157.09		Glucose		
3	133.03		1''	98.83	5.74
4	178		2''	78.65	3.59
5	161.84		3''	77.54	3.59
6	98.27	6.18	4''	70.42	3.31
7	164.27		5''	76.99	3.31
8	93.14	6.38	6''	61.22	3.59
9	157.04		Rhamnose		
10	104.57		1'''	101.22	5.22
1'	121.71		2'''	70.99	4.00
2',6'	130.74	8.04	3'''	70.88	3.8
3',5'	114.67	6.89	4'''	78.64	3.37
4'	159.93		5'''	68.51	4.00
			6'''	16.12	0.99

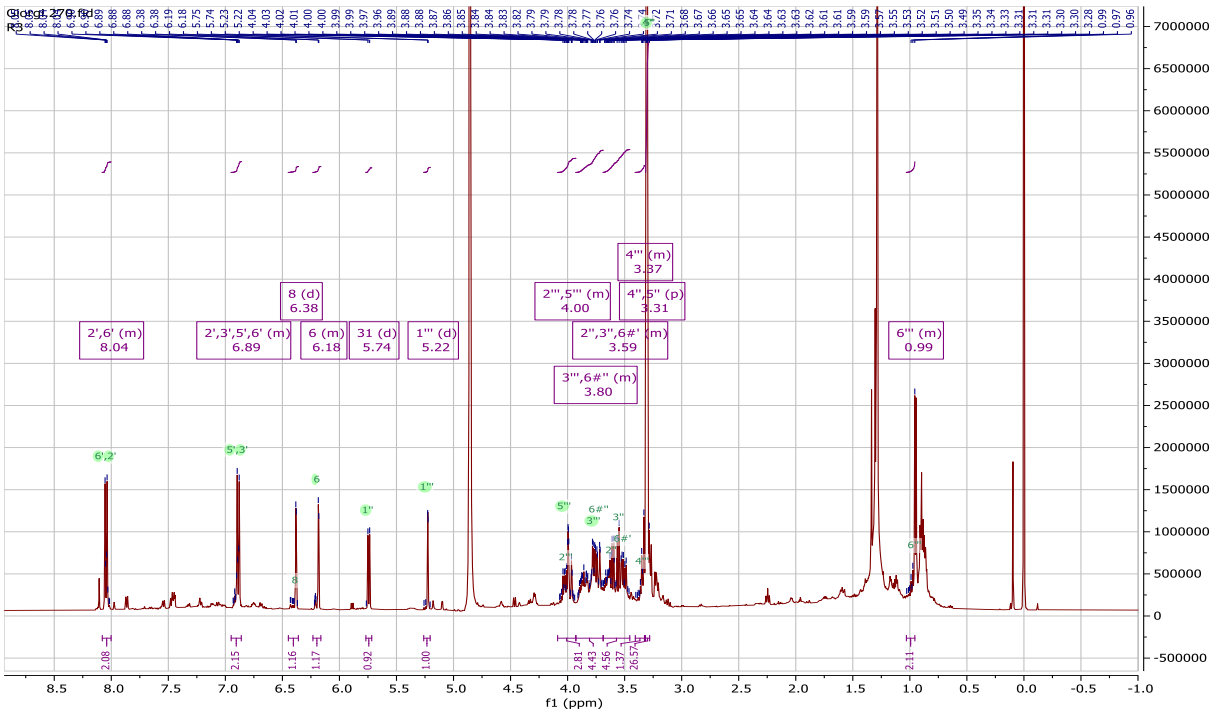


Figure 46. ^{1}H Spectrum of kaempferol 3-O-neohesperidoside.

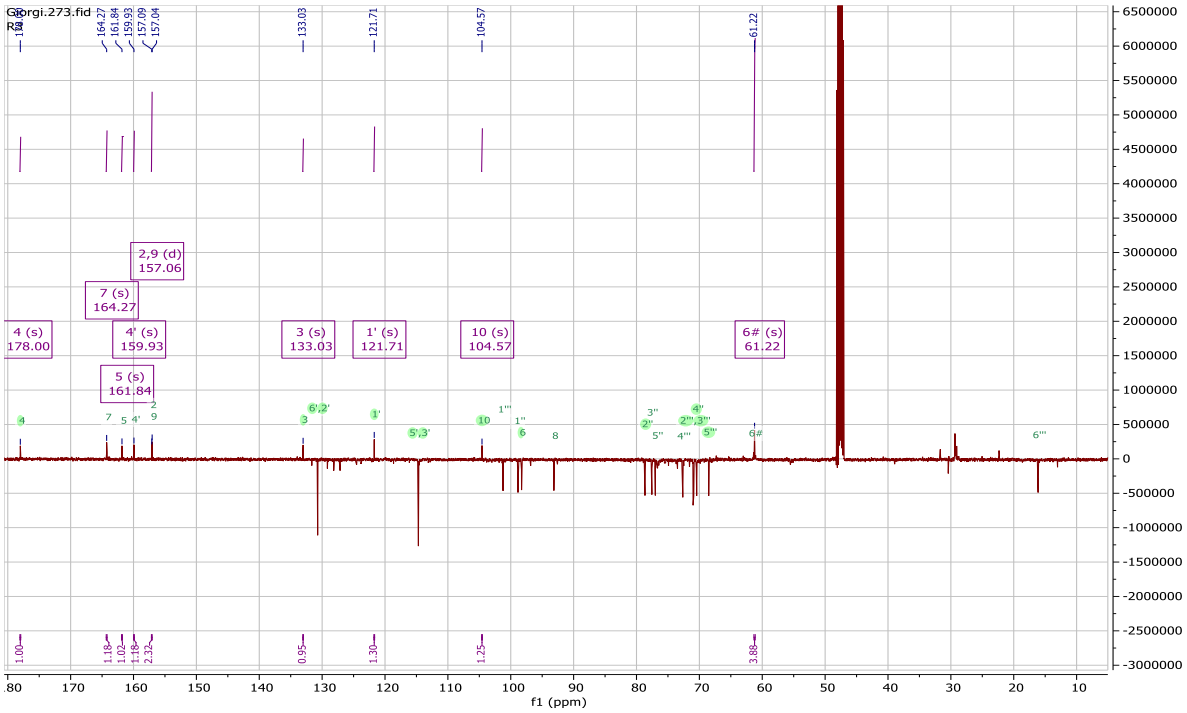


Figure 47. ^{13}C Spectrum of kaempferol 3-O-neohesperidoside.

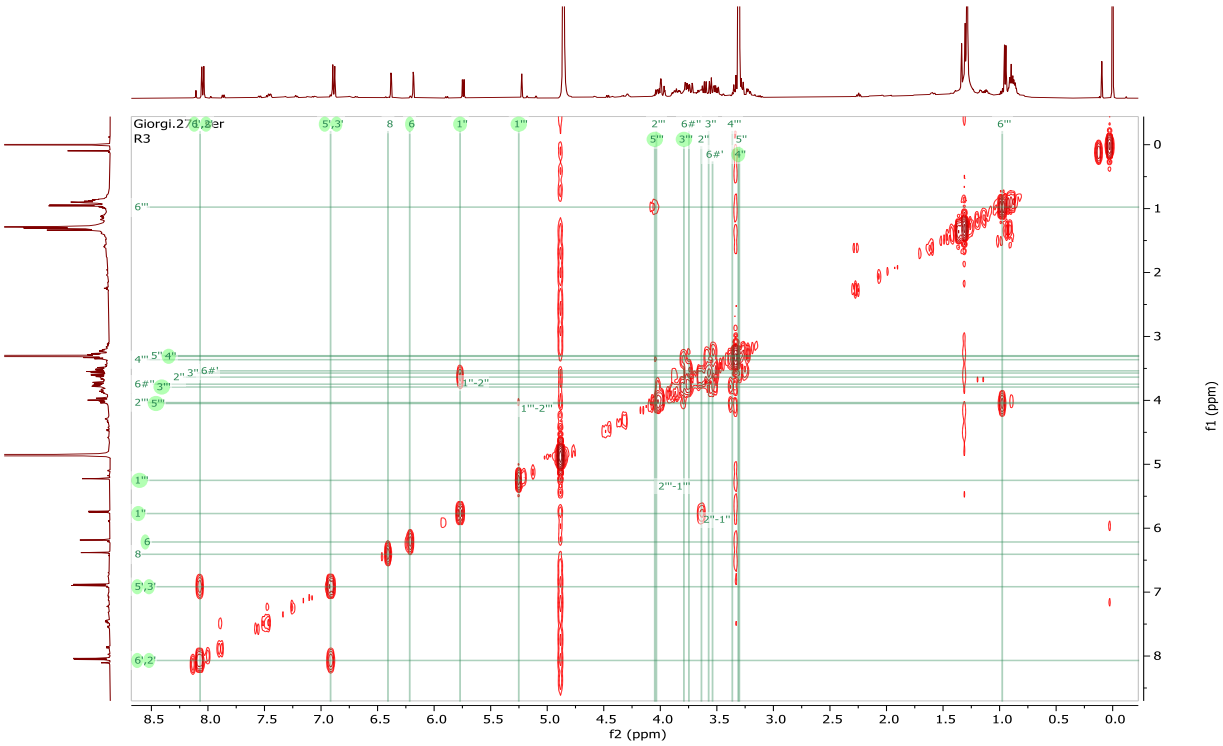


Figure 48. COSY Spectrum of kaempferol 3-O-neohesperidoside.

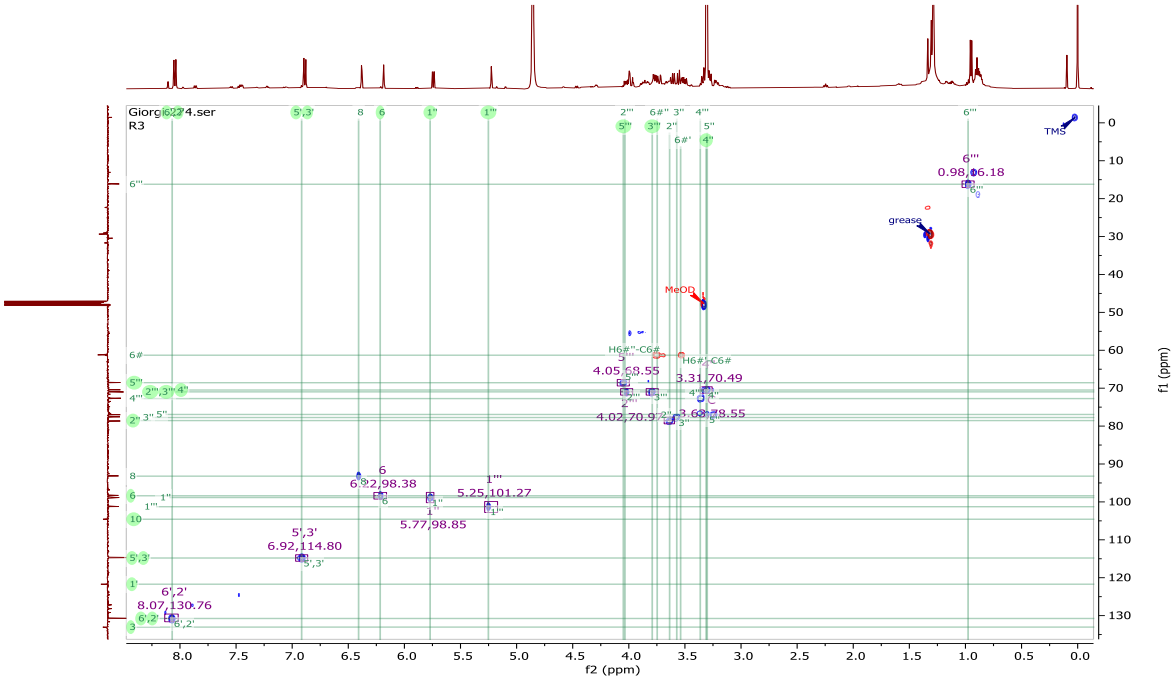


Figure 49. HSQC Spectrum of kaempferol 3-O-neohesperidoside.

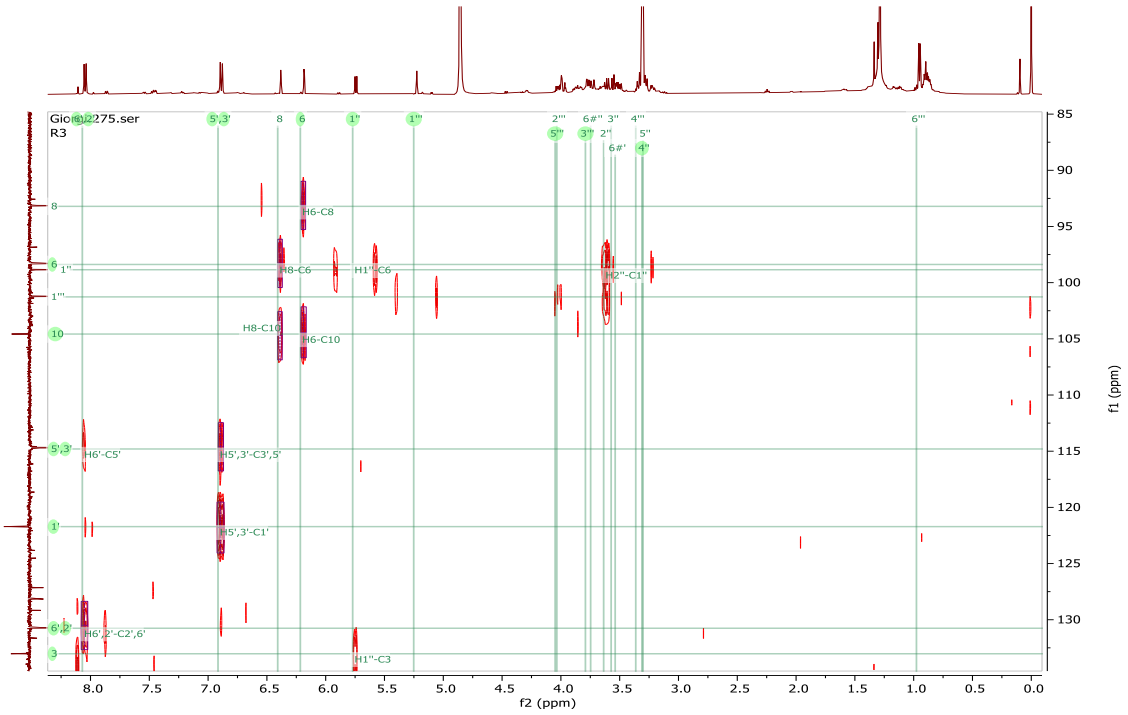


Figure 50. HMBC Spectrum of kaempferol 3-O-neohesperidoside.

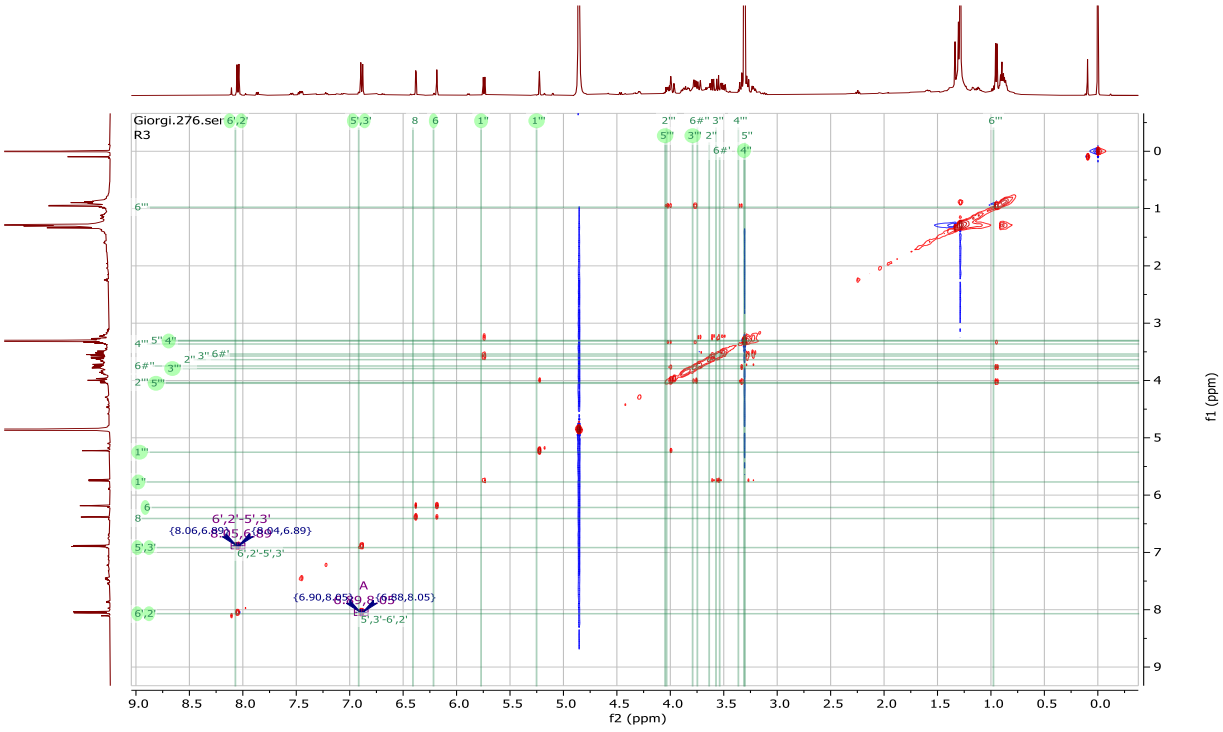


Figure 51. TOCSY Spectrum of kaempferol 3-O-neohesperidoside.

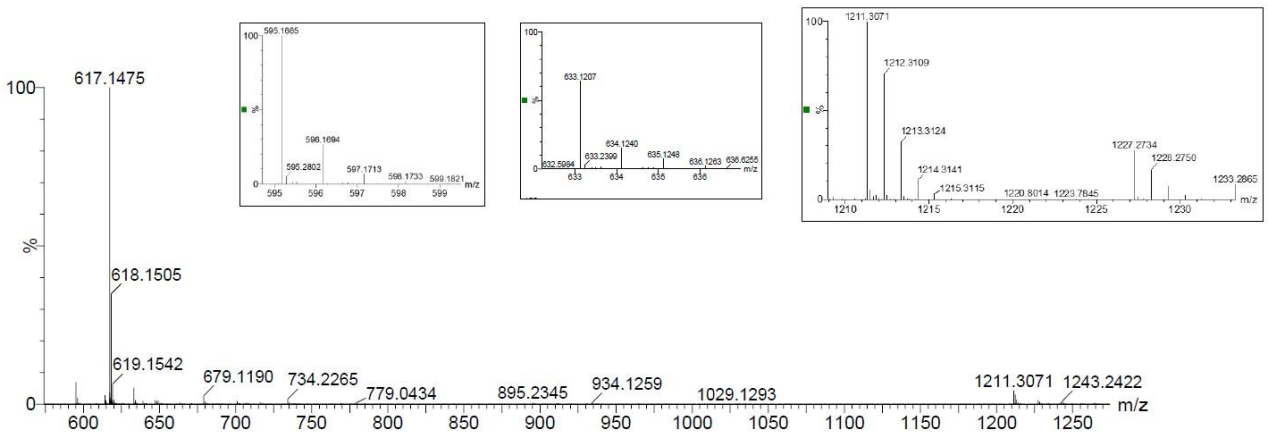


Figure 52. MS fragmentation of kaempferol 3-O-neohesperidoside.

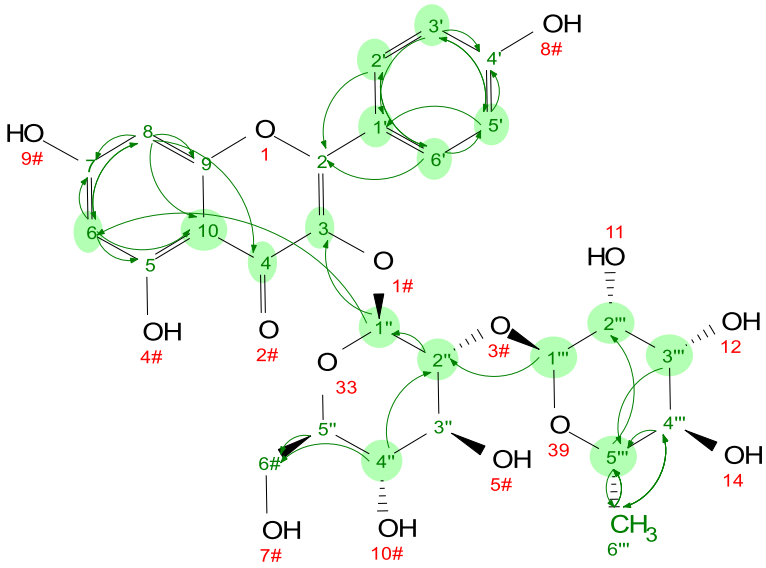


Figure 53. Structure of kaempferol 3-O-neohesperidoside with HMBC correlations.

Conclusion

^{13}C NMR spectrum of compound 2 showed 32 resonances. Obtained signals at d 5.74 (d, J = 7.5 Hz) and 5.22 (d, J = 1.3 Hz) revealed the presence of two sugar moieties that present in one case the α-configuration and in the other one the β-configuration. Complete assignments of each sugar proton system were achieved by considering TOCSY and ^{1}H–^{1}H COSY spectra. The presence of a glucose unit was confirmed by the large vicinal coupling among ring protons due to the trans-diaxial orientation.

The ^{1}H NMR spectrum showed two coupled doublets with a very small J-value, typical of the two meta-related H-6 and H-8 protons of the A ring of the flavonoid unit and an AA'BB' system (d 8.04 and 6.89, J = 8.9 Hz) that is due to the B ring of the same flavonoid unit. This lead to a kaempferol aglycon, as for compound 1. The proton and carbon data showed this time the presence of two sugars moieties, one corresponding to glucose, the other to rhamnose. The position of the sugar moieties are confirmed by the existence of HMBC correlations between H-1" (glucose) and C-3 and between H-1"' (rhamnose) and C-2" (glucose). Then the glucose unit, that was directly connected to kaempferol, was linked through position 2 to rhamnose. Obtained NMR data was in correlation with the data given in the literature[268]. The combination of NMR data and the results of MS analysis helped to elucidate the structure of compound 2 as kaempferol 3-O-neohesperidoside. NMR data of sample was compared to data in literature to confirm its structure [262]. This compound was isolated from *Paris verticillate* M. v. BIEB for first time [269]. Among *Allium* species this compound was isolated from *A. ursinum* [270].

Compound 3 – was isolated from 100% methanolic fraction of *A. saxatile* – as white amorphous powder, with a melting temperature of 298-300 °C; a molecular weight of 885.4833 m/z [M+H] corresponding to a Molecular formula of $C_{45}H_{72}O_{17}$ (theoretical isotopic mass of 884.4770). Chemical shift of carbon and hydrogen atoms are given in table 15.

Table 15. Chemical shift of ^{1}H and ^{13}C of **Compound 3.**

Aglycon			Sugar		
C-pos.	δC	δH	C-pos.	δC	δH
1	38.6	1.07; 1.87	Glucose I		
2	30.8	1.6; 1.88	1	100.6	4.48
3	78.8	3.35	2	78.4	4.20
4	39.6	2.44; 2.3	3	78.8	4.20
5	142.0		4	81.05	4.20
6	122.6	5.38	5	76.34	3.86
7	32.9	1.28; 1.55	6	62.7	3.64; 3.83
8	32.7	1.64			
9	51.7	0.96	Rhamnose		
10	38.2		1	100.2	5.19
11	22.1	1.55	2	72.54	4.74
12	40.8	1.19; 1.77	3	73.79	4.58
13	41.9	-	4	74.61	4.34
14	57.9	1.14	5	70.32	4.94
15	32.9	1.28; 1.99	6	18.0	1.23
16	82.5	4.38			
17	64.5	1.74	Glucose II		
18	16.8	0.83	1	104.6	4.22
19	19.8	1.05	2	78.4	3.31
20	41.2	2.17	3	88.3	4.21
21	16.1	1.00	4	77.99	4.25
22	114.1	-	5	68.35	3.97
23	30.8	1.29; 1.6	6	62.5	3.64; 3.83
24	29.0	1.15			
25	35.0	1.73			
26	75.7	3.38; 3.73			
27	17.4	0.95			

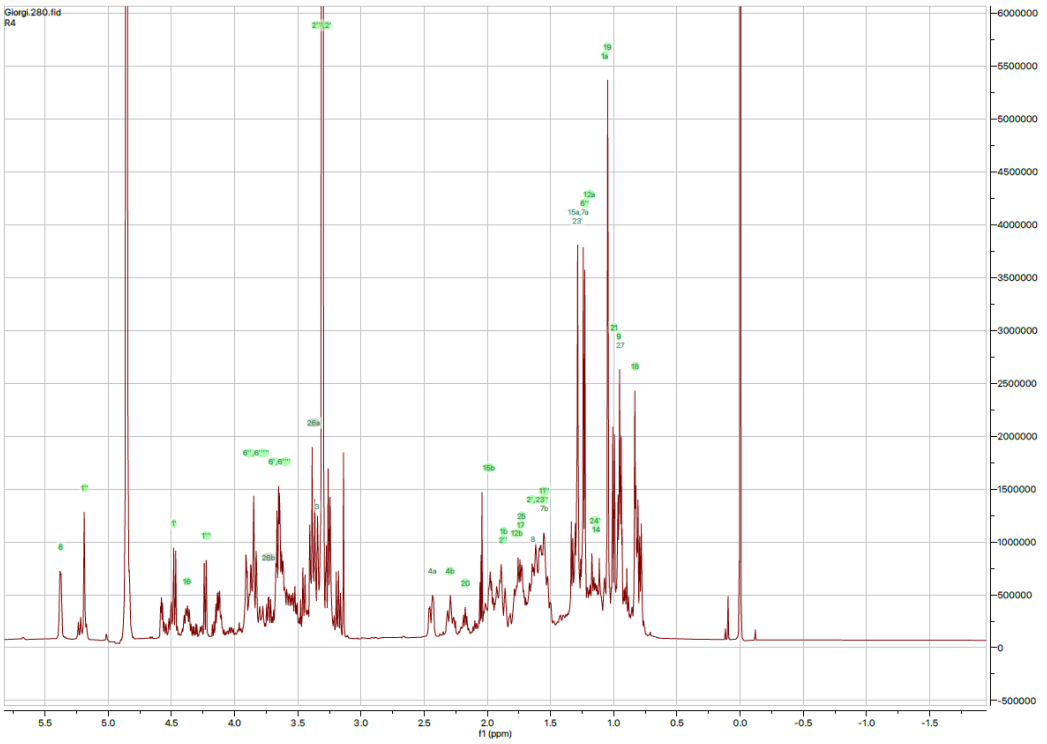


Figure 54. ^{1}H spectrum of Compound 3

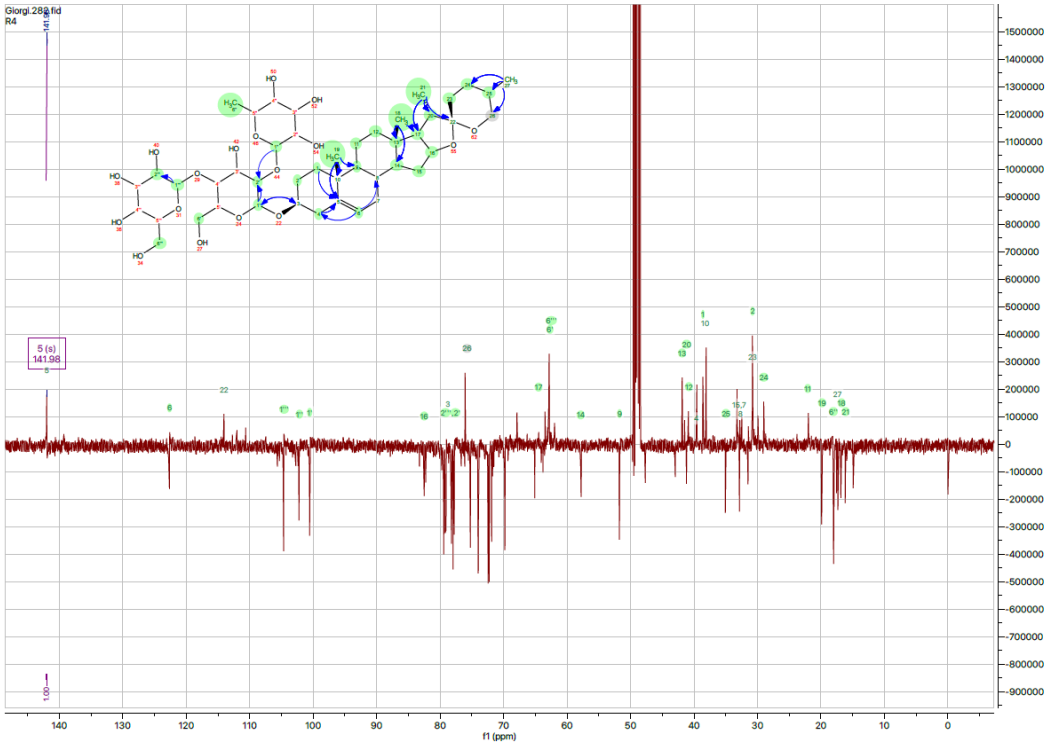


Figure 55. ^{13}C spectrum of Compound 3

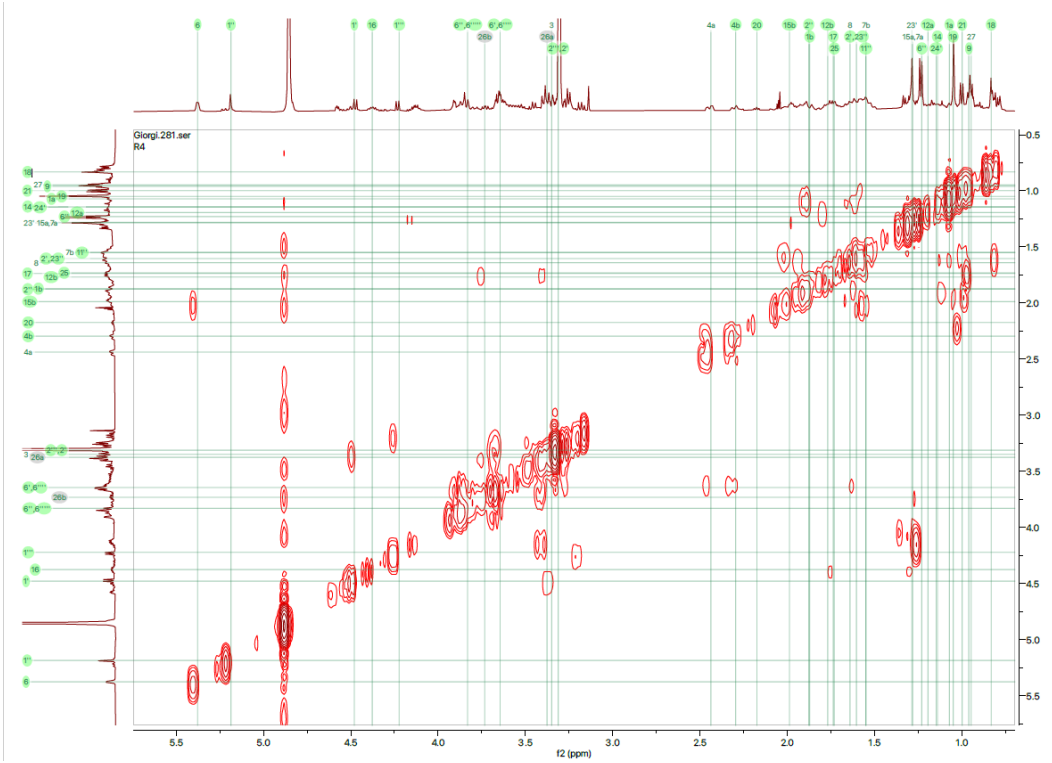


Figure 56. COSY spectrum of Compound 3

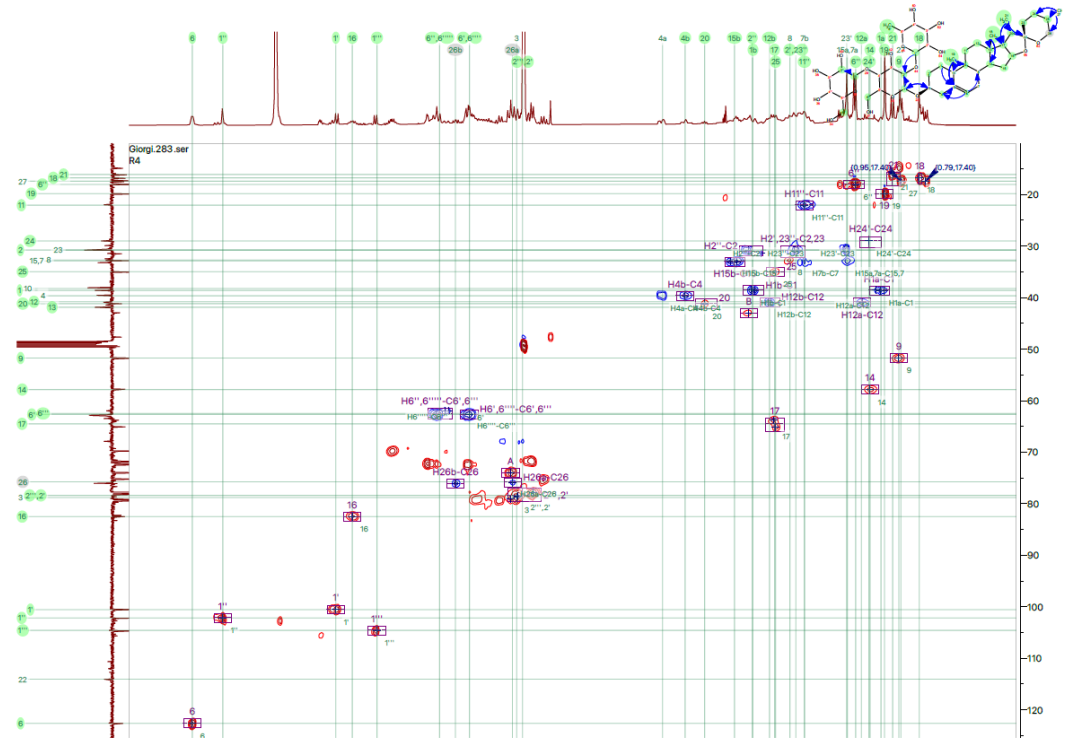


Figure 57. HSQC spectrum of Compound 3

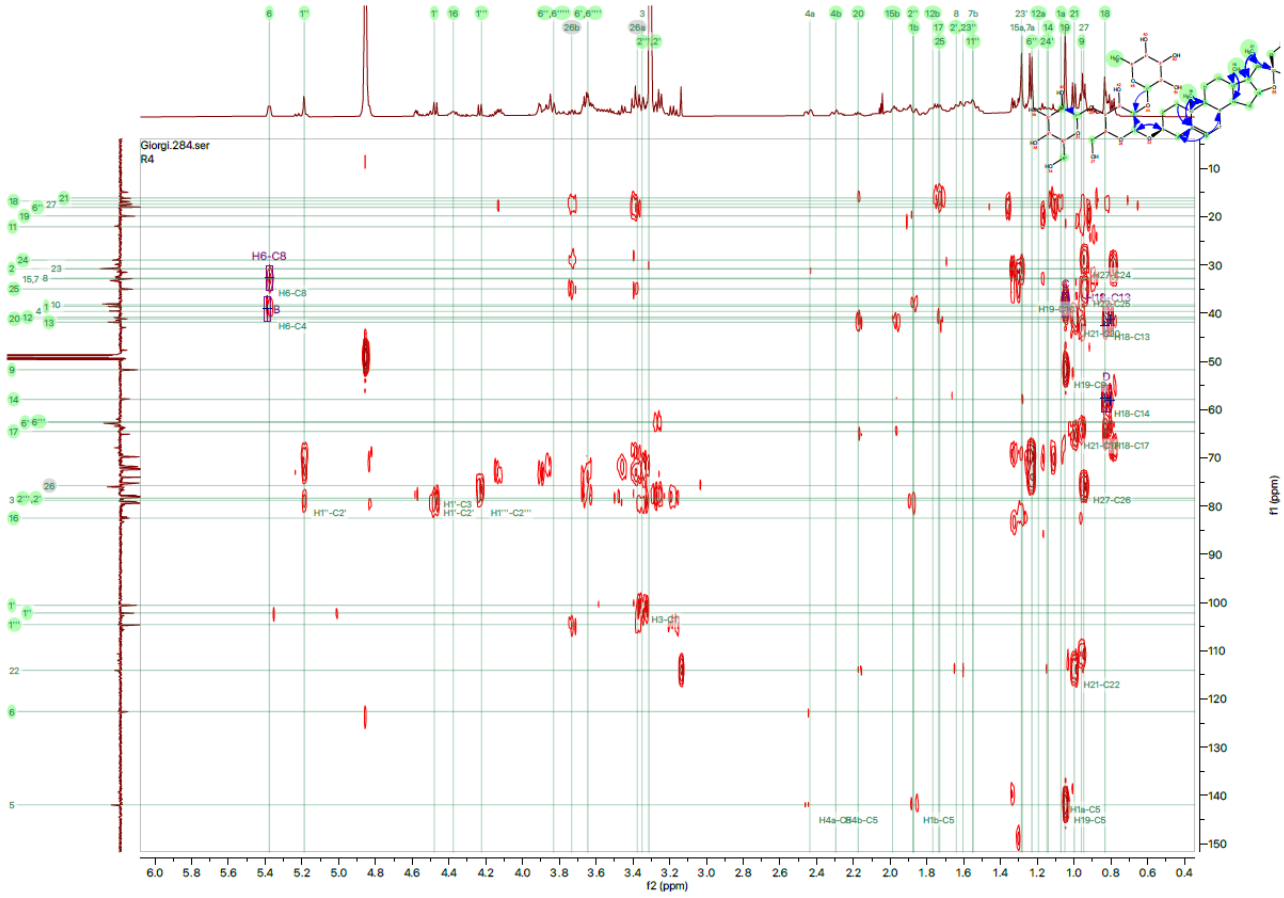


Figure 58. HMBC spectrum of Compound 3

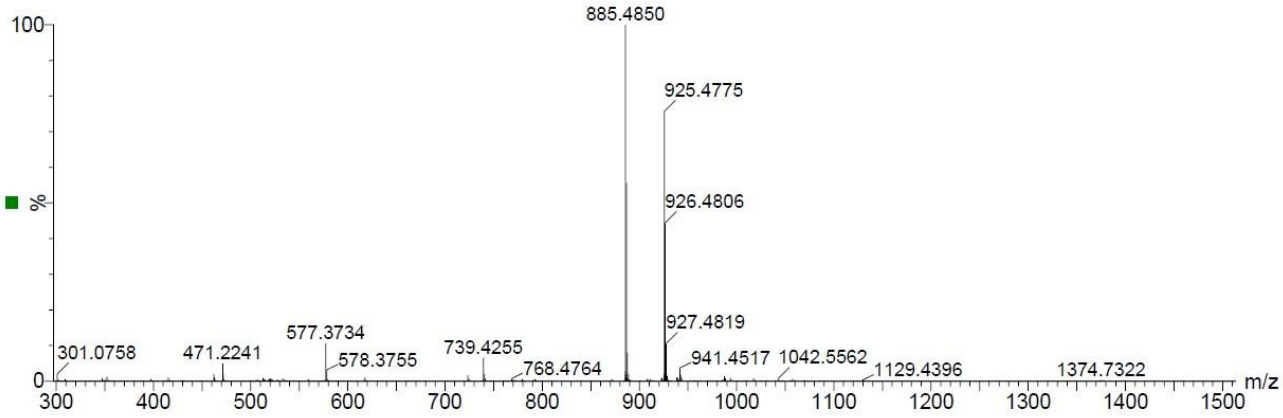


Figure 59. MS of the peak of Compound 3 in positive mode. 885 m/z corresponds to compound 3. Whereas 925 m/z corresponds to additive of potassium [M+K].

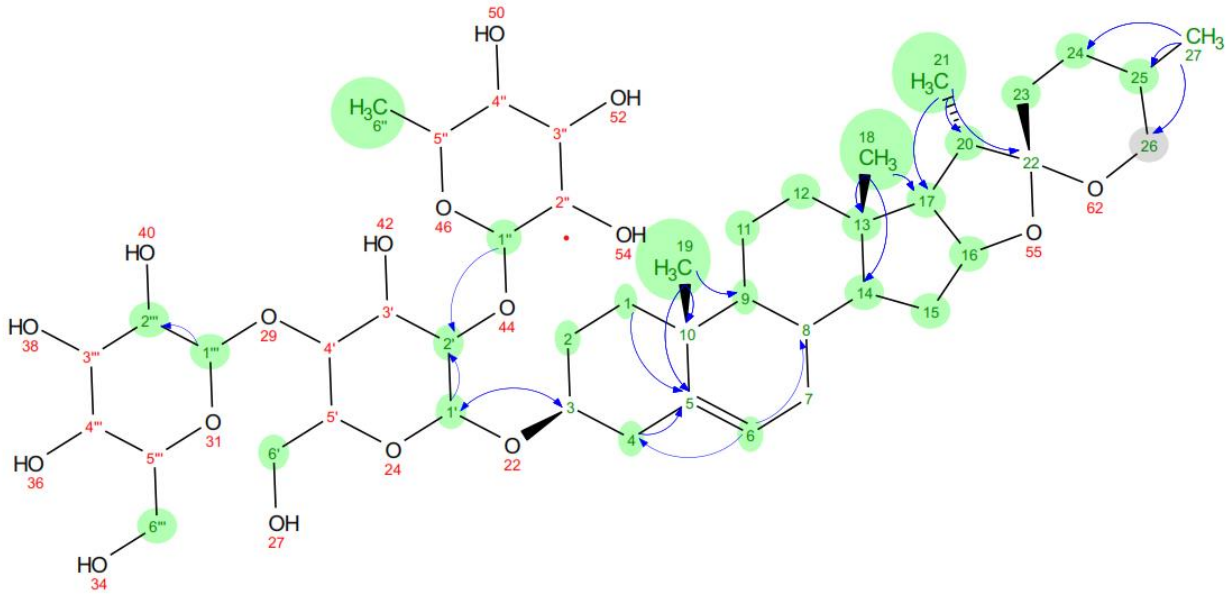


Figure 60. Structure of Compound 3.

Conclusion

Compound 3 was shown to contain three sugar residues from the HSQC spectrum. The anomeric protons at δ 4.48 (s), 5.19 (s) and 4.22 (s) giving correlations with carbon signals at δ 100.6, 100.2, and 104.6, respectively, were assigned to anomeric protons of β-glucopyranose. α-rhamnopyranose and β-glucopyranose, respectively. Furthermore, NMR data suggested a branched oligosaccharide chain for compound 3. Thus, in the 1H- 1H COSY spectrum, from the anomeric proton Glc H-1 (δ 4.48) it was possible to assign H-2 (δ 4.20), H-3 (δ 4.20), H-4 (δ 4.20), H-5 (δ 3.86), and H-6 (δ 3.64/3.83). The point of attachment of this trisaccharide to the aglycon was then deduced from the HMBC spectrum of 3 which showed a cross peak between the Glc I H-1 at δ 4.48 and C-3 of the aglycon at δ 78.8. Other correlations between Rha H-1 (δ 5.19) and Glc I C-2 (δ 78.4) and between Glc II H-1 (δ 4.22) and Glc I C-4 (δ 81.05) indicated that the rhamnose was linked to the glucose I by a 1→2 linkage and the glucose II moiety to the glucose I by a 1→4 linkage[271].

Specific rotation of the isolated compound was compared to the data given in the literature to confirm the structure. The structure of aglycon was determined with proton/carbon shifts at 5.38 ppm/121 ppm, which is typical for Diosgenin. All the values are coherent with literature. On the basis of comparison above results and the results obtained with MS to bibliography, structure of compound 3 was determined as (3β,25R)-Spirost-5-en-3-yl-O-α-L-rhamnopyranosyl-(1→2)-O-[β-Ds-glucopyranosyl-(1→4)]-β-D-glucopyranoside (Deltonin), previously isolated from other *Allium* species[272].

3.2.6. Quantification of secondary metabolites in the two *Allium* species

3.2.6.1. Quantification of furostanolic saponins in studied objects.

For the quantitative determination of furostanol saponins in research objects, we used the spectrophotometric method (Method 2.3.8), according to the article[273]. With calibration curve of Protodioscin, we calculated content of furostanolic saponins in plant materials and crude extracts of both plants. We have carried out this quantitative determination on both wild and cultivated plant raw materials (Table 16).

Table 16. Quantitative content of furosanol saponins in plant raw materials and crude extract of the study objects (%).

		A. saxatile	*A. ponticum*
Wild plant	Plant material	1.69 %	0.37 %
	Crude extract	37.15 %	11.54 %
Cultivated	Plant material	1.55 %	0.25 %
	Crude extract	35.84 %	9.9 %

Conclusions

Quantitative content of furostanol saponins is determined in plant raw materials and crude extracts of the study objects. Furostanolic saponin content in plant material of *A. saxatile* was

0.69%, in crude extract was 37.15 %; Content of furostanolic saponins was decreased in *A. ponticum* plant material was 0.37%, in crude extract was 11.54%. The content of furostanolic saponins in plant raw materials and crude extracts of cultivated studied objects is relatively low.

3.2.6.2. Quantification of astragalin and ferulic acid in plants raw material and total extracts

To determine content of astragalin and ferulic acid in plants raw material, we have developed and validated a HPLC method during the research (Method 2.3.9.). Using high performance liquid chromatography we determined the quantitative content of dominant phenolic compounds in the study objects. In case of *A. saxatile* dominant compound is flavonoid Astragalin, and in case of *A. ponticum* dominant compound was Ferulic acid (Figure 50). Ferulic acid was identified with HPLC method, Standard solution was used as a reference.

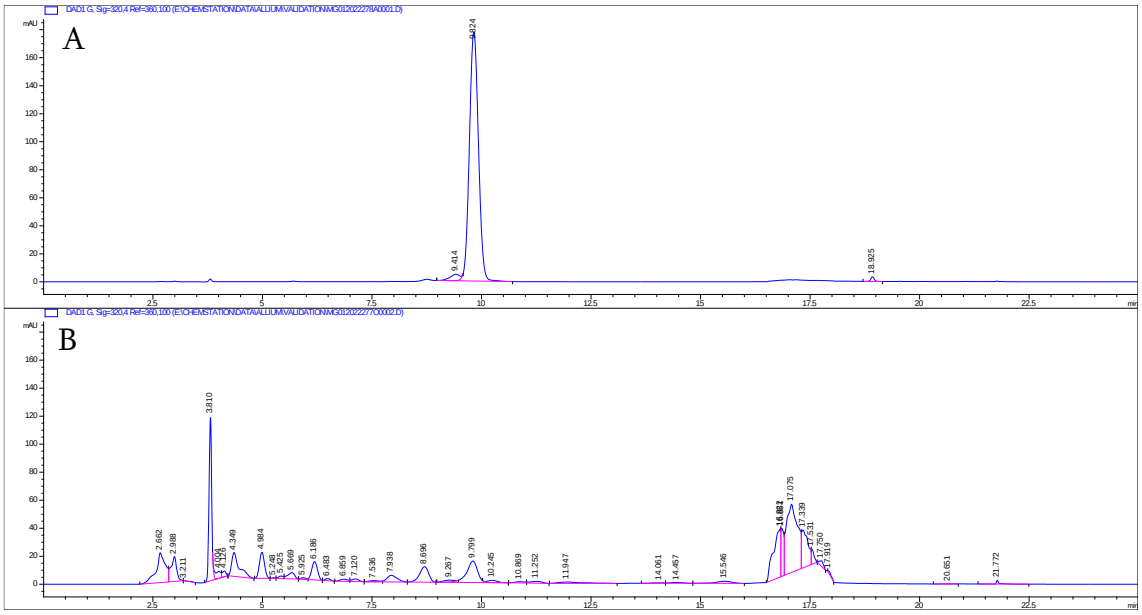


Figure 61. HPLC chromatogram of A-Ferulic acid reference standard and B-crude extract of *A. ponticum*.

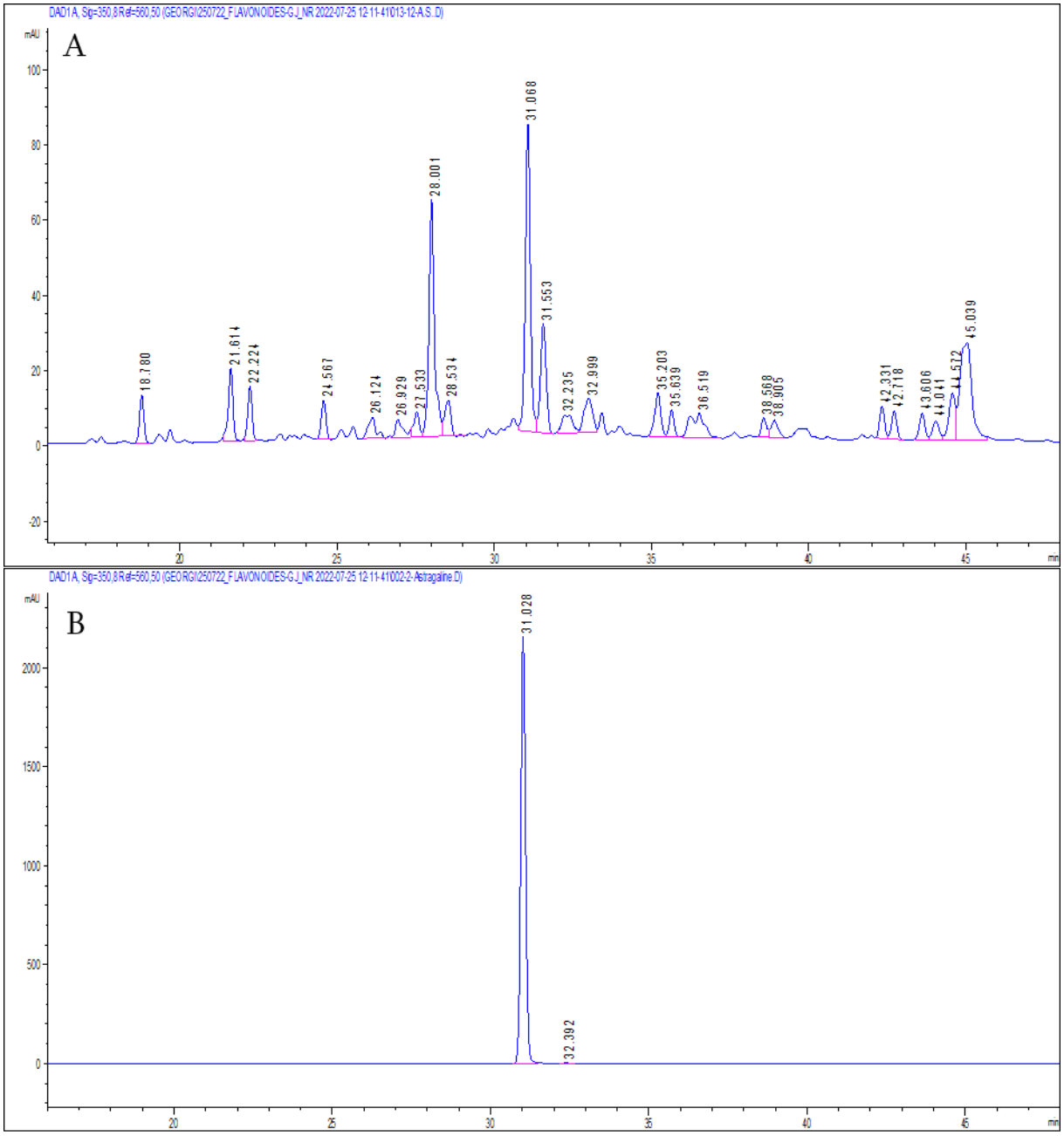


Figure 62. HPLC chromatogram of A-Crude extract of *A. saxatile* and B-Astragalin.

Method validation

The HPLC system consisted of an Agilent Technologies Model 1260 infinity liquid chromatography, equipped with a vacuum degasser, a binary pump, an auto-sampler, and a photodiode array detector (DAD).

Mobile phase: Water (0.1% Acetic acid) and Acetonitrile (77-23, v/v);

Column: Eclipse plus C-18 (4.6 x 250 mm; 5 µm); Temp : 25 °C ;

Detector: For *A. saxatile* crude extr.– 320 nm, for *A. ponticum* crude extr.– 280 nm;

Flow rate: For *A. saxatile* crude extr.– 0.8 ml/min, for *A. ponticum* crude extr.– 0.7 ml/min;

Preparation of solutions:

1 g of powdered plant material of *A. saxatile* was dissolved in 100 ml of 50% methanol, 1 ml of this solution was filtered through 0.45 µm membrane filter (Millipore, ref HVPL04700), for HPLC analysis.

10.0 mg of *A. saxatile* crude extr. was dissolverd in 1 ml 50% methanol and filtered through 0.45 µm membrane filter (Millipore, ref HVPL04700).

2 g of powdered plant material of *A. ponticum* was dissolved in 100 ml of 50% methanol, 1 ml of this solution was filtered through a 0.45 µm membrane filter (Millipore, ref HVPL04700), for HPLC analysis.

20.0 mg of *A. ponticum* crude extract was dissolverd in 1 ml of 50% methanol and filtered through 0.45 µm membrane filter (Millipore, ref HVPL04700).

Standard solution of astragalin and ferulic acid were prepared in 50% methanol with 1.0 mg/ml concentration separately. A series of dilutions of astragalin and ferulic acid (n=5) was prepared to obtain samples with various concentration. All prepared standard solutions were filtered through 0.45 µm membrane filter (Millipore, ref HVPL04700) before HPLC analysis.

Dilutions were prepared with 1 mg/ml; 0.5 mg/ml; 0.25 mg/ml; 0.125 mg/ml; 0.0625 mg/ml; 0.03125 mg/ml and 0.015625 mg/ml concentrations and the peak areas with HPLC.

The HPLC method was validated according to the ICH guidelines. The linearity of the method for astragalin and ferulic acid was determined using five different concentrations, and calibration curves were constructed. Five different concentrations were prepared and analyzed in

triplicate for each concentration. The concentration range was 0.06 to 1.0 mg/mL for both compounds. Calibration curves were constructed by plotting peak areas against concentrations, and the linearity was assessed using least squares regression to calculate the slope, y-intercept, and coefficient of correlation (R^2). The limits of detection and quantification were determined based on signal-to-noise ratios of 3:1 and 10:1, respectively.

The precision of the method was evaluated for both intra-day and inter-day precision, and the accuracy of the method was evaluated using the recovery test. Intra-day precision was calculated from the analyses of six sample solutions prepared independently on one day. Inter-day precision was evaluated by repeating the same procedure on 3 other days, the standard deviation, and the RSD (relative standard deviation) values were calculated for each day. The recovery test is a method used to evaluate the accuracy of an analytical method. In this case, known quantities of astragalin and ferulic acid standard solutions were added to the real samples at three different concentration levels: 50%, 100%, and 150%. The spiked samples were then analysed in triplicates using the HPLC method previously described. The recovery was calculated as the ratio of the measured concentration of the spiked sample to the expected concentration, expressed as a percentage. The calibration curves for the crude extract of *A. saxatile* and *A. ponticum* were linear within the concentration range (Table 1). All correlation coefficients were greater than 0,999. The inter-day % RSD was 3.34% for *A. saxatile* and 1.9% for *A. ponticum*, which proves a good precision of the method (Table 17). Parameters are given in table 17.

Table 17. Validation parameters, accuracy and precision of methods.

	A. saxatile	*A. ponticum*
Range (mg/ml)	0.06-1.0	0.06-1.0
Calibration curves and R2	y=2363.9x	y=12328x
	R^2=0.9996	R^2=0.9997
Acuracy	100.02±0.93%	100.43±0.47%
Intermediate precision %RSD	3.34%	1.9%

Graph 7. A – Calibration curve of Astragalin; B – Calibration curve of Ferulic acid.

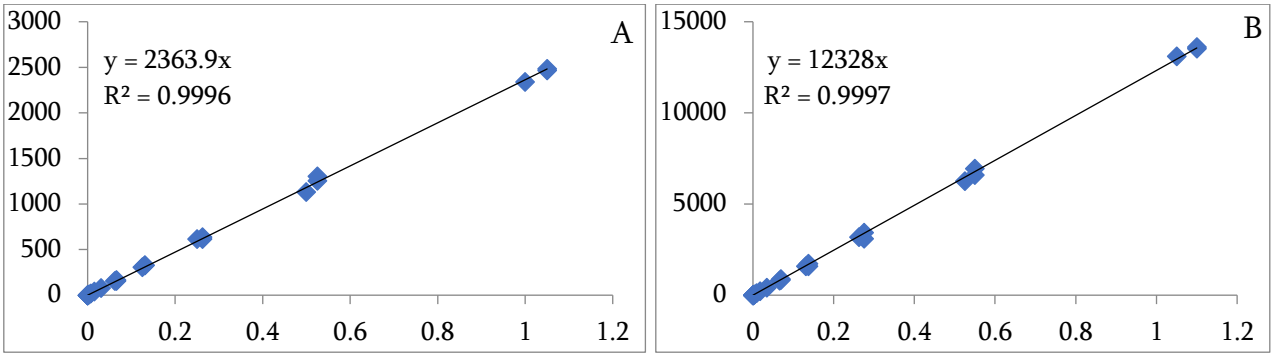


Table 18. Quantitative content of phenolic compounds in plant raw materials and total extract of the study objects.

		A. saxatile	*A. ponticum*
Wild plant	Plant material	0.06%	0.004 %
	Crude extract	0.87 %	0.08 %
Cultivated	Plant material	0.05 %	0.003 %
	Crude extract	0.75 %	0.07 %

Conclusion

Quantitative content of phenolic compounds is determined in plant raw materials and crude extracts of the study objects. Quantitative content of flavonoid astragalin in *A. saxatile* in plant material was 0.06%, in crude extract_0.87%; Quantitative content of Ferulic acid in *A. ponticum* plant material was 0.004%, and in crude extract was 0.08%. The content of phenolic compounds in plant raw materials and crude extracts of cultivated studied objects is relatively low.

3.3. Pharmacological activity of crude extracts, fractions, and individual compounds of studied objects in *in vitro* and *in vivo* experiments.

3.3.1. Cytotoxic activity of studied objects in *in vitro* experiment.

Cytotoxic activity of crude extracts of both plants obtained with 80% ethanol, fractions obtained after separation with open column chromatography (Water Methanol in gradient condition), and individual compounds was evaluated on human melanoma (A2058) and breast cancer (MDA-MB-231) cells. Doxorubicin was used as a positive control (Method 2.3.11.2). Results of cytotoxic activity of research objects is given in Table 19-21.

Table 19. Cytotoxic activity of crude extracts

Research object	IC_{50} µg/ml (A2058)	IC_{50} µg/ml (MDA-MB-231)
A. saxatile	37,62±4.2	17,52±3.5
A. ponticum	>100	>100
Doxorubicin	2.7	2.7

n=3

Table 20. Cytotoxic activity of fractions.

Research object	IC_{50} µg/ml (A2058)	IC_{50} µg/ml (MDA-MB-231)
A. saxatile F1 (100% water)	>50	>50
A. saxatile F2 (50% MeOH:50% water)	>50	>50
A. saxatile F3 (100% MeOH)	4.04±1.2	14.17±3.02
A. ponticum F1 (100% water)	>50	>50
A. ponticum F2 (50% MeOH:50% water)	>50	>50
A. ponticum F3 (100% MeOH)	35.68±2.7	29.12±2.5
A. ponticum F4 (100% Ethyl acetate)	>50	>50
Doxorubicin	2.7	2.7

n=3

Table 21. Cytotoxic activity of individual compounds obtained from the fractions.

Research object	IC_{50} µg/ml (A2058)	IC_{50} µg/ml (MDA-MB-231)

Compound 3	4.3 μM	4.31 μM
Doxorubicin	5 μM	5 μM

n=3

Conclusion

Results have determined that crude extract and 100% methanolic fraction (A.S.F3) of *A. saxatile* have expressed cytotoxic activity against both Human melanoma and Breast cancer cell strains. In case of *A. ponticum* only 100% methanolic fraction (A.P.F3) was biologically active against both cell strains.

Studies also have determined cytotoxic activity of individual **compound 3,** identified as (3β,25R)-Spirost-5-en-3-yl-O-α-L-rhamnopyranosyl-(1→2)-O-[β-Ds-glucopyranosyl-(1→4)]-β-D-glucopyranoside (Deltonin), isolated from *A. saxatile* against both cell strains.

Deltonin is isolated from plant *Dioscorea parviflora*. Among *Allium* species this compound is isolated from: *A. gramineum*[116], *A. shoenoprasum*[135], *A. narcissiflorum*[274], *A. vineale*[132].

Deltonin is characterized with wide range of biological activities. This compound inhibits colon cancer cell growth *in vitro* and tumor growth *in vivo* via induction of apoptosis and antiangiogenesis[275]. Also this compound induces apoptosis of MDA-MB-231 [276]. Deltonin induces inhibition of Angiogenesis via regulation of VEGFR2 gene in endothelial cells[277]. Deltonin is chracterised with neuroprotective activity, and ameliorates Cerebral ischemia/reperfusion [278]. Deltonin has also hepatoprotective activity, it decreases Hydrogen Peroxide-Induced Hepatotoxicity in HepG2 Cells[279].

3.3.2. Antiprotozoal activity of research objects in *in vitro* experiment

Antiprotozoal activity of studied objects was evaluated on *P. falciparum* 3D7 strain. Artemisinin was used as a positive control (Method 2.3.11.5).

Table 22. Antiprotozoal activity of studied objects

Studying objects	IC_{50} μg/ml
A.S. Crude extr.	>100
A.P. Crude extr.	>100
Artemisinin	0.009±0.003

n=3

Conclusion

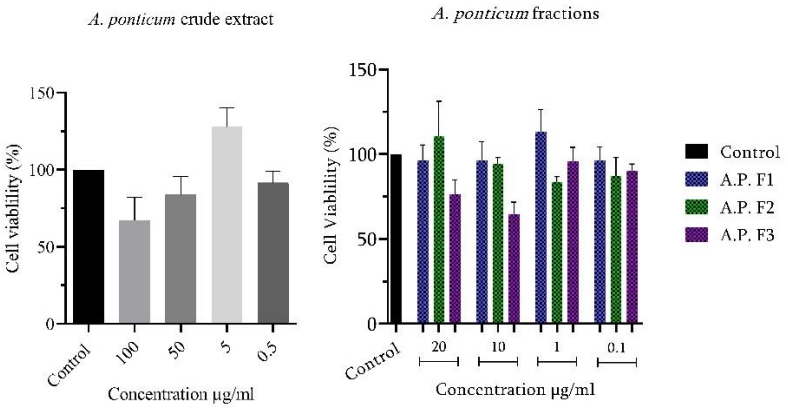
According to results, crude extracts of the two plants do not express antiprotozoal activity against *P. falciparum* 3D7 strain. The decision was made to discontinue the investigation of the the antiplasmodial activity of fractions and pure compounds.

3.3.3. Antioxidant activity of research objects in *in vitro* experiment.

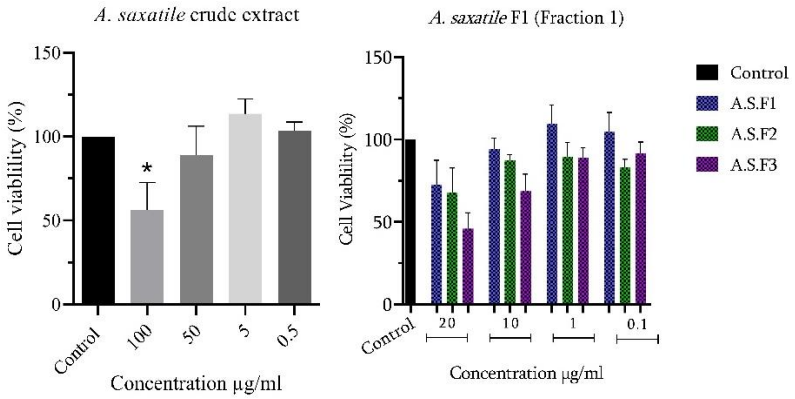
Antioxidant activity of studied objects was determined using *in vitro* experiments on RAW 264.7 macrophages (Methods 2.3.11.3., 2.3.11.4.).

First, we evaluated "self" toxicity of the products. Toxicity of the crude extract and fractions were investigated on murine RAW264.7 macrophages. Cell viability was evaluated using MTT test.

Graph 8. Toxicity of crude extract and fraction of *A. ponticum*.



Graph 9. Toxicity of crude extract and fraction of *A. saxatile.*



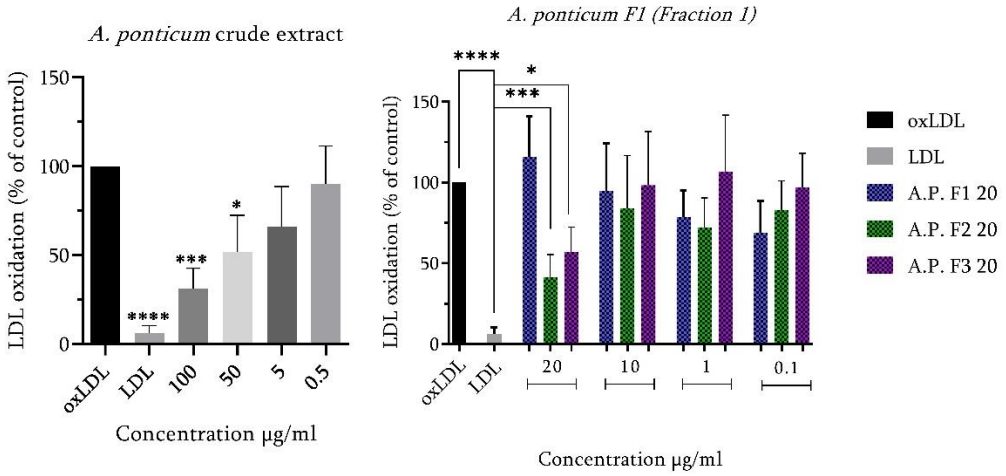
For *A. ponticum* only methanol fraction (A.P. F3) showed low toxicity with 20% inhibition at 20 µg/mL in comparison with untreated control (Graph 9). *A. saxatile* crude extract showed 45% inhibition and methanolic fraction (A.S.F3) showed 55% inhibition at 20 µg/mL and 30% inhibition at 10 µg/ml (Graph 10).

We evaluated the antioxidant activity of crude extracts and fractions of *A. ponticum* and *A. saxatile* using *in vitro* experiment with two different approaches:

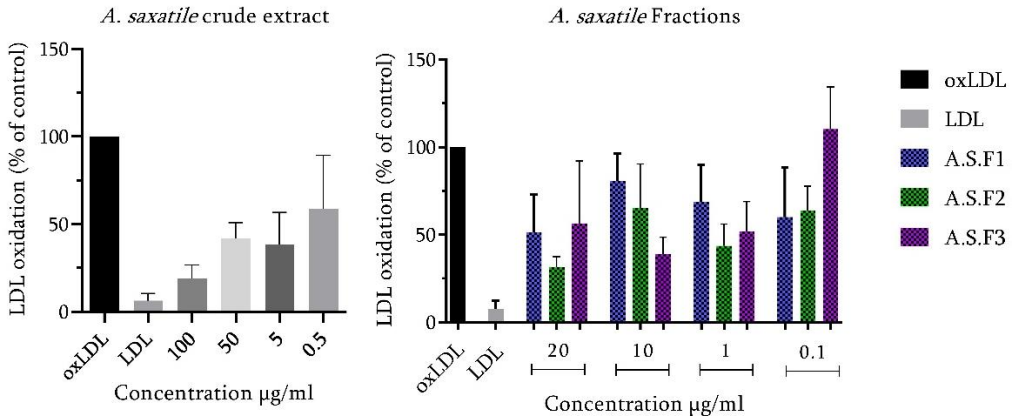
1. Firstly, a non-cellular assay, in the culture medium, where we added LDL, $CuSO_4$ and extracts and fractions of the plants, with increasing concentration, dissolved in DMSO. $CuSO_4$ induces oxidation of LDL. In this case, we wanted to see if the studied products would prevent LDL oxidation directly via biochemical pathways.
2. Secondly a cellular assay on RAW 264.7 cells, incubated with LDL, $CuSO_4$ and extracts and fractions of the plants, with increasing concentration, dissolved in DMSO.

3.3.3.1. Antioxidant activity of crude extracts and fractions of studied objects in non-cellular assay

Graph 10. Antioxidant activity of crude extract and fractions of *A. ponticum* in non-cellular model.



Graph 11. Antioxidant activity of crude extract and fractions of *A. saxatile* in non-cellular model.



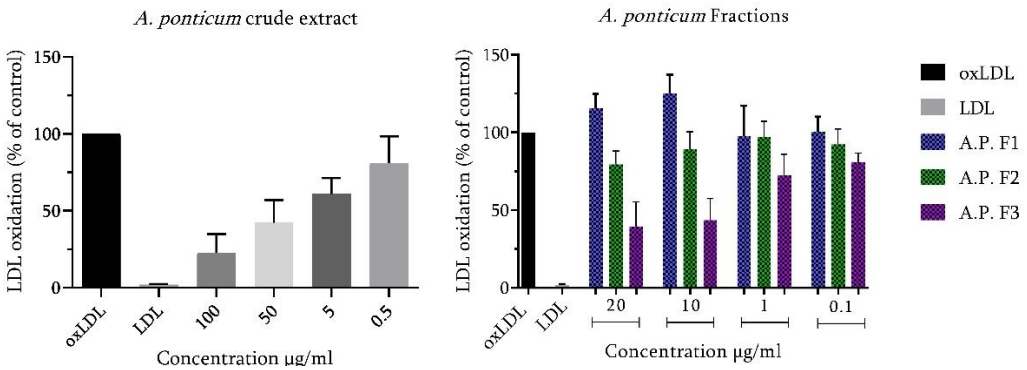
Results of present studies have shown that RAW264.7 induces LDL oxidation and TBARS content increase in the culture medium.

According to the results obtained, the crude extract of the plant *A. Ponticum* inhibited oxidation of LDL. Concerning the fractions, *A. ponticum* F2 and *A. ponticum* F3 showed antioxidant activity in the non-cellular assay with IC_{50} of 14.78 µg/mL and 24.53 µg/mL respectively (Table 17).

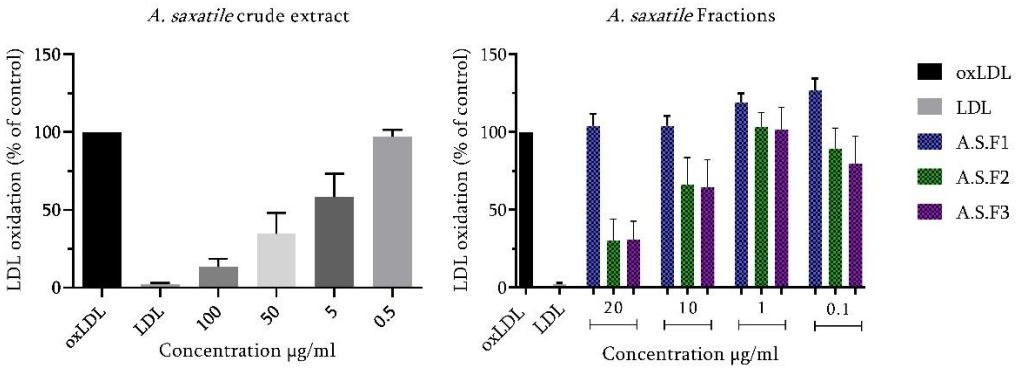
In the case of *A. saxatile*, the activity of the crude extract and fractions was higher than for the crude extract and fractions of *A. ponticum*. All the fractions have expressed antioxidant activity, particularly fraction of *A. saxatile* F2 (eluted with 50% methanol) had better activity than others, with IC_{50} value of 2.93 µg/mL (Table 17).

3.3.3.2. Antioxidant activity of crude extracts and fractions of studied objects in cellular assay

Graph 12. Antioxidant activity of crude extract and fractions of *A. ponticum* in cellular model.



Graph 13. Antioxidant activity of crude extract and fractions of *A. saxatile* in cellular model *A. saxatile*.



Regarding the cellular assay, we tested the capacity of crude extract and fractions of *A. ponticum* and *A. saxatile* to limit cell-induced LDL oxidation. According to the results, crude extract of A. ponticum and fraction A.P.F3 (eluted with 100% Methanol) have shown better activity than in non-cellular experiment with IC_{50} of 42.16 µg/mL and 12.15 µg/mL respectively (Table 23).

Table 23. Antioxidant activity of studied objects.

	Antioxidant activity. Non-cellular assay_ IC_{50} µg/ml	Antioxidant activity. Cellular assay_ IC_{50} µg/ml
A.S. Crude extr.	3.5±1.6	40.13±3.3
A.S.F1	16.41±3.2	>50
A.S.F2	2.93±0.8	14.42±1.9
A.S.F3	8.2±1.2	14.13±2.7
A.P. Crude extr.	58.48±5.4	42.16±4.2
A.P.F1	>50	>50
A.P.F2	14.78±2.1	>50
A.P.F3	24.53±3.6	12.15±1.4

n=3

Conclusion

According to the results, 50% methanolic fraction (A.S.F2 and A.P.F2) and 100% methanolic fraction (A.S.F3 and A.P.F3) of both plants are characterised with important antioxidant activity, with IC_{50} value: A.S.F2 2.93±0.8 µg/ml, A.S.F3 8.2±1.2 µg/ml, A.P.F2

14.78±2.1 μg/ml and A.P.F3 24.53±3.6 μg/ml. Among the fractions, 50% methanolic fractions are more active than 100% methanolic fractions. In case of cellular assay, 50% methanolic and 100% methanolic fractions express antioxidant activity with IC_{50} value: A.S.F2 14.42±1.9 μg/ml, A.S.F3 14.13±2.7 μg/ml. Comparison of cellular and non-cellular assay shows that antioxidant activity of *A. saxatile* is decreasing, while activity of crude extract and 100% methanolic fraction of *A. ponticum* increases between non-cellular and cellular assays. Activity of *A. ponticum* in non-cellular assay, IC_{50} value: crude extract 58.48±5.4 μg/ml, A.P.F3 24.53±3.6 μg/ml. In cellular assay, IC_{50} value: crude extract 42.16±4.2 μg/ml, A.P.F3 12.15±1.4 μg/ml. The ROS can be produced from either endogenous or exogenous sources. The endogenous sources of ROS include different cellular organs such as mitochondria, peroxisomes and endoplasmic reticulum, where the oxygen consumption is high[280]. Thus, compounds that target specific sources of free radicals, such as ROS generated by mitochondria, may have different effects in cells compared to biological assay without cells, depending on the relative contribution of each source to lipid peroxidation[281]. An example of intracellular antioxidant activity is the role of glutathione, which is an important antioxidant that is synthesized in cells. Glutathione helps to protect cells from oxidative damage by neutralizing ROS and maintaining a reducing environment within cells. Glutathione is found in high concentrations in the cytoplasm and mitochondria of cells and can help to protect these organelles from oxidative damage[282]. Studies suggest that dietary supplementation with *Allium hookeri*, can restore the glutathione level[283].

3.3.4. Analgesic activity of research objects in *in vivo* experiments.

Analgesic activity of studied objects was studied using Hot-plate assay(Method 2.3.12.2). The hot-plate latency in the experimental groups was 11.5 seconds before the intraperitoneal administration of 50 mg/kg of crude extracts of *A. saxatile* (A.S. crude extr.) and *A. ponticum* (A.P. Crude extr.) and 25 mg/kg and 50 mg/kg of fractions: A.S.F2, A.S.F3, A.P.F2, A.P.F3 (Table 24).

Table 24. The hot-plate latency of crude extracts and fractions.

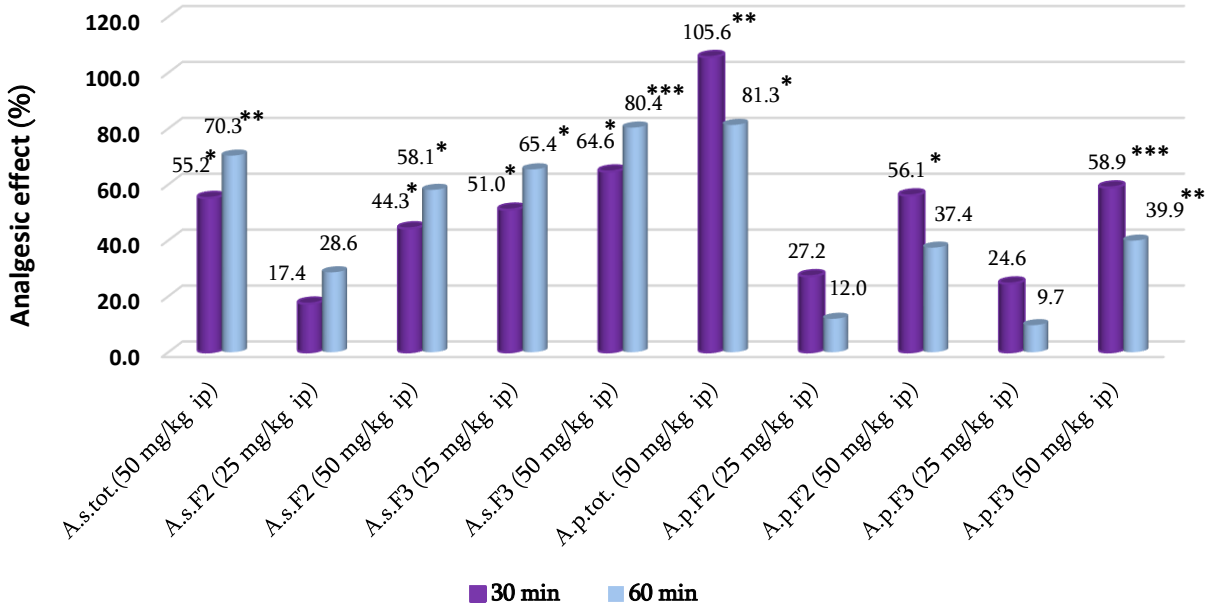
	Baseline latency #	30 min	60 min
Control		11.6±2.6	11.8±2.4
A.S. Crude extr. (50 mg/kg I/P)		17.9±5.6*	20.1±7.3**
A.S. F2 (25 mg/kg I/P)		13.5±5.3	15.1±5.6
A.S. F2 (50 mg/kg I/P)		16.6±2.3*	18.6±2.5**
A.S. F3 (25 mg/kg I/P)		17.4±5.4	19.5±7.2*
A.S. F3 (50 mg/kg I/P)	11.5±3.5	19.0±5.4*	21.2±6.7***
A.P. Crude extr. (50 mg/kg I/P)		23.7±6.7**	21.3±6.4*
A.P. F2 (25 mg/kg I/P)		14.6±2.9	13.2±2.6
A.P. F2 (50 mg/kg I/P)		18.0±4.5*	16.2±4.4
A.P. F3 (25 mg/kg I/P)		14.3±3.5	12.9±3.1
A.P. F3 (50 mg/kg I/P)		18.3±2.5***	16.5±2.2*

Data in sec are represented as mean (n=6) ±S.E.M. #Baseline latency calculated as mean±S.E.M. from all groups; * - $p<0.05$; ** - $p<0.01$; * - $p<0.001$;

According to the results, analgesic activity of the *A. saxatile* crude extr. ascends with time and reaches its maximum at 60 min after the administration. Differently, the analgesic activity of *A. ponticum* crude extr. revealed short duration but faster onset analgesic activity. Hot-plate latency for the *A. saxatile* crude extr. was 17.9 and 20 seconds on the 30th and 60th minutes after the injection respectively, in case of *A. ponticum* crude extr, latency time was 23.7 and 23.3 seconds, respectively, yielding the analgesic effect 55.2 % after 30 minutes and 70.3% after 60 minutes for *A. saxatile* crude extract, and for *A. ponticum* the effect was 105.6 % and 81.3 % respectively (Graph 14).

Similar tendency was observed when studying the efficacy of fractions obtained from total extracts. Moreover, fractions obtained from both *A. saxatile* and *A. ponticum* showed dose-dependent activity (Graph 14).

Graph 14. Analgesic effects of *A. saxatile* and *A. ponticum* total extracts and fractions.



Conclusion

Studies have concluded that analgesic activity of the *A. saxatile* crude extr. ascends with time and reaches its maximum at 60 min after the administration. Crude extract of *A. ponticum,* is characterised with fast analgesic activity, which decreases with the time.

Fractions obtained from both *A. saxatile* and *A. ponticum* demonstrated dose-dependent analgesic activity. Analysis of the obtained data, especially that A.S.F3 fraction has even higher activity than crude extract, allows to conclude that this fraction contains compound(s) responsible for analgesic effect. Phytochemical studies have shown that this fraction is rich with saponins.

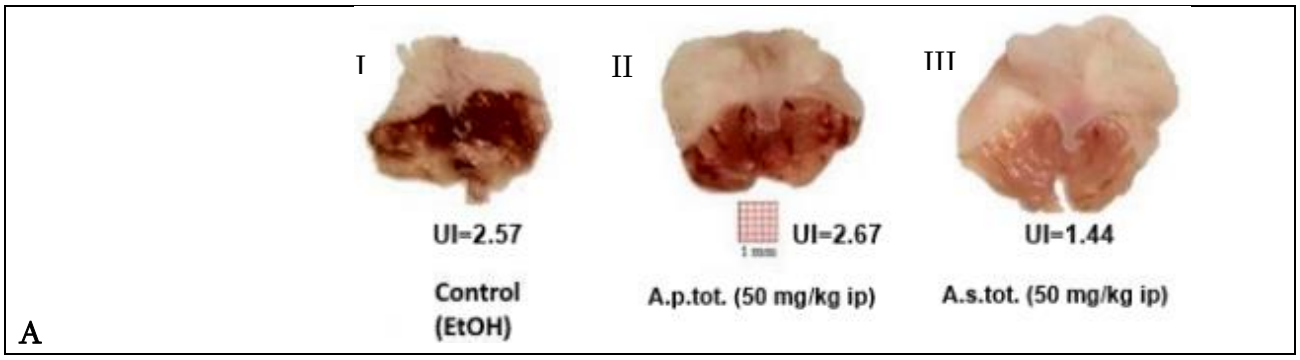
3.3.5. Gastroprotective activity of research objects in *in vivo* experiment.

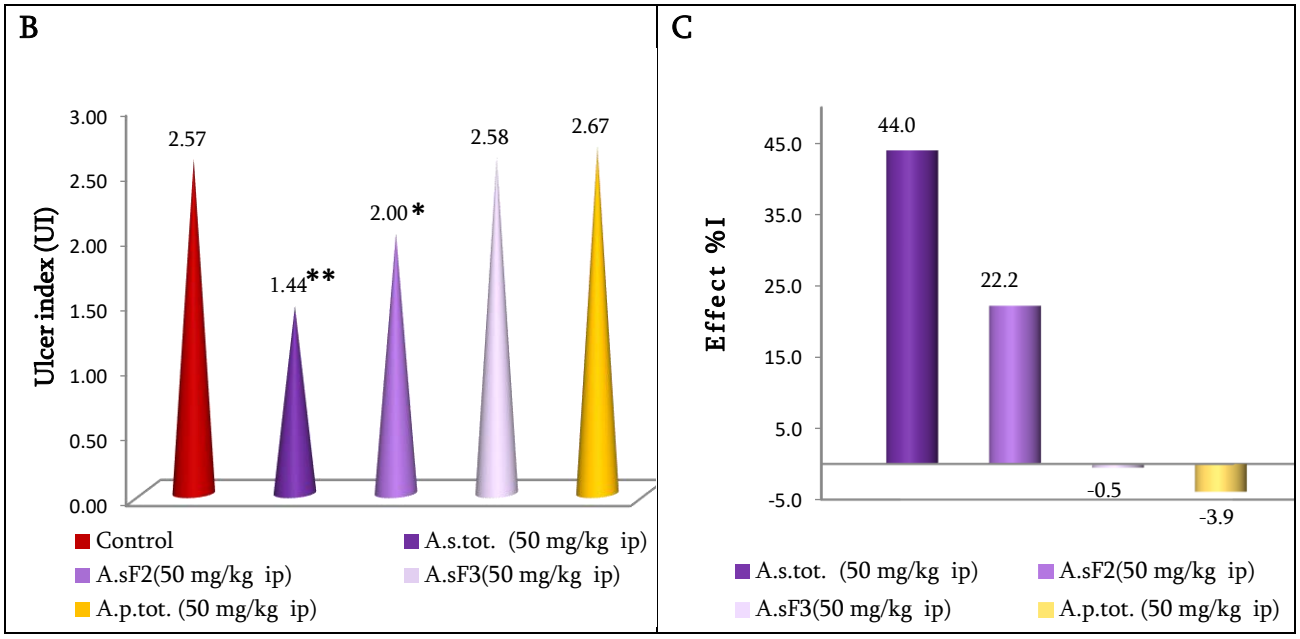
Gastroprotective activity of crude extract and fractions was evaluated using methanol induced ulcer assay (Method 2.3.12.3) In untreated animals, administration of absolute ethanol induced marked gross mucosal lesions, including full length haemorrhagic streaks along the longitudinal axis of the glandular part of stomach and petechial lesions (Graph 17, A I). In mice which were given 50 mg/kg i.p. of crude extract of *A. saxatile,* only single petechial lesions were present (Graph 17, A III), whereas in animals pre-treated with 50 mg/kg crude extract of *A.*

ponticum mostly continuous linear haemorrhagic lesions along the entire length of the glandular part of stomach, were observed (Graph 17, A II). Correspondingly, the UI was significantly reduced in animals pre-treated with crude extract of *A. saxatile* (UI=1.44; %I=44, $p<0.05$) compared with untreated mice (UI=2.57). Administration of a crude extract of *A. ponticum* (UI=2.67; %I=-3.9), apparently, increased haemorrhagic lesions.

Taking above into consideration we proceeded only with fractions obtained from *A. saxatile* - A.S.F2 and A.S.F3 in a dose 50 mg/kg ip. The results showed that fraction A.S.F2 has a moderate gastroprotective effect of 22.2 %, whereas fraction A.S.F3 appeared to be inactive. (Graph 15. B, C).

Graph 15. Gastroprotective effect of A.S. and A.P. crude extracts. A – Ethanol induced ulcer lesions in control (I) and mice treated with crude extract of *A. ponticum* (II) and crude extract of *A. saxatile* (III); B – Ulcer index (UI); C - Efficacy of extracts (%I). Data represented as mean (n=6). * - $p<0.05$; **- $p<0.01$.





Conclusion

Studies have determined that ulcer index (UI) is decreasing in mice treated with crude extract of *A. saxatile.* In the contrary, administration of crude extract of *A. ponticum* increases ulcer index (UI).

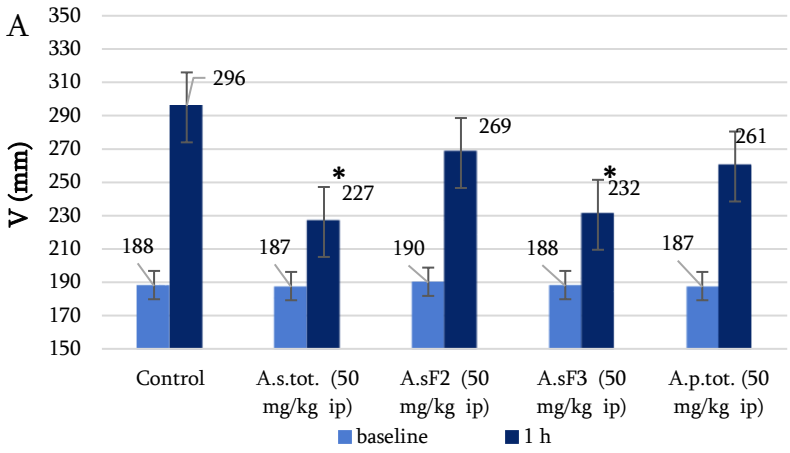
Studies on the fractions of *A. saxatile* showed that fraction A.S.F2 has a moderate gastroprotective effect, while fraction A.S.F3 appeared to be inactive.

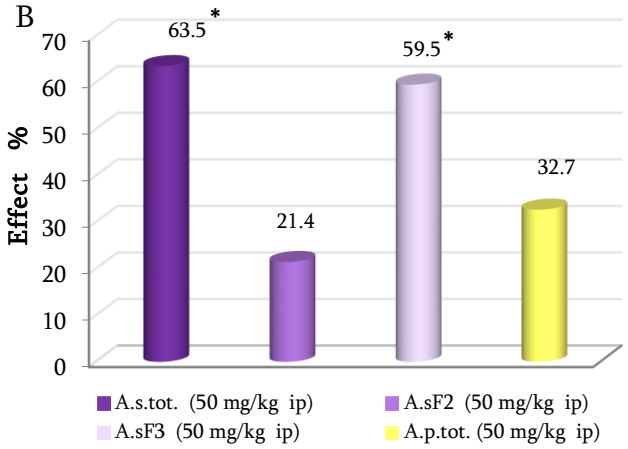
3.3.6. Anti-inflammatory activity of research objects in *in vivo* experiment.

Anti-inflammatory activity of crude extracts and fractions of research objects was evaluated using Carrageenan-induced Paw oedema assay (Method 2.3.12.4).

In experimental animals, 1 hour after the carrageenan administration, in groups that received crude extract of *A. saxatile,* paw thickness was increased with 39.4 μm and in groups treated with crude extract of *A. ponticum* paw thickness was increased with 72.7 μm, which was less than in control group animals (80.2 μm). The efficacy of crude extract of *A. saxatile* and crude extract of *A. ponticum* was 63.5% and 32.7%, respectively, evidencing a notable anti-inflammatory activity of *A. saxatile* (Graph 16. A, B). As *A. saxatile* extract was significantly more active than *A. ponticum*, further study was continued only on the *A. saxatile* fractions A.S.F2 and A.S.F3, and revealed their efficacy equal to 21.4% and 59.5% respectively. Thus, it is likelihood that fraction A.s.F3 contains compound(s) responsible for the observed anti-inflammatory activity (Graph 16. A, B).

Graph 16. Anti-inflammatory effect of *A. saxatile* and *A. ponticum* crude extracts and fractions. A - thickness of the paw, of control and experimental groups. B - Efficacy of the extracts and fractions. Data represented as mean (n=6). * - p<0.01 vs control group.





Conclusion

Studies have concluded that crude extract of *A. saxatile* has better anti-inflammatory activity than crude extract of *A. ponticum.*

Among the fractions, fraction A.S.F3 has expressed better anti-inflammatory activity, thus, it is possible that fraction A.S.F3 contains compound(s) responsible for the observed activity.

4. General discussion

During the research, several analyses were performed on species *A. saxatile* and *A. ponticum* of genus *Allium*, family *Alliaceae*. This genus includes species which have been cultivated since ancient times. Plants of genus *Allium* has long history of traditional use. Wide range of biological activities of the *Allium* species are associated with their phytochemical compounds. The aim of this study was to investigate phytochemical composition of *A. saxatile* and *A. ponticum* and determine their biological activities.

Result of microscopic characterization revealed key differences between these plants, which can help to identify them.

Phytochemical studies of both plants, showed that, these species are rich saponins and flavonoids, which is common for the species of genus *Allium*[111], [284]. TLC screening of the research objects showed that, these plants do not contain Alkaloids, Cardiac glycosides, Antraglycosides and Tannins.

During the research, Cytotoxic, antioxidant, anti-protozoal, gastroprotective, analgesic and anti-inflammatory activities of crude extract and fractions of studied objects was evaluated.

Cytotoxic study of research objects revealed that fractions obtained from *A. saxatile* and *A. ponticum* can inhibit the growth of human melanoma and breast cancer cells.

In vitro studies have revealed that some saponins extracted from the plant genus *Allium* have a cytotoxic effect on human and animal cancer cells. For example: Spirostanol saponins, extracted from *Allium leucanthum,* have a cytotoxic effect against adenocarcinoma (A549) and colorectal adenocarcinoma (DLD-1) cells[118]. Compounds identified in *Allium gramineum* has strongly inhibited the growth of breast adenocarcinoma cell lines, with an IC50 of 4.5 ± 0.7μg/mL for MDAMB-231 and 4.8 ± 0.9μg/mL for MCF-7 cells[116]. Furostanol saponins, from *Allium chinense,* have induced apoptosis of HepG2 cells[285]. Biological research on steroidal saponins extracted from *Allium flavum* exhibited moderate cytotoxicity against a human colorectal cancer cell line (SW480)[142]. Steroidal glycoside from *Allium macrostemon* has also shown moderate cytotoxicity against A549 and SK-MEL-2 cells [286].

During the research, biologically active compound Deltonin has been isolated from *A. saxatile*. This compound is well known with its wide range of biological activities, most important, this compound is characterized with cytotoxic activity against colon cancer cells, head and neck squamous carcinoma FaDu cell, and etc[275], [287]. Same effect was observed while studying cytotoxic activity of Compound 3 against Human melanoma and breast adenocarcinoma cells, which confirms that isolated compound is Deltonin. The data obtained from literature and from the experiments can explain that probably Deltonin is responsible on cytotoxic activity of *A. saxatile* crude extract and fraction.

Antioxidant studies demonstrated that, crude extract and 50% methanolic fraction of both plants are characterized with antioxidant activity. The free radical scavenging activity of the extract of *Allium* species is well known. Antioxidant activity is described in onion, garlic, and in many other species of the genus *Allium* (*A. roseum; A. subhirsutum; A. neapolitanum*) [288]–[291]. For example, ethanol extract from *Allium saralicum* has the capability to neutralize ROS in a dose-depending manner[161]. Antioxidant potential of *Allium* species are mostly associated to high amount of polyphenols and flavonoids, found in these species, which are natural antioxidants[153]. The flavonoids extracted from *Allium cepa* have exhibited antioxidant activity [292]. The ethanol extract of *A. ursinum* leaves showed antioxidant activity of 77% with an EC_{50} value of 322 g/mL in DPPH assay [293]. The activity was influenced by the presence of phenolic compounds and flavonoids in these plants. Existence of these compound was confirmed with TLC and HPLC studies. Also, two flavonoid compounds were isolated during the research from *A. saxatile:* Astragalin and Kaempferol 3-O-neohesperidoside. Astragalin is well known for its diversified pharmacological applications such as anti-inflammatory, antioxidant, neuroprotective, cardioprotective, antiobesity, antiosteoporotic, anticancer, antiulcer, and antidiabetic properties[266].

Studies have shown that, these compounds do not express antiprotozoal activity.

Allium species have been shown to have anti-inflammatory and analgesic properties[294]. Methanol extract of red cultivar *A. cepa* bulb significantly increases the percentage inhibition of oedema formation [223].

Methanolic extract of *A. paradoxum* has also significant analgesic activity. The activity was evaluated by Hot plate and acetic acid induced writhing test in male Balb/C mice. In both models, the extracts demonstrated significant analgesic activity [220].

Our data shows that, both plants express analgesic activity. The activity of *A. saxatile* increases with time and reaches its maximum in 60 min, in case of *A. ponticum* analgesic activity is stronger but with short duration. Same tendency is observed among the fractions. Also, their activity is dose dependent.

Concerning anti-inflammatory activity, this effect is higher in crude extract of *A. saxatile.* Among the fractions, obtained from *A. saxatile,* 100% methanolic fraction is more active.

The anti-inflammatory activity of *Allium* plants is described in other species also, for example *A. subhirsutum* [199], *A. cepa* [81], *A. sativum* [158] and etc.

Gastroprotective and antiulcerogenic activity of *Allium* species is well studied. Garlic extract has shown effective preventive activity[212]. Researchers have investigated antiulcerogenic activity of steroidal saponin isolated from *A. ampeloprasum* by measuring acute gastric lesions induced by acidified ethanol. Studied compound has shown significant reduction in gastric hyperemia and severity of lesions[295].

Gastroprotective sutied of the research objects have shown that, crude extract of *A. saxatile* decreases ulcer index, while crude extract of *A. ponticum*, expresses ulcerogenic activity.

The study investigated the phytochemical composition and biological activities of *A. saxatile* and *A. ponticum* of the *Allium* genus. The plants were found to be rich in saponins and flavonoids, and the study revealed their cytotoxic, antioxidant, gastroprotective, analgesic, and anti-inflammatory activities (Table 24). The study supports the traditional use of *Allium* species and highlights their potential as a source of natural compounds with various biological activities.

Table 25. Summary of pharmacological activities of crude extracts and fractions.

	Studied objects

Studied biological activity	A.P. Crd. extr.	A.P.F1	A.P.F2	A.P.F3	A.S.Crd. Extr.	A.S.F1	A.S.F2	A.S.F3
Cytotoxic	-	-	-	✓	✓	-	-	✓
Antiprotozoal	-	-	-	-	-	-	-	-
Antioxidant	✓	-	✓	✓	✓	-	✓	✓
Analgesic	✓	-	✓	✓	✓	-	✓	✓
Gastroprotective	-	-	-	-	✓		✓	
Anti-inflammatory	-	-	-	-	✓	-	✓	✓

Obtained data from the experiments, was used to write temporary monograph of both plants: *A. saxatile* and *A. ponticum,* for their standardization (Temporary monographs of both plant are given in Annex1 and Annex 2.

5. General conclusions and perspectives

In conclusion, this study focused on the microstructural characteristics of the vegetative and generative organs of *A. saxatile* and *A. ponticum*. The optimal conditions for the extraction of secondary metabolites from these plants and their subsequent fractionation were elaborated. The qualitative content of the secondary metabolites in crude extracts of *A. saxatile* and *A. ponticum* was thoroughly investigated. Furthermore, individual compounds were successfully isolated from these plants, and their chemical structures were elucidated. The identified compounds included Kaempferol 3-O-glucoside (Astragalin), kaempferol 3-O-neohesperidoside, and (3β,25R)-Spirost-5-en-3-yl-O-α-L-rhamnopyranosyl-(1→2)-O-[β-Ds-glucopyranosyl-(1→4)]-β-D-glucopyranoside (Deltonin).

Additionally, the study determined the quantitative content of furostanolic saponins and phenolic compounds, such as Astragalin and Ferulic acid in the plant raw material and crude extracts of *A. saxatile* and *A. ponticum*. The cytotoxic and antioxidant activities of the crude extracts and fractions of these plants were established through *in vitro* experiments. Moreover, *in vivo* experiments demonstrated the analgesic, gastroprotective, and anti-inflammatory activities of the crude extracts and fractions of *A. saxatile* and *A. ponticum*.

Finally, based on the findings of this study, temporary normative-technical documentation of the plant materials of *A. saxatile* and *A. ponticum* was compiled.

While the research has provided valuable insights into the phytochemistry and potential therapeutic applications of *A. saxatile* and *A. ponticum*, there are several aspects that require further exploration and should be studied in future work. These areas of investigation are crucial for a more comprehensive understanding of the plants and for maximizing their potential, leading to new avenues of exploration and advancements in the field of drug discovery.

In future perspective, one aspect that requires further exploration is the elucidation of the underlying mechanisms of action for the observed biological activities. While the study has demonstrated cytotoxic, antioxidant, analgesic, gastroprotective, and anti-inflammatory activities, understanding the precise molecular pathways through which these effects are

mediated would be invaluable. Future research should aim to unravel the exact targets and modes of action of the bioactive compounds present in these plants.

Additionally, the study has identified several biologically active compounds, such as Deltonin, Kaempferol 3-O-neohesperoside, and Astragalin, but further investigation is needed to fully characterize and evaluate their therapeutic potential. Future work should involve detailed studies on the pharmacokinetics, bioavailability, and toxicity profiles of these compounds to assess their suitability for drug development.

One potential extension of this work lies also in the identification and isolation of additional bioactive compounds from *A. saxatile* and *A. ponticum*. Which will allow the discovery of new and diverse compounds with potential therapeutic applications. This extension could contribute to expanding the library of bioactive compounds available for drug development, benefiting not only this study but also the wider scientific community.

Furthermore, while the research has conducted *in vitro* and *in vivo* experiments, there may be additional *in vitro* assays and *in vivo* models that could provide a more comprehensive evaluation of the biological activities. Exploring other cell lines or animal models relevant to specific diseases or conditions could provide further insights into the therapeutic potential of *A. saxatile* and *A. ponticum*.

Ideally, future studies should also explore the formulation and delivery methods of the bioactive compounds to optimize their efficacy and ensure their stability. This would involve investigating different extraction techniques, encapsulation methods, and formulation strategies to enhance the bioavailability and therapeutic effects of the isolated compounds.

Moreover, expertise in pharmacology and toxicology could play a vital role in further elucidating the pharmacokinetics, toxicity profiles, and potential drug-drug interactions of the identified bioactive compounds. This knowledge would not only enhance the value of this research but would also provide valuable insights for other studies and researchers working in the area of drug development.

Furthermore, this research can provide valuable contributions to other studies by expanding the knowledge base, enhancing the identification of potential drug targets.

By addressing these aspects, researchers can maximize the potential of *A. saxatile* and *A. ponticum* in drug discovery and pave the way for their eventual translation into practical applications.

6. References

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ANNEXES

Annex 1

Temporary monograph

Allium saxatile - plant

Following temporary monograph expands on - *Allium saxatile,* Familly - *Alliaceae* plant raw material.

Appearance. Stem with 10-50 cm length; Leaves 5-7 grooved, shorter than the trunk. Inflorescence is spherical, or semi-spherical. Anthophore is 1.5-3 times longer than perianth. Pointed egg/bell-shaped perianth with pale pink color. Filaments are 1.5-2 times longer than perianth. Style of pistil is stick out from perianth. Bulbs elongated in shape, dry, with leathery shell.

Powdered plant raw material: Aerial parts of the plant grinded to 1 mm particles.

Microscopy. Examination of superficial section of leaf shows following diagnostic characters: The basal cells of the epidermal tissue of the leaf of *A. saxatile*, are lock-stitched in a row, the cell membrane straight linear, longitudinally elongated, narrowly spindle-shaped in configuration. The stomata is equal to the epidermal tissue. In the mesophyll of the leaf, large and small conductive vessels are arranged alternatively; Between two large conducting vessels, 2 or 3 smaller conducting vessels are differentiated.

The basal cells of the epidermis of the petals are arranged in a row, elongated, straight epidermal cells are present. On the cross-section of the leaf of the perianth, significantly thin, single-rowed, pitted epidermal cells and monochromatic, thin-walled, densely interconnected structure of the mesophyll, can be seen.

Identification:

Flavonoids. Plant extract is obtained as described in "*Quantification"* section.

Thin-Layer Chromatography

Raw material

Test solution: plant extract

Reference solution: Dissolve 1 mg of astragalin in 1 ml of 50% methanol.

Plate: silica gel 60F254 TLC plates

Application volume: 50µl, as band of 1 cm applied at 1 cm from the bottom edge of the plate.

Migration distance: 18 cm

Developing solvent system: mixture of Ethyl acetate : Formic acid : Glacial acetic acid : Water (100:11:11:26 v/v)

TLC chamber: 20x20 cm saturated for at least 40 minutes.

Revelation: 2% 2-Aminoethyl diphenylborinate solution in methanol.

Detection: Observe the plate at 366 nm.

Numerial indication. Moisture not more than 8%; Total ash, not more than 10%; Astragalin _ in plant dry raw material, not less than 0,06%; Organic matter, not more than 0,5 %; Mineral matter, not more than 0,5%;

Quantification.

Astragalin. Content of Astragalin in *A. saxatile* plant material is quantified with High Performance Liquid Chromatography, using following parameter:

Mobile phase: Water (0.1% Acetic acid) and Acetonitrile (77-23, v/v);

Column: Eclipse plus C-18 (4.6 x 250 mm; 5 µm);

Column temp: 25 °C ;

Detector: 320 nm wavelength

Flow rate: 0.8 ml/min

Injection: 10 µl. for standard solution and test solution

The HPLC system: Agilent Technologies Model 1260 infinity liquid chromatography, equipped with a vacuum degasser, a binary pump, an auto-sampler, and a photodiode array detector (DAD).

Chemical marker of *A. saxatile* is Astragalin.

Standard solution preparation: Standard solution of astragalin is prepared in 50% methanol with 1 mg/ml; 0.5 mg/ml; 0.25 mg/ml; 0.125 mg/ml; 0.0625 mg/ml; 0.03125 mg/ml and 0.015625 mg/ml concentrations.

Test solution preparation: 2 g of powdered plant material of *A. saxatile* is transferred into 250 ml volumetric flask and extracted with 100 ml Methanol for one hour. After 1 hour, the solution was cooled to room temperature and filtered in 0.45 μm filter.

Packaging. In bags.

Storage. Dry place, protected from sun light.

Annex 2

Temporary monograph

Allium ponticum - plant

Following temporary monograph expands on - *Allium ponticum,* Familly - *Alliaceae* plant raw material.

Appearance. Stem with 30-70 cm length; Leaves 3-4 grooved, shorter than the trunk, without hollow. Inflorescence semi-spherical, anthophore are 2-3 times longer than perianth. Spherical/bell-shaped perianth with dark red color petals. Filaments as long as perianth or shorter. filaments are split into three parts, lateral parts are higher than middle. Style of carpel is long, higher than petals.

Powdered plant raw material: Aerial parts of the plant grinded to 1 mm particles.

Microscopy. Examination of superficial section of leaf shows following diagnostic characters: The leaf of *A. ponticum* is flat, grooved, bifacial. Covered with unicellular, cone shaped trichomes. The covering tissue of the leaf is sharply cutinized, the cell membrane of the single-row epidermal tissue is strongly thickened. Stomata is immersed in the epidermal tissue. On the basal cells of leaf epidermis, paracytic type of stomata is differentiated. The thickening of the sheath of conducting vessels in stem, is spiral.

On the cross section of the petals, single layered, papilliform cells of epidermis and densely interconnected structure of mesophyll is presented. In the texture of the shell of the bulb different size of crystals are concentrated.

Identification:

Flavonoids. Plant extract is obtained as described in "*Quantification"* section.

Thin-Layer Chromatography

Raw material

Test solution: plant extract

Reference solution: Dissolve 1 mg of Ferulic acid in 1 ml of 50% methanol.

Plate: silica gel 60F254 TLC plates

Application volume: 50µl, as band of 1 cm applied at 1 cm from the bottom edge of the plate.

Migration distance: 18 cm

Developing solvent system: mixture of Ethyl acetate : Formic acid : Methanol : Water (20:0.5:2.5:2 v/v)

TLC chamber: 20x20 cm saturated for at least 40 minutes.

Revelation: 2% 2-Aminoethyl diphenylborinate solution in methanol.

Detection: Observe the plate at 366 nm.

Numerial indication. Moisture not more than 8%; Total ash, not more than 5%; Ferulic acid _ in plant dry raw material, not less than 0,004%; Organic matter, not more than 0,5 %; Mineral matter, not more than 0,5%;

Quantification.

Ferulic acid. Content of Ferulic acid in *A. ponticum* plant material is quantified with High Performance Liquid Chromatography, using following parameter:

Mobile phase: Water (0.1% Acetic acid) and Acetonitrile (75-25, v/v);

Column: Eclipse plus C-18 (4.6 x 250 mm; 5 µm);

Column temp: 25 °C ;

Detector: 280 nm wavelength

Flow rate: 0.7 ml/min

Injection: 10 µl. for standard solution and test solution

The HPLC system: Agilent Technologies Model 1260 infinity liquid chromatography, equipped with a vacuum degasser, a binary pump, an auto-sampler, and a photodiode array detector (DAD).

Chemical marker of *A. ponticum* is Ferulic acid.

Standard solution preparation: Standard solution of Ferulic acid is prepared in 50% methanol with 1 mg/ml; 0.5 mg/ml; 0.25 mg/ml; 0.125 mg/ml; 0.0625 mg/ml; 0.03125 mg/ml and 0.015625 mg/ml concentrations.

Test solution preparation: 2 g of powdered plant material of *A. ponticum* is transferred into 250 ml volumetric flask and extracted with 100 ml Methanol for one hour. After 1 hour, the solution was cooled to room temperature and filtered in 0.45 μm filter.

Packaging. In bags.

Storage. Dry place, protected from sun light.

Annex 3

Published articles

1) Article title: Pharmacological Assessment of Constituents of Species *Allium Saxatile* and *Allium Ponticum* Growing in Georgia

Journal: Experimental and Clinical Medicine Georgia

DOI: https://doi.org/10.52340/jecm.2022.06.04

2) Article title: Microstructural features of generative and vegetative organs of *Allium ponticum* growing in Georgia

Journal: Georgian Scientists

DOI: https://doi.org/10.52340/gs.2022.04.04.32

3) Article title: Microstructural features of generative and vegetative organs of *Allium saxatile* growing in Georgia

Journal: Georgian Scientists

DOI: https://doi.org/10.52340/gs.2022.04.04.33

4) Article title: Evaluation of Antioxidant and Cytotoxic Activity of Plants *Allium Ponticum* and *Allium Saxatile*, Growing in Georgia

Journal: The Georgian Biomedical News

DOI: https://doi.org/10.52340/GBMN.2023.01.01.02

5) Article title: Dosage of furostanol and flavonoid glycosides of Plants of the Allium Genus, Growing in Georgia

Journal: TSMU Collection of Scientific Works.

DOI: https://doi.org/10.52340/csw

Published articles as co-author

1) Article title: Exploration by molecular networking of *Strychnos* alkaloids reveals the unexpected occurrence of strychnine in seven *Strychnos* species

Journal: Toxicon

DOI: https://doi.org/10.1016/j.toxicon.2022.06.002

2) Article title: Biologically Active Alkaloids of Some Species of Plants Widespread In Georgia

Journal: Georgian Scientists

DOI: https://doi.org/10.52340/gs.2023.05.01.09

Annex 4

Communications

1) Conference name: AFERP – rencontres virtuelles 2022

Title of oral communication: Phytochemical study of secondary metabolites of plants genus *Allium*, growing in Georgia and determination of their biological activity

Place and date of conference: 12-13 July, 2022. Liege, Belgium (Online).

Text of the oral communication:

Phytochemical study of secondary metabolites of plants genus *Allium*, growing in Georgia and determination of their biological activity

JGERENAIA Giorgi[1,2], FREDERICH Michel[2], MSKHILADZE Lasha[1]

[1]*Department of Pharmacognosy, Direction of Pharmacognosy and pharmaceutical Botany, Faculty of Pharmacy, Tbilisi State Medical, University, 33, Vazha Pshavela Ave., Tbilisi, Georgia*

[2]*Laboratory of Pharmacognosy, Center of Interdisciplinary Research on Medicines (CIRM), University of Liège, B36, 4000 Liège, Belgium.*

The genus *Allium* belongs to family Alliaceae. This genus involves up to 1233 species, growing especially in the northern hemisphere. The flora of the Caucasus, and especially that of Georgia, is highly endemic. In the region, about 21% of the flora (900 species) are endemic, about 600 are Caucasian endemic species and about 300 species are endemic to Georgia. The objects of this research were to study the plants *A. saxatile* and *A. ponticum* growing in Georgia. Powdered plants were extracted with 80% EtOH, using an ultrasonic water bath at 50 ˚C. Dried extracts of each plant were subjected to Diaion HP-20 column chromatography. The mobile phase was H_2O-MeOH in gradient condition (100:0; 50:50; 0:100 v/v) and finally EtOAc to give 4 enriched fractions of each plant (A.S.F1_H_2O; A.S.F2_MeOH-50%; A.S.F3_MeOH-100%; A.S.F4_EtOAc; A.P.F1_H_2O; A.P.F2_MeOH-50%; A.P.F3_MeOH-100%; A.P.F4_EtOAc). Cytotoxic, anti-inflammatory, analgesic, gastroprotective and antioxidant activities of total extracts of both plants and these fractions were evaluated using different assays. During the research, the total furostanolic content was quantified, in the plant material and crude extract of both plants. Cytotoxicity was studied on melanoma cells and according to the results, it was found that total extract of *A. saxatile* and 100%-MeOH(A.S.F3 and A.P.F3) fraction of both plats exhibit cytotoxic

activity. Results of other biological tests have shown that total extracts of both plants and fractions A.S.F2, A.S.F3, A.P.F2, A.P.F3 have analgesic activity. Anti-inflammatory activity and gastroprotective activity are mostly exhibited in the plant *A. saxatile* and fraction A.S.F3_MeOH-100%. Fractions A.S.F2 and A.P.F2 have shown antioxidant activity. Individual compounds were isolated from these active fractions and determination of their chemical structure is ongoing.

All these obtained results are important contribution to study these species which were never studied and to find a new sources of biologically active compounds.

Attendance certificate:

université PARIS-SACLAY | FACULTÉ DE PHARMACIE

aferp
Association Francophone pour l'Enseignement et la Recherche en Pharmacognosie

Pr. Pierre Champy
Laboratoire de Pharmacognosie, UMR CNRS 8076 BioCIS
UFR Pharmacie, Université Paris-Saclay,
Bâtiment Moissan, 17 avenue des Sciences,
91400, Orsay, France

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pierre.champy@universite-paris-saclay.fr

Je soussigné Pierre Champy, président de l'Association Francophone pour l'Enseignement et la Recherche en Pharmacognosie, atteste du fait que

M. Giorgi Jgerenaia

a participé au colloque organisé en visioconférence les 12 et 13 juillet 2022 par l'association, intitulé « *AFERP – rencontres virtuelles 2022* ».

X **avec une communication orale**

☐ avec une communication flash

☐ sans communication

Pr. Pierre Champy

Laboratoire de Pharmacognosie - Chimie des substances naturelles
CNRS UMR 8076 BioCIS
UFR Pharmacie
Université Paris-Sud / Paris-Saclay

2) Conference name: INTERNATIONAL SCIENTIFIC-PRACTICAL CONFERENCE

"Georgian Scientific Pharmacy: Past and Present"

Title of oral communication Pharmacognostic and pharmacological aspects of plants genus *Allium* growing in Georgia

Place and date of conference: 1-2 October 2022. Tbilissi, Georgia

Text of oral communication:

PHYTOCHEMICAL STUDY OF SECONDARY METABOLITES OF PLANTS OF THE GENUS ALLIUM, GROWING IN GEORGIA AND DETERMINATION OF THEIR BIOLOGICAL ACTIVITY

Giorgi Jgerenaia[1,3], Natela Gogitidze[2], Nadezhda Mushkiashvili[2], Michel Frederich[3], Lasha Mskhiladze[1]

[1]Department of Pharmacognosy, Direction of Pharmacognosy and pharmaceutical Botany, Faculty of Pharmacy, Tbilisi State Medical University, [2] Department of Preclinical Pharmacological Research, I.Kutateladze Institute of Pharmacochemistry, Tbilisi State Medical University, Tbilisi, Georgia; [3]Laboratory of Pharmacognosy, Center of Interdisciplinary Research on Medicines (CIRM), University of Liège, Belgium.

Corresponding author e-mail g.jgerenaia@tsmu.edu

The genus *Allium* belongs to the former family Alliaceae (now included in Amaryllidaceae). This genus involves up to 1233 species. Plants of the genus *Allium* have a long history of traditional uses worldwide. The story of *Allium* cultivation starts over 4000 years ago in ancient Egypt. *Allium* species are widely used in Georgian traditional medicine as antifungal, antiseptic and antibacterial remedy. 36 species of genus *Allium* are described in Georgia. Among them 5 species are endemic of Georgia and 2 of Caucasus region.

The aim of this project was the phytochemical study of secondary metabolites of species of the genus *Allium*, namely *A. saxatile* and *A. ponticum* growing in Georgia. To obtain crude extract of these plants, powdered plants were extracted with 80% EtOH, using an ultrasonic water bath heated at 50˚C. Dried extracts of each plant were subjected to Diaion HP-20 column chromatography. The mobile phase was H_2O-MeOH in gradient condition (100:0; 50:50; 0:100 v/v) and EtOAc to give 4 enriched fractions of each plant (A.S.F1_H_2O; A.S.F2_MeOH-50%; A.S.F3_MeOH-100%; A.S.F4_EtOAc; A.P.F1_H_2O; A.P.F2_MeOH-50%; A.P.F3_MeOH-100%; A.P.F4_EtOAc).

Analgesic, anti-inflammatory and gastroprotective activity of the aforementioned fractions were evaluated in rodents using "Hot plate", carrageenan induced paw edema, and ethanol induced ulcer assays, respectively.

A. saxatile total extract reveals analgesic activity reaching its maximum at 60 min after the administration. Differently, *A. ponticum* total extract revealed faster onset but shorter duration of action. Similar tendency was observed when studying the efficacy of fractions obtained from total extracts. The fact that A.S.F3 fraction has even higher activity than total extract, allows to conclude that this fraction contains compound(s) responsible for analgesic effect. In ethanol induced ulcer model, only crude extract of *A. saxatile* has moderate gastroprotective effect (Table 1).

Table 1. Pharmacological assessment of total extracts and fractions of A. *saxatile* and *A. ponticum*

Extracts & fractions (50 mg/kg i.p.)	Effects of objects compared to control and studying groups (%) analgesic at 30'	at 60'	anti-inflammatory	gastroprotective
Allium saxatile	55.2	70.3	63.5	37.3
A.s. F2	44.3	58.1	21.4	22.2
A.s. F3	64.6	80.4	59.5	0
Allium ponticum	105.6	81.3	32.7	-15
A.p. F2	56.1	37.4	n/d	n/d
A.p. F3	58.9	39.9	n/d	n/d

Moreover, the crude extract of *A. ponticum* revealed ulcerogenic properties increasing the ulcer index over one in control animals. Assessment of anti-inflammatory activity revealed a notable efficacy of A.S.tot and A.P.tot extracts (63.5% and 32.7%, respectively). Similarly, to analgesic assay, 100% methanolic fraction (A.S.F3) showed the pronounced activity.

The obtained results will contribute to the phytochemistry of *Allium* species already studied and give a strong background for further investigation of active fractions to isolate the individual compounds responsible for the detected activity.

Attendance certificate:

ISPC-2022

CERTIFICATE

is awarded to

Giorgi JGERENAIA

for oral presentation at the

International Scientific-Practical Conference "Georgian Scientific Pharmacy: Past and Present"
dedicated to TSMU I.Kutateladze Institute of Pharmacochemistry 90th and
Academician Iovel Kutateladze 135th anniversary

October 1 – 2, 2022 Tbilisi, Georgia

(TSMU Professional Development Council, meeting minutes #2, 21.06.2022)

N 043

Z. Vadachkoria
Rector of Tbilisi State
Medical University

L. Mskhiladze
Chair of ISPC-2022
Organizing Committee

Best presentation award certificate:

ISPC-2022

International Scientific-Practical Conference
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dedicated to TSMU I.Kutateladze Institute of Pharmacochemistry 90th and
Academician Iovel Kutateladze 135th anniversary.

BEST PRESENTATION

AWARD CERTIFICATE

Presented to

Giorgi Jgerenaia

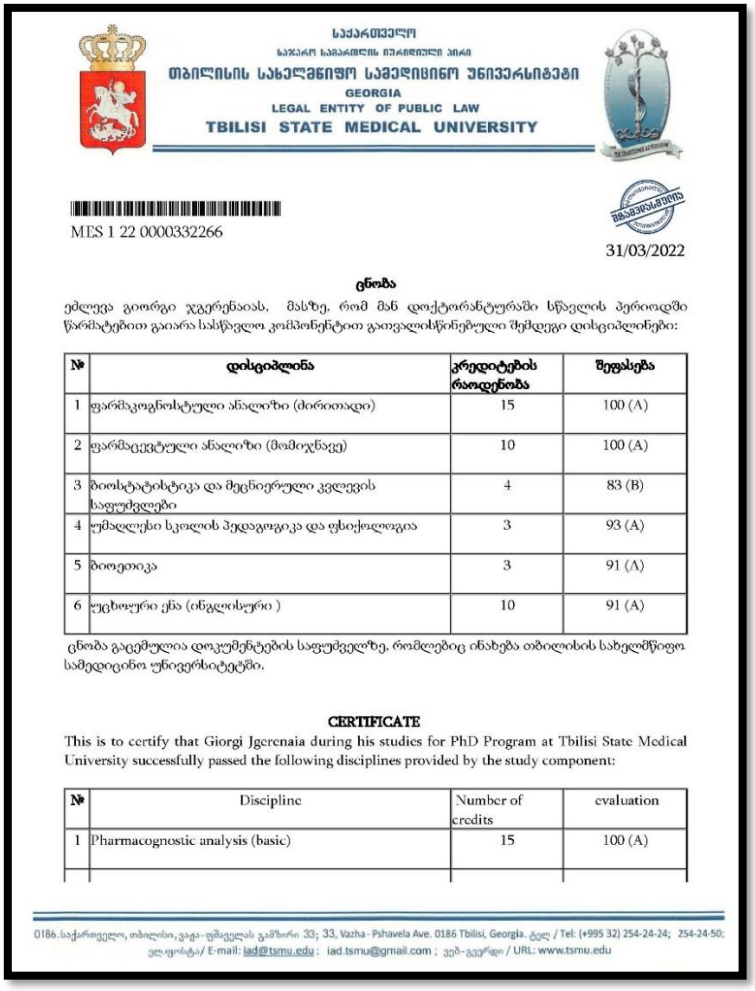
October 1 - 2, 2022, Tbilisi, Georgia

A. Geronikaki
Chair of Juries

L. Mskhiladze
Chair of ISPC-2022
Organizing Committee

Annex 5

Trainings at Tbilisi State Medical University



საქართველო
საჯარო სამართლის იურიდიული პირი
თბილისის სახელმწიფო სამედიცინო უნივერსიტეტი
GEORGIA
LEGAL ENTITY OF PUBLIC LAW
TBILISI STATE MEDICAL UNIVERSITY

MES 1 22 0000332266

31/03/2022

ცნობა

ემლევა გიორგი ჯგერენაიას, მასზე, რომ მან დოქტორანტურაში სწავლის პერიოდში წარმატებით გაიარა სასწავლო კომპონენტით გათვალისწინებული შემდეგი დისციპლინები:

№	დისციპლინა	კრედიტების რაოდენობა	შეფასება
1	ფარმაკოგნოსტული ანალიზი (ძირითადი)	15	100 (A)
2	ფარმაცევტული ანალიზი (მომიჯნავე)	10	100 (A)
3	ბიოსტატისტიკა და მეცნიერული კვლევის საფუძვლები	4	83 (B)
4	უმაღლესი სკოლის პედაგოგიკა და ფსიქოლოგია	3	93 (A)
5	ბიოეთიკა	3	91 (A)
6	უცხოური ენა (ინგლისური)	10	91 (A)

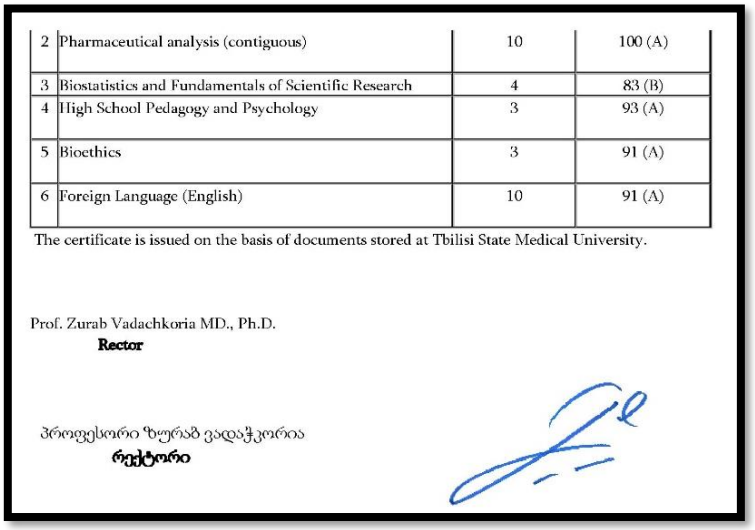
ცნობა გაცემულია დოკუმენტების საფუძველზე, რომლებიც ინახება თბილისის სახელმწიფო სამედიცინო უნივერსიტეტში.

CERTIFICATE

This is to certify that Giorgi Jgerenaia during his studies for PhD Program at Tbilisi State Medical University successfully passed the following disciplines provided by the study component:

№	Discipline	Number of credits	evaluation
1	Pharmacognostic analysis (basic)	15	100 (A)

0186. საქართველო, თბილისი, ვაჟა - ფშაველას გამზირი 33; 33, Vazha - Pshavela Ave. 0186 Tbilisi, Georgia. ტელ / Tel: (+995 32) 254-24-24; 254-24-50; ელ.ფოსტა/ E-mail: iad@tsmu.edu ; iad.tsmu@gmail.com ; ვებ-გვერდი / URL: www.tsmu.edu



2	Pharmaceutical analysis (contiguous)	10	100 (A)
3	Biostatistics and Fundamentals of Scientific Research	4	83 (B)
4	High School Pedagogy and Psychology	3	93 (A)
5	Bioethics	3	91 (A)
6	Foreign Language (English)	10	91 (A)

The certificate is issued on the basis of documents stored at Tbilisi State Medical University.

Prof. Zurab Vadachkoria MD., Ph.D.
Rector

პროფესორი ზურაბ ვადაჭკორია
რექტორი

Annex 6

Trainings

1) Training name: Principes de la communication scientifique

Place of training: University of Liege Belgium

LIÈGE université

ATTESTATION

JGERENAIA Giorgi

a participé à la formation transversale / *participated in the transversal training*
Principes de la communication scientifique (S&T)

mardi 30 novembre 2021
- 10:00 -> 12:00 (2h)

nombre de crédit.s suggéré.s / *number of suggested credit.s*
0,5

intervenant.e.s / speaker.s
Madame Caroline COLLETTE

Le cas échéant, nous vous invitons à soumettre cette attestation à votre Collège de Doctorat afin de faire valider les crédits qui pourraient vous être accordés suite à cette participation. Aucun crédit n'est suggéré pour les formations qui durent moins d'une-demi journée.
If applicable, we invite you to submit this certificate to your Doctoral College in order to validate the credits that may be granted following your participation. No credit suggested for trainings lasting less than half a day.

Fait à Liège, le / *Done in Liège on*
02/12/2021

p.o. V. Dubuy

Prof. Dominique LONGRÉE
Président du C.U.F.D.D.

Conseil Universitaire de la Formation Doctorale et du Doctorat

2) Training Name: Vers l'innovation : introduction à la propriété intellectuelle

Place of training: University of Liege Belgium

LIÈGE université

ATTESTATION

JGERENAIA Giorgi

a participé à la formation transversale / *participated in the transversal training*
Vers l'innovation : introduction à la propriété intellectuelle

Mardi 16 novembre 2021
- 9:00 -> 12:30 (3h30)

nombre de crédit.s suggéré.s / *number of suggested credit.s*
0,5

intervenant.e.s / speaker.s
NAVEAU Elodie (Interface)

Le cas échéant, nous vous invitons à soumettre cette attestation à votre Collège de Doctorat afin de faire valider les crédits qui pourraient vous être accordés suite à cette participation. Aucun crédit n'est suggéré pour les formations qui durent moins d'une-demi journée.
If applicable, we invite you to submit this certificate to your Doctoral College in order to validate the credits that may be granted following your participation. No credit suggested for trainings lasting less than half a day.

Fait à Liège, le / *Done in Liège on*
29/11/2021
p.o.
V. Dubuy
Prof. Dominique LONGRÉE
Président du C.U.F.D.D.

Conseil Universitaire de la Formation Doctorale et du Doctorat

3) Training Name : Vers l'innovation : introduction à la valorisation des résultats

Place of training: University of Liege Belgium

LIÈGE université

ATTESTATION

JGERENAIA Giorgi

a participé à la formation transversale / *participated in the transversal training*
Vers l'innovation : introduction à la valorisation des résultats

Mercredi 17 novembre 2021
- 9:00 -> 12:30 (3h30)

nombre de crédit.s suggéré.s / *number of suggested credit.s*

0,5

intervenant.e.s / speaker.s

Olivier GILLIEAUX
Technology Transfer Officer,
Interface Entreprises-ULiège

Le cas échéant, nous vous invitons à soumettre cette attestation à votre Collège de Doctorat afin de faire valider les crédits qui pourraient vous être accordés suite à cette participation. Aucun crédit n'est suggéré pour les formations qui durent moins d'une-demi journée.
If applicable, we invite you to submit this certificate to your Doctoral College in order to validate the credits that may be granted following your participation. No credit suggested for trainings lasting less than half a day.

Fait à Liège, le / *Done in Liège on*
29/11/2021

p.o.
V. Dubuy

Prof. Dominique LONGRÉE
Président du C.U.F.D.D.

Conseil Universitaire de la Formation Doctorale et du Doctorat

4) Training name: Basics of Nuclear Magnetic Resonance

Training place: Online; Université de Lille

FUN FRANCE UNIVERSITÉ NUMÉRIQUE

Université de Lille

ATTESTATION DE SUIVI AVEC SUCCÈS

Giorgi JGERENAIA
a suivi avec succès le MOOC*

Basics of Nuclear Magnetic Resonance
proposé par Université de Lille
et diffusé sur la plate-forme FUN
Le 17/11/2021

Enseignants

Olivier Lafon
Professor

Cédric Lion
Assistant professor

https://www.fun-mooc.fr

* MOOC : cours en ligne

La présente attestation n'est pas un diplôme et ne confère pas de crédits (ECTS).
Elle n'atteste pas que le participant était inscrit à/au Université de Lille .
L'identité du participant n'a pas été vérifiée.

5) Workshop name: Pharmaceutical Sciences

Place of training: Online; University of Liege

Certificate of Participation

Awarded to

JGERENAIA Giorgi

for attending the 3 day workshop

Pharmaceutical Sciences

held in GIGA (ULiège), Liège, 14-16 December 2020

LIÈGE université
GIGA institute

GIGA Doctoral School
for Health Sciences

Annex 7

Internship at the University of Liege

06/01/2020-06/07/2020

LIÈGE université

Relations internationales

Contact person : Anne-Laure Villeminot
+32 4 366 58 59
anne-laure.villeminot@uliege.be

Liège, 15/11/2019

Scholarship certificate
University of Liège

I undersigned Monique Marcourt, General Director for Teaching and Training, attest and certify:

Mr Giorgi JGERENAIA
Birthdate: 23-11-1994
Birthplace: Martvili
Nationality: Géorgie

Will benefit from a scholarship "Erasmus + International Credit Mobility" awarded by the University of Liège on behalf of the European Commission, for his stay as doctoral student in following research departement : MEDECINE (Pharma) - M. FREDERICH. This scholarship is awarded for his stay from **06 01 2020** to **06 07 2020.**

This scholarship is not subject to social security or taxation.

The scholarship will be allocated for **6 months and 1 day(s), 850 € each month**, according the Erasmus+ rules. The student will be exempted from paying tuition fees. The student will be exempted from paying tuition fees and will receive an amount of **530 €** as participation in the transport costs.

All details regarding the payment modalities will be described in a grant agreement, which will be signed on the day of registration at the University of Liège.

Monique MARCOURT
General Director

Place du 20-Août, 7 — B-4000 Liège — Belgique
anne-laure.villeminot@uliege.be - Tél. +32/(0)4 366 58 59

Annex 8

Internship at the University of Liege

06/09/2021-06/12/2021

LIÈGE université

Erasmus+

Relations internationales

ERASMUS – Séjour académique 2020-2021
Attestation de séjour

A TRANSMETTRE AU RESPONSABLE ERASMUS
DE VOTRE ETABLISSEMENT EN BELGIQUE DES VOTRE RETOUR

A faire compléter dans la semaine qui suit votre arrivée dans l'institution d'accueil *(date d'arrivée pour le 1er cours ou la journée d'accueil ou les cours intensifs de langue)* et durant la semaine qui précède la fin de votre séjour d'études au sein de l'université *(date du dernier jour de présence au sein de l'Université)*.
Les dates de séjour doivent comporter un jour, un mois et une année.
Elles correspondent à la **date d'arrivée dans l'institution d'accueil** (période de préparation linguistique comprise) et à la **date réelle de départ de l'institution d'accueil**.

Ce document est OBLIGATOIRE.
Un oubli pourrait entraîner l'obligation de rembourser votre bourse.

Student family name and first name: JGERENAIA Giorgi
Home Institution: TBILISSI MEDICAL UNIVERSITY
Receiving Institution: U Liège **Faculty:** Medicine **Country:** Belgium
Duration: 3 months **Period of study: from** 06/09/2021 **to** 06/12/2021

Arrival form

We confirm that the above mentioned student has arrived at our institution *(for the 1st course, for the welcoming event organised by the host institution or for language and intercultural courses)*
on 06/09/2021 (day/month/year) and will study for 3 months in our faculty.
Signature :
Name : Anne-Laure VILLEMINOT
Position : International Office
Date: 07/09/2021
Stamp :

Departure form

We confirm that the above mentioned student is leaving our institution *(last day the student has been present at the receiving institution i.e. the end of the exams period, courses or mandatory sitting period and NOT the actual date of departure of the student)* on 06/12/2021 (day/month/year).
Signature :
Name : Anne-laure Villeminot
Position : International Office
Date : 03/12/2021
Stamp :

Place du 20-Août, 7 — B-4000 Liège — Belgique
anne-laure.villeminot@uliege.be - Tél. +32/(0)4 366 58 59

Annex 9

Internship at the University of Liege

01/05/2022-31/07/2022

LIÈGE université

Relations internationales

Contact person : Anne-Laure Villeminot
+32 4 366 58 59
anne-laure.villeminot@uliege.be

Liège, 22/04/2022

Scholarship certificate
University of Liège

I undersigned Patricia PETIT, Director of International Office, attest and certify:

Mr Giorgi JGERENAIA
Birthdate: 23-11-1994
Birthplace: Martvili (Géorgie)
Nationality: géorgienne

Will benefit from a scholarship "Erasmus + International Credit Mobility" awarded by the University of Liège on behalf of the European Commission, for his stay as doctoral student in following research departement : MEDECINE - Michel FREDERICH. This scholarship is awarded for his stay from **01/05/2022** to **31/07/2022.**

This scholarship is not subject to social security or taxation.

The scholarship will be allocated for **3 months and 0 day(s)**, **850 € each month**, according the Erasmus+ rules. The student will be exempted from paying tuition fees. The student will be exempted from paying tuition fees and will receive an amount of **530 €** as participation in the transport costs.

All details regarding the payment modalities will be described in a grant agreement, which will be signed on the day of registration at the University of Liège.

Patricia PETIT
Director of International Office

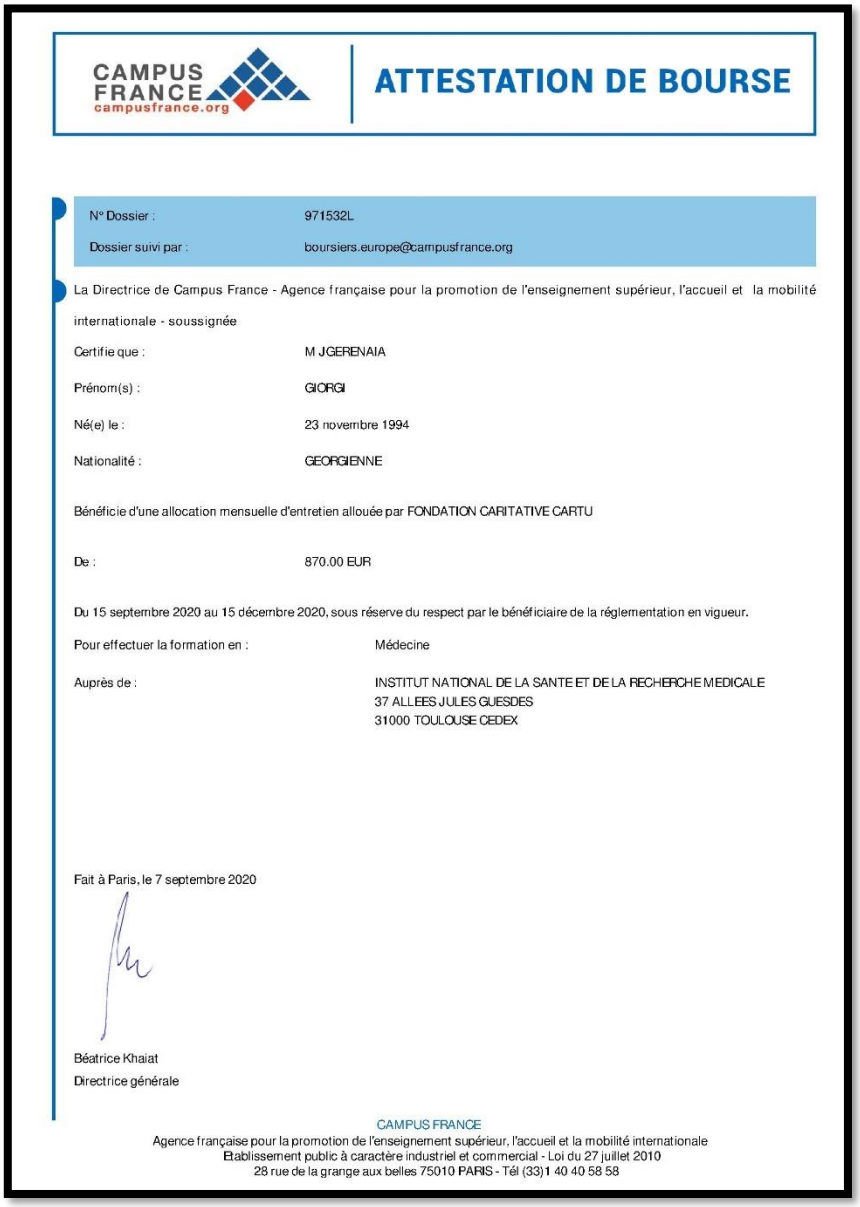
Place du 20-Août, 7 — B-4000 Liège — Belgique
anne-laure.villeminot@uliege.be - Tél. +32/(0)4 366 58 59

Annex 10

Scholarship certificate of "Campus France"

Internship at The Institute of Metabolic and Cardiovascular Diseases (I2MC). Toulouse, France.

15/09/2020-15/12/2020



CAMPUS FRANCE
campusfrance.org

ATTESTATION DE BOURSE

N° Dossier :	971532L
Dossier suivi par :	boursiers.europe@campusfrance.org

La Directrice de Campus France - Agence française pour la promotion de l'enseignement supérieur, l'accueil et la mobilité internationale - soussignée

Certifie que :	M JGERENAIA
Prénom(s) :	GIORGI
Né(e) le :	23 novembre 1994
Nationalité :	GEORGIENNE

Bénéficie d'une allocation mensuelle d'entretien allouée par FONDATION CARITATIVE CARTU

De : 870.00 EUR

Du 15 septembre 2020 au 15 décembre 2020, sous réserve du respect par le bénéficiaire de la réglementation en vigueur.

Pour effectuer la formation en : Médecine

Auprès de : INSTITUT NATIONAL DE LA SANTE ET DE LA RECHERCHE MEDICALE
37 ALLEES JULES GUESDES
31000 TOULOUSE CEDEX

Fait à Paris, le 7 septembre 2020

Béatrice Khaiat
Directrice générale

CAMPUS FRANCE
Agence française pour la promotion de l'enseignement supérieur, l'accueil et la mobilité internationale
Etablissement public à caractère industriel et commercial - Loi du 27 juillet 2010
28 rue de la grange aux belles 75010 PARIS - Tél (33)1 40 40 58 58

Annex 11

Participating institutions

- Tbilisi State Medical University, Direction of Pharmacognosy and Pharmaceutical botany;
- Tbilisi State Medical University Scientific-Research and Practical Skills Laboratory of the faculty of Pharmacy;
- Tbilisi State Medical University, I Kutateladze Institute of Pharmacochemistry;
- Laboratory of Pharmacognosy, Department of Pharmacy, University of Liège, Liège, Belgium.
- The Institute of Metabolic and Cardiovascular Diseases (I2MC). Toulouse, France.