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ЕЖЕМЕСЯЧНЫЙ НАУЧНЫЙ ЖУРНАЛ

Медицинские новости Грузии

საქართველოს სამედიცინო სიახლენი

GEORGIAN MEDICAL NEWS

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GMN: Georgian Medical News is peer-reviewed, published monthly journal committed to promoting the science and art of medicine and the betterment of public health, published by the GMN Editorial Board since 1994. GMN carries original scientific articles on medicine, biology and pharmacy, which are of experimental, theoretical and practical character; publishes original research, reviews, commentaries, editorials, essays, medical news, and correspondence in English and Russian.

GMN is indexed in MEDLINE, SCOPUS, PubMed and VINITI Russian Academy of Sciences. The full text content is available through EBSCO databases.

GMN: Медицинские новости Грузии - ежемесячный рецензируемый научный журнал, издаётся Редакционной коллегией с 1994 года на русском и английском языках в целях поддержки медицинской науки и улучшения здравоохранения. В журнале публикуются оригинальные научные статьи в области медицины, биологии и фармации, статьи обзорного характера, научные сообщения, новости медицины и здравоохранения. Журнал индексируется в MEDLINE, отражён в базе данных SCOPUS, PubMed и ВИНИТИ РАН. Полнотекстовые статьи журнала доступны через БД EBSCO.

GMN: Georgian Medical News – საქართველოს სამედიცინო სიახლენი – არის ყოველთვიური სამეცნიერო სამედიცინო რეცენზირებადი ჟურნალი, გამოიცემა 1994 წლიდან, წარმოადგენს სარედაქციო კოლეგიისა და აშშ-ის მეცნიერების, განათლების, ინდუსტრიის, ხელოვნებისა და ბუნებისმეტყველების საერთაშორისო აკადემიის ერთობლივ გამოცემას. GMN-ში რუსულ და ინგლისურ ენებზე ქვეყნდება ექსპერიმენტული, თეორიული და პრაქტიკული ხასიათის ორიგინალური სამეცნიერო სტატიები მედიცინის, ბიოლოგიისა და ფარმაციის სფეროში, მიმოხილვითი ხასიათის სტატიები.

ჟურნალი ინდექსირებულია MEDLINE-ის საერთაშორისო სისტემაში, ასახულია SCOPUS-ის, PubMed-ის და ВИНИТИ РАН-ის მონაცემთა ბაზებში. სტატიების სრული ტექსტი ხელმისაწვდომია EBSCO-ს მონაცემთა ბაზებიდან.

WEBSITE

www.geomednews.com

К СВЕДЕНИЮ АВТОРОВ!

При направлении статьи в редакцию необходимо соблюдать следующие правила:

1. Статья должна быть представлена в двух экземплярах, на русском или английском языках, напечатанная через **полтора интервала на одной стороне стандартного листа с шириной левого поля в три сантиметра**. Используемый компьютерный шрифт для текста на русском и английском языках - **Times New Roman** (**Кириллица**), для текста на грузинском языке следует использовать **AcadNusx.** Размер шрифта - **12**. К рукописи, напечатанной на компьютере, должен быть приложен CD со статьей.

2. Размер статьи должен быть не менее десяти и не более двадцати страниц машинописи, включая указатель литературы и резюме на английском, русском и грузинском языках.

3. В статье должны быть освещены актуальность данного материала, методы и результаты исследования и их обсуждение.

При представлении в печать научных экспериментальных работ авторы должны указывать вид и количество экспериментальных животных, применявшиеся методы обезболивания и усыпления (в ходе острых опытов).

4. К статье должны быть приложены краткое (на полстраницы) резюме на английском, русском и грузинском языках (включающее следующие разделы: цель исследования, материал и методы, результаты и заключение) и список ключевых слов (key words).

5. Таблицы необходимо представлять в печатной форме. Фотокопии не принимаются. **Все цифровые, итоговые и процентные данные в таблицах должны соответствовать таковым в тексте статьи**. Таблицы и графики должны быть озаглавлены.

6. Фотографии должны быть контрастными, фотокопии с рентгенограмм - в позитивном изображении. Рисунки, чертежи и диаграммы следует озаглавить, пронумеровать и вставить в соответствующее место текста **в tiff формате**.

В подписях к микрофотографиям следует указывать степень увеличения через окуляр или объектив и метод окраски или импрегнации срезов.

7. Фамилии отечественных авторов приводятся в оригинальной транскрипции.

8. При оформлении и направлении статей в журнал МНГ просим авторов соблюдать правила, изложенные в «Единых требованиях к рукописям, представляемым в биомедицинские журналы», принятых Международным комитетом редакторов медицинских журналов - http://www.spinesurgery.ru/files/publish.pdf и http://www.nlm.nih.gov/bsd/uniform_requirements.html
В конце каждой оригинальной статьи приводится библиографический список. В список литературы включаются все материалы, на которые имеются ссылки в тексте. Список составляется в алфавитном порядке и нумеруется. Литературный источник приводится на языке оригинала. В списке литературы сначала приводятся работы, написанные знаками грузинского алфавита, затем кириллицей и латиницей. Ссылки на цитируемые работы в тексте статьи даются в квадратных скобках в виде номера, соответствующего номеру данной работы в списке литературы. Большинство цитированных источников должны быть за последние 5-7 лет.

9. Для получения права на публикацию статья должна иметь от руководителя работы или учреждения визу и сопроводительное отношение, написанные или напечатанные на бланке и заверенные подписью и печатью.

10. В конце статьи должны быть подписи всех авторов, полностью приведены их фамилии, имена и отчества, указаны служебный и домашний номера телефонов и адреса или иные координаты. Количество авторов (соавторов) не должно превышать пяти человек.

11. Редакция оставляет за собой право сокращать и исправлять статьи. Корректура авторам не высылается, вся работа и сверка проводится по авторскому оригиналу.

12. Недопустимо направление в редакцию работ, представленных к печати в иных издательствах или опубликованных в других изданиях.

При нарушении указанных правил статьи не рассматриваются.

REQUIREMENTS

Please note, materials submitted to the Editorial Office Staff are supposed to meet the following requirements:

1. Articles must be provided with a double copy, in English or Russian languages and typed or compu-ter-printed on a single side of standard typing paper, with the left margin of **3** centimeters width, and **1.5** spacing between the lines, typeface - **Times New Roman** (**Cyrillic**), print size - **12** (referring to Georgian and Russian materials). With computer-printed texts please enclose a CD carrying the same file titled with Latin symbols.

2. Size of the article, including index and resume in English, Russian and Georgian languages must be at least 10 pages and not exceed the limit of 20 pages of typed or computer-printed text.

3. Submitted material must include a coverage of a topical subject, research methods, results, and review.

Authors of the scientific-research works must indicate the number of experimental biological species drawn in, list the employed methods of anesthetization and soporific means used during acute tests.

4. Articles must have a short (half page) abstract in English, Russian and Georgian (including the following sections: aim of study, material and methods, results and conclusions) and a list of key words.

5. Tables must be presented in an original typed or computer-printed form, instead of a photocopied version. **Numbers, totals, percentile data on the tables must coincide with those in the texts of the articles.** Tables and graphs must be headed.

6. Photographs are required to be contrasted and must be submitted with doubles. Please number each photograph with a pencil on its back, indicate author's name, title of the article (short version), and mark out its top and bottom parts. Drawings must be accurate, drafts and diagrams drawn in Indian ink (or black ink). Photocopies of the X-ray photographs must be presented in a positive image in **tiff format**.

Accurately numbered subtitles for each illustration must be listed on a separate sheet of paper. In the subtitles for the microphotographs please indicate the ocular and objective lens magnification power, method of coloring or impregnation of the microscopic sections (preparations).

7. Please indicate last names, first and middle initials of the native authors, present names and initials of the foreign authors in the transcription of the original language, enclose in parenthesis corresponding number under which the author is listed in the reference materials.

8. Please follow guidance offered to authors by The International Committee of Medical Journal Editors guidance in its Uniform Requirements for Manuscripts Submitted to Biomedical Journals publication available online at: http://www.nlm.nih.gov/bsd/uniform_requirements.html
http://www.icmje.org/urm_full.pdf
In GMN style for each work cited in the text, a bibliographic reference is given, and this is located at the end of the article under the title "References". All references cited in the text must be listed. The list of references should be arranged alphabetically and then numbered. References are numbered in the text [numbers in square brackets] and in the reference list and numbers are repeated throughout the text as needed. The bibliographic description is given in the language of publication (citations in Georgian script are followed by Cyrillic and Latin).

9. To obtain the rights of publication articles must be accompanied by a visa from the project instructor or the establishment, where the work has been performed, and a reference letter, both written or typed on a special signed form, certified by a stamp or a seal.

10. Articles must be signed by all of the authors at the end, and they must be provided with a list of full names, office and home phone numbers and addresses or other non-office locations where the authors could be reached. The number of the authors (co-authors) must not exceed the limit of 5 people.

11. Editorial Staff reserves the rights to cut down in size and correct the articles. Proof-sheets are not sent out to the authors. The entire editorial and collation work is performed according to the author's original text.

12. Sending in the works that have already been assigned to the press by other Editorial Staffs or have been printed by other publishers is not permissible.

Articles that Fail to Meet the Aforementioned Requirements are not Assigned to be Reviewed.

ავტორთა საყურადღებოდ!

რედაქციაში სტატიის წარმოდგენისას საჭიროა დავიცვათ შემდეგი წესები:

1. სტატია უნდა წარმოადგინოთ 2 ცალად, რუსულ ან ინგლისურ ენებზე,დაბეჭდილი სტანდარტული ფურცლის 1 გვერდზე, 3 სმ სიგანის მარცხენა ველისა და სტრიქონებს შორის 1,5 ინტერვალის დაცვით. გამოყენებული კომპიუტერული შრიფტი რუსულ და ინგლისურენოვან ტექსტებში - **Times New Roman (Кириллица)**, ხოლო ქართულენოვან ტექსტში საჭიროა გამოვიყენოთ **AcadNusx.** შრიფტის ზომა – 12. სტატიას თან უნდა ახლდეს CD სტატიით.

2. სტატიის მოცულობა არ უნდა შეადგენდეს 10 გვერდზე ნაკლებს და 20 გვერდზე მეტს ლიტერატურის სიის და რეზიუმეების (ინგლისურ, რუსულ და ქართულ ენებზე) ჩათვლით.

3. სტატიაში საჭიროა გაშუქდეს: საკითხის აქტუალობა; კვლევის მიზანი; საკვლევი მასალა და გამოყენებული მეთოდები; მიღებული შედეგები და მათი განსჯა. ექსპერიმენტული ხასიათის სტატიების წარმოდგენისას ავტორებმა უნდა მიუთითონ საექსპერიმენტო ცხოველების სახეობა და რაოდენობა; გაუტკივარებისა და დაძინების მეთოდები (მწვავე ცდების პირობებში).

4. სტატიას თან უნდა ახლდეს რეზიუმე ინგლისურ, რუსულ და ქართულ ენებზე არანაკლებ ნახევარი გვერდის მოცულობისა (სათაურის, ავტორების, დაწესებულების მითითებით და უნდა შეიცავდეს შემდეგ განყოფილებებს: მიზანი, მასალა და მეთოდები, შედეგები და დასკვნები; ტექსტუალური ნაწილი არ უნდა იყოს 15 სტრიქონზე ნაკლები) და საკვანძო სიტყვების ჩამონათვალი (key words).

5. ცხრილები საჭიროა წარმოადგინოთ ნაბეჭდი სახით. ყველა ციფრული, შემაჯამებელი და პროცენტული მონაცემები უნდა შეესაბამებოდეს ტექსტში მოყვანილს.

6. ფოტოსურათები უნდა იყოს კონტრასტული; სურათები, ნახაზები, დიაგრამები - დასათაურებული, დანომრილი და სათანადო ადგილას ჩასმული. რენტგენოგრამების ფოტოასლები წარმოადგინეთ პოზიტიური გამოსახულებით **tiff** ფორმატში. მიკროფოტოსურათების წარწერებში საჭიროა მიუთითოთ ოკულარის ან ობიექტივის საშუალებით გადიდების ხარისხი, ანათალების შეღებვის ან იმპრეგნაციის მეთოდი და აღნიშნოთ სურათის ზედა და ქვედა ნაწილები.

7. სამამულო ავტორების გვარები სტატიაში აღინიშნება ინიციალების თანდართვით, უცხოურისა – უცხოური ტრანსკრიპციით.

8. სტატიას თან უნდა ახლდეს ავტორის მიერ გამოყენებული სამამულო და უცხოური შრომების ბიბლიოგრაფიული სია (ბოლო 5-8 წლის სიღრმით). ანბანური წყობით წარმოდგენილ ბიბლიოგრაფიულ სიაში მიუთითეთ ჯერ სამამულო, შემდეგ უცხოელი ავტორები (გვარი, ინიციალები, სტატიის სათაური, ჟურნალის დასახელება, გამოცემის ადგილი, წელი, ჟურნალის №, პირველი და ბოლო გვერდები). მონოგრაფიის შემთხვევაში მიუთითეთ გამოცემის წელი, ადგილი და გვერდების საერთო რაოდენობა. ტექსტში კვადრატულ ფჩხილებში უნდა მიუთითოთ ავტორის შესაბამისი N ლიტერატურის სიის მიხედვით. მიზანშეწონილია, რომ ციტირებული წყაროების უმეტესი ნაწილი იყოს 5-6 წლის სიღრმის.

9. სტატიას თან უნდა ახლდეს: ა) დაწესებულების ან სამეცნიერო ხელმძღვანელის წარდგინება, დამოწმებული ხელმოწერითა და ბეჭდით; ბ) დარგის სპეციალისტის დამოწმებული რეცენზია, რომელშიც მითითებული იქნება საკითხის აქტუალობა, მასალის საკმაობა, მეთოდის სანდოობა, შედეგების სამეცნიერო-პრაქტიკული მნიშვნელობა.

10. სტატიის ბოლოს საჭიროა ყველა ავტორის ხელმოწერა, რომელთა რაოდენობა არ უნდა აღემატებოდეს 5-ს.

11. რედაქცია იტოვებს უფლებას შეასწოროს სტატია. ტექსტზე მუშაობა და შეჯერება ხდება საავტორო ორიგინალის მიხედვით.

12. დაუშვებელია რედაქციაში ისეთი სტატიის წარდგენა, რომელიც დასაბეჭდად წარდგენილი იყო სხვა რედაქციაში ან გამოქვეყნებული იყო სხვა გამოცემებში.

აღნიშნული წესების დარღვევის შემთხვევაში სტატიები არ განიხილება.

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STUDY OF THE BIOACTIVE COMPOUND COMPOSITION, ANTIMICROBIAL, AND CYTOTOXIC ACTIVITIES OF ENDEMIC PLANT SPECIES OF ADJARA-LAZETI

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Abstract.

The flora of Adjara (Southern Colchis) is characterized by high biodiversity, a significant proportion of endemism, and unique forest ecosystems. Our study aimed to analyze the biologically active compounds in leaves of five Adjara-Lazeti endemic species: *Astragalus sommieri, Quercus petraea subsp. Dshorochensis, Amaracus rotundifolius, Rhododendron smirnovii,* and *Rhododendron ungernii*. Methanolic leaf extracts were prepared and analyzed using GC-MS to identify bioactive constituents. Antimicrobial (specifically fungicidal) activity was evaluated in vitro against *Colletotrichum gloeosporioides, Alternaria alternata*, and *Fusarium solani* using the agar well diffusion method. Cytotoxicity was assessed on human lung carcinoma (A-549) and normal skin fibroblasts (WS-1) using resazurin and Hoechst assays. For GC–MS analysis and cytotoxicity assays, methanolic extracts were prepared, the aqueous and 40% ethanolic extracts were used exclusively for antifungal activity evaluation. GC-MS analyses revealed a diverse array of bioactive compounds, including phenolic acids, flavonoids, terpenoids, sterols, etc. *Rhododendron ungernii* extract exhibited the highest selective cytotoxicity against A-549 cells (IC_{50} = 12.4 ± 0.5 µg/ml; SI = 9.68). Strong antifungal activity was observed in R. ungernii, Astragalus sommieri, and R. smirnovii extracts. These results suggest the potential of these endemic species as sources of selective anticancer and antimicrobial agents. All experiments were performed in triplicate. Results are expressed as mean ± standard deviation (SD). Statistical analysis was carried out using one-way analysis of variance (ANOVA). Differences were considered statistically significant at $p < 0.05$.

Key words. Adjara-Lazeti endemics, GC-MS, bioactive compounds, antifungal, cytotoxicity, selective anticancer activity.

Introduction.

Adjara, or the South Colchic floristic region, occupies a noteworthy place due to the exceptional diversity of its vegetation cover, the high proportion of endemic species, and the uniqueness of its forest ecosystems. The flora of Adjara is distinguished by remarkable diversity and originality, which is conditioned by ancient plant communities, endemics, and relicts that were formed as early as the Tertiary period (Paleogene). According to geographic structure, the endemic flora of Adjara includes Caucasian, Georgian, Colchic, Adjara–Lazeti, and Adjara local endemic plant species [1-3].

Comprehensive study of endemic plants with localized distribution is of great importance. In earlier years, the endemic plant species of Adjara and Adjara–Lazeti had been insufficiently investigated. We initiated their study not only from biodiversity and botanical perspectives but also in terms of their phytochemical composition and biological activity.

The objective of the present research was to determine the content of bioactive compounds in the leaves of five endemic species of Adjara–Lazeti belonging to the genera *Quercus* L., *Rhododendron* L., *Astragalus* L., and *Amaracus* Hill. (*Origanum* L.); and to assess the antimicrobial (specifically, fungicidal) and cytotoxic activities of extracts obtained from their leaves. According to scientific literature, species representing these botanical genera are known to contain biologically active compounds and have practical applications in pharmacy, medicine, cosmetology, agriculture, and other fields [4-8]. Therefore, we attach great significance to the study of previously unexplored endemic representatives of these genera in Adjara–Lazeti.

Based on the aim of the research, the objectives of the study were:

1. To collect analytical plant material of the target species in the subalpine and alpine zones of Adjara;
2. To process the collected raw material and prepare extracts for determining the content of biologically active compounds and for assessing antimicrobial and cytotoxic activities under *in vitro* conditions;

To determine fungicidal and cytotoxic activities.

Materials and Methods.

Plant Material: The research objects were five endemic plant species of Adjara–Lazeti: *Astragalus sommieri* Freyn, *Quercus petraea* subsp. *dshorochensis* (K.Koch) Menitsky, *Amaracus rotundifolius* (Boiss.) Briq., *Rhododendron smirnovii* Trautv., and *Rhododendron ungernii* Trautv. *Astragalus sommieri* is a perennial herbaceous plant, whereas the remaining species are woody trees and shrubs. Both *Rhododendron* species are evergreen shrubs growing in the subalpine zone of the Adjara floristic region.

Plant material was collected during field expeditions and processed at the Department of Biodiversity Monitoring and Conservation, Institute of Phytopathology and Biodiversity, Batumi Shota Rustaveli State University.

Preparation of Plant Extracts:

Different extraction protocols were applied depending on the type of analysis and biological activity evaluated.

For GC–MS analysis and cytotoxicity assays, methanolic extracts were prepared.

Briefly, 5 g of air-dried and powdered leaf material were mixed with 25 mL of methanol and extracted by maceration.

After filtration, the solvent was evaporated under reduced pressure.

For GC–MS analysis, 50–55 µL of BSTFA/EtAc (40:10) was added to the dried residue, followed by heating at 70 °C for 20 min.

After cooling, 1 µL of the derivatized sample was injected into the GC–MS system.

GC/MS Conditions: Agilent Technologies 7000 GC/MS Triple Quad; column: Elite 5-MS, 30 m × 250 µm × 0.25 µm; oven temperature: 60–310°C (programmed mode); injector: 250°C; transfer line: 310°C; carrier gas: helium, 1 mL/min; ionization: EI 70 eV; scan mode: TIC. Relative quantification of the identified compounds was performed based on the percentage of peak area (% Area) in the total ion chromatogram (TIC). Peak identification was performed by comparing mass spectra to the NIST database.

For antifungal activity assays, aqueous and 40% (v/v) ethanolic extracts were prepared independently. Aqueous extracts were obtained by extracting 10 g of dried plant material with distilled water (1:10, w/v) at elevated temperature, followed by filtration. Ethanolic extracts were prepared by macerating 10 g of dried plant material in 40% ethanol at room temperature. The extracts were filtered and used for antifungal testing at different dilutions.

Methanolic extracts were used exclusively for GC–MS analysis and cytotoxicity assays, whereas aqueous and 40% ethanolic extracts were used exclusively for antifungal activity evaluation.

Fungicidal Activity Assay:

The antifungal activity of the extracts was evaluated *in vitro* using the Agar Well Diffusion method. Phytopathogenic fungi (*Colletotrichum gloeosporioides*, *Alternaria alternata*, *Fusarium solani*) were obtained from the Institute's culture collection. Sterile Potato Dextrose Agar (PDA) was poured into Petri dishes, and 6 mm wells were cut and filled with extracts. Plates were incubated at room temperature for 24 hours to allow diffusion. Fungal test cultures were inoculated around the wells, and plates were incubated at 25°C. Distilled water was used as a control. Each The fungicidal activity was evaluated by measuring the inhibition of fungal colony growth compared to controls. All experiments were performed in triplicate. Results are expressed as mean ± standard deviation (SD). Statistical analysis was carried out using one-way analysis of variance (ANOVA). Differences were considered statistically significant at $p < 0.05$ [9-10].

Cytotoxicity Assay:

The cytotoxic activity of methanolic extracts was tested against human skin fibroblasts WS1 (ATCC CRL-1502) and human lung carcinoma A-549 (ATCC CCL-185).

Cell Culture: Cells were grown in DMEM supplemented with 10% fetal bovine serum, 1× sodium pyruvate, 1× vitamins, 1× non-essential amino acids, 100 IU/mL penicillin, and 100 µg/mL streptomycin. Cultures were maintained in a humidified incubator at 37°C with 5% CO_2.

Assay Procedure: Cells were seeded in 96-well plates at 5×10^3 cells/well and allowed 24 h for adhesion. Test extracts, dissolved in DMSO (final concentration 0.5%), were added to each well. After 48 h, cytotoxicity was assessed using Resazurin and Hoechst staining. Fluorescence was measured with a plate reader (FLTM, Labsystems, Milford, MA, USA) at an excitation wavelength of 530 nm and emission of 590 nm. Cytotoxicity was expressed as the IC_{50}, i.e., the concentration inhibiting 50% of cell growth.

Results.

Content of Biologically Active

Compounds in the Studied Species:

GC-MS analysis of the leaves of the endemic species *Astragalus sommieri*, revealed the presence of 21 biologically active compounds. The identified compounds belonged to several chemical classes: Organic acids: malic acid; Fatty acids: oleic acid; Phenolic acids: benzoic acid; Sugars and sugar alcohols: xylose, ribitol, galactopyranoside, D-tagatofuranose, D-psicofuranose, D-lyxofuranose, D-pinitol, L-fucopyranose, dulcitol; Polyphenolic compounds: chromone derivatives; Coumarins: knidimin, archangelicin; Flavonoids: quercetin, kaempferol; Triterpenoids: lupeol, α-amyrin derivatives , ursolic acid; Steroidal compounds: campesterol, stigmasterol, β-sitosterol. The major dominant compounds were halfordine and β-sitosterol (Figure 1 and Table 1). These compounds are known for their potential pharmacological activity, including antioxidant, antimicrobial, and cytotoxic properties, which supports the relevance of *A. sommieri* in further biological studies.

GC-MS analysis of the endemic species *Quercus petraea* subsp. *dshorochensis* revealed the presence of 55 compounds. The identified compounds were categorized as follows: Carboxylic, fatty, and phenolic acids: threonine, citric acid, malic acid, shikimic acid, traumatic acid, quinic acid, gallic acid, palmitic acid; Sugars and sugar alcohols: anhydroglucitol, ribitol, arabinofuranose, allofuranose, fructose, galactopyranose, mannopyranose, tagatofuranose, rhamnose; Benzylquinol derivatives; Coumaroylquinic acid derivatives; Pentacyclic triterpenoids: friedelin; Polyphenolic compounds: catechin, epigallocatechin.

The dominant compounds were catechin, the triterpenoid friedelan-3-1, malic acid and citric acid (Figure 2 and Table 2). These bioactive compounds are known for their antioxidant, antimicrobial, and cytotoxic properties, indicating the pharmacological potential of this endemic oak species.

GC-MS analysis of the endemic species *Rhododendron smirnowii*, identified 41 compounds. The detected compounds were classified as follows: Carboxylic, organic, fatty, and phenolic acids: lactic acid, malic acid, succinic acid, valeric acid, quinic acid, citric acid, palmitic acid, lilonelin, jasmonic acid; Amino acids: L-leucine; Sugars: ribofuranose, galactopyranose; Benzodihydropyridine derivatives; Steroidal compounds: α-sitosterol. The dominant compound was the sesquiterpenoid ledol (Figure 3 and Table 3). These bioactive constituents suggest potential pharmacological activities, including antimicrobial and cytotoxic effects.

In the GC-MS analysis of *Rhododendron ungernii*, a total of 26 compounds were identified. These include carbon, various organic and fatty acids, and phenolic acids such as lactic, malic, quinic, citric, protocatechuic, lignoceric, gallic, palmitic, and linolenic acids. Among sugars, arabitol, fructofuranose, and d-erythritol were detected. Sesquiterpene compounds included

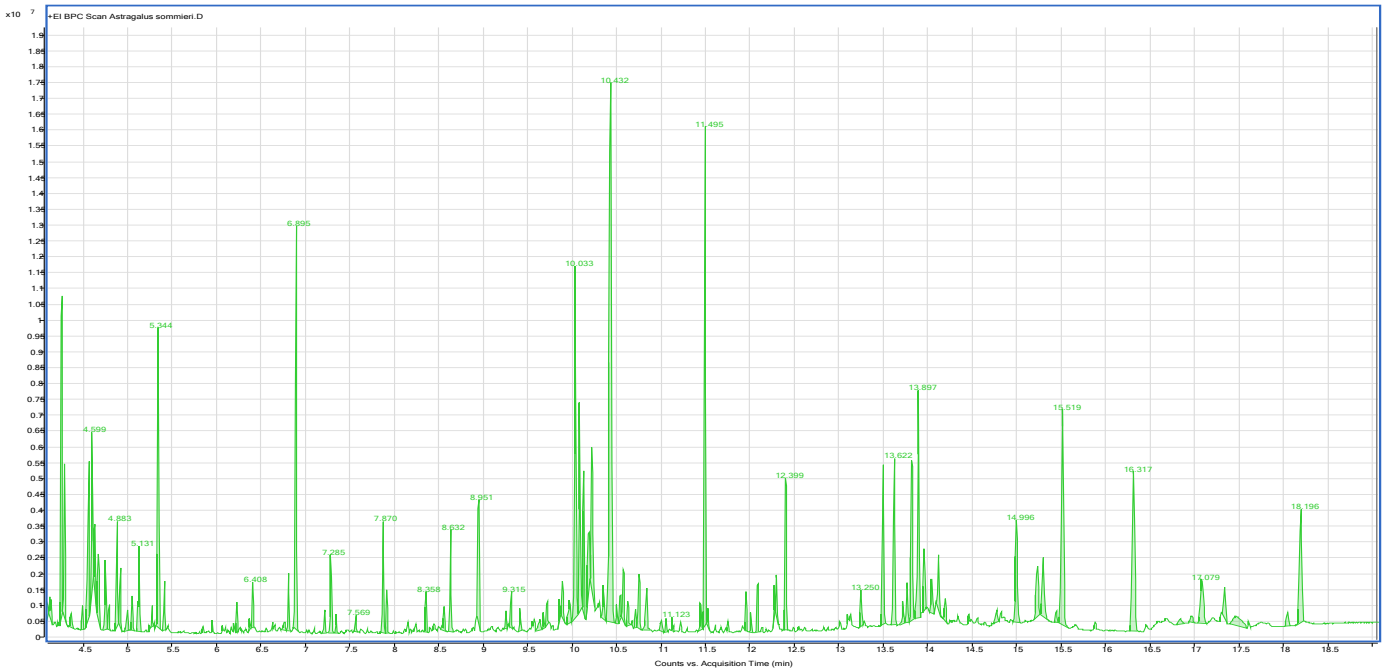

Figure 1. *GC–MS chromatogram of Astragalus sommieri extract.*

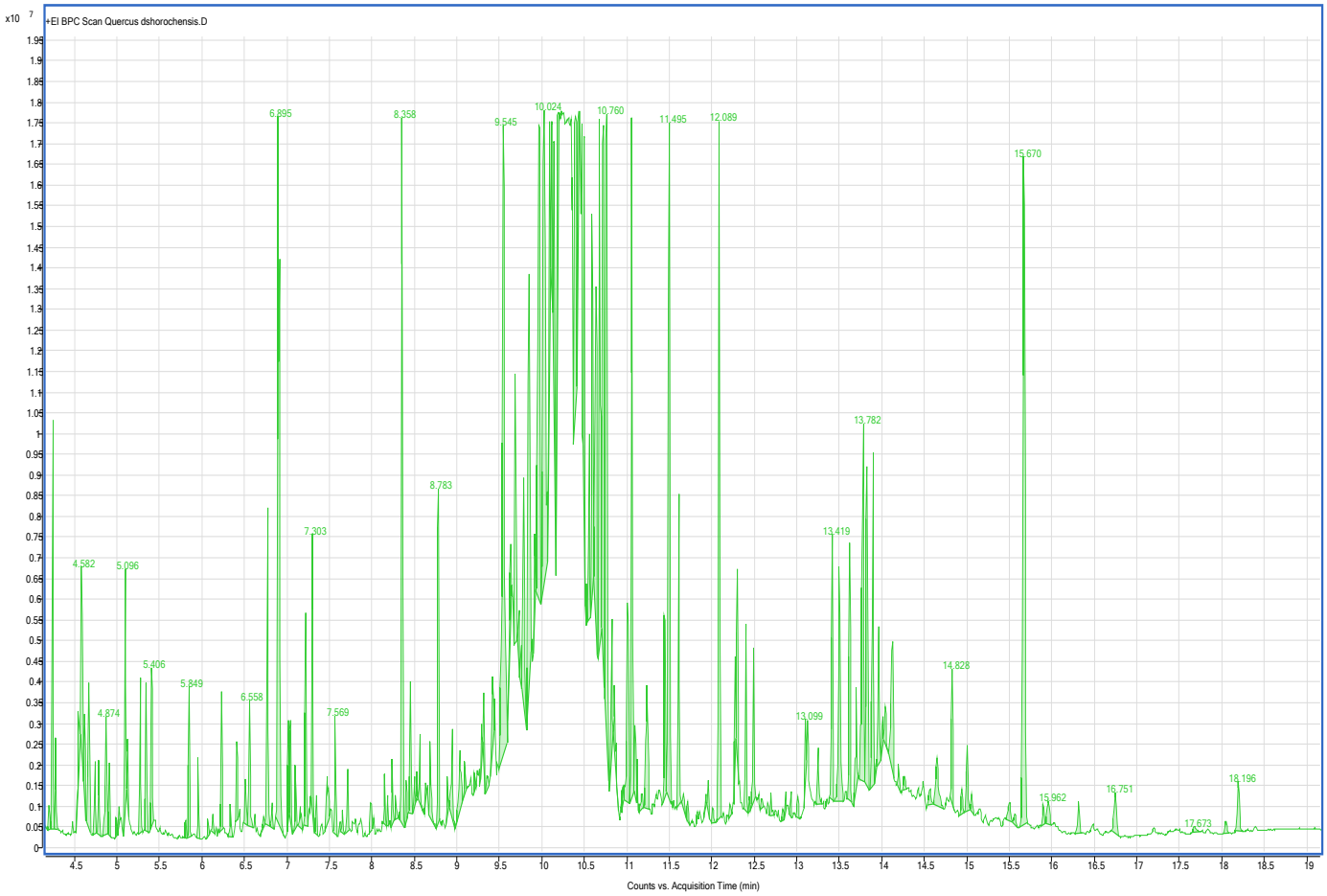

Figure 2. *GC–MS chromatogram of Quercus petraea subsp. dshorochensis extract.*

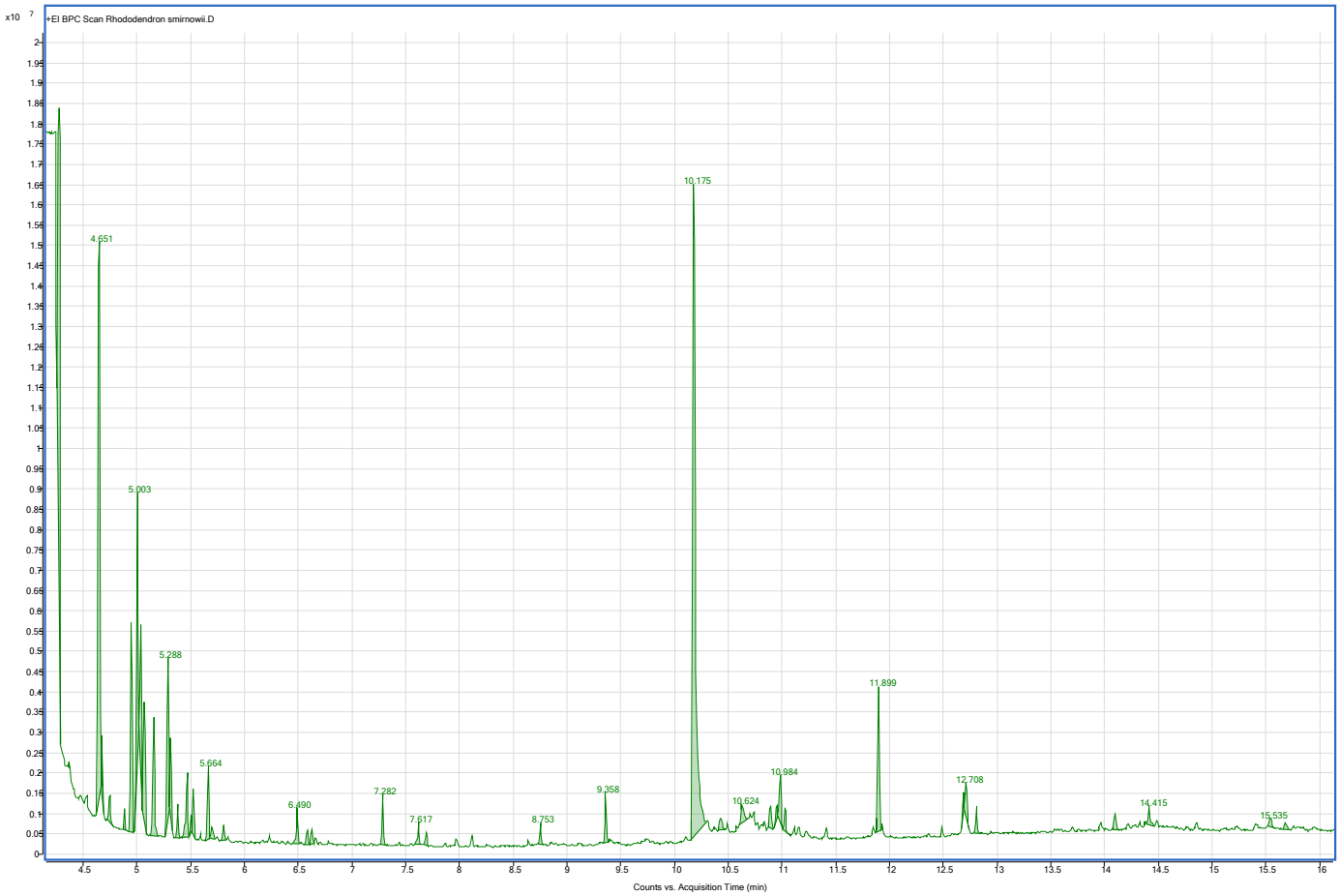

Figure 3. *GC–MS chromatogram of Rhododendron smirnovii extract.*

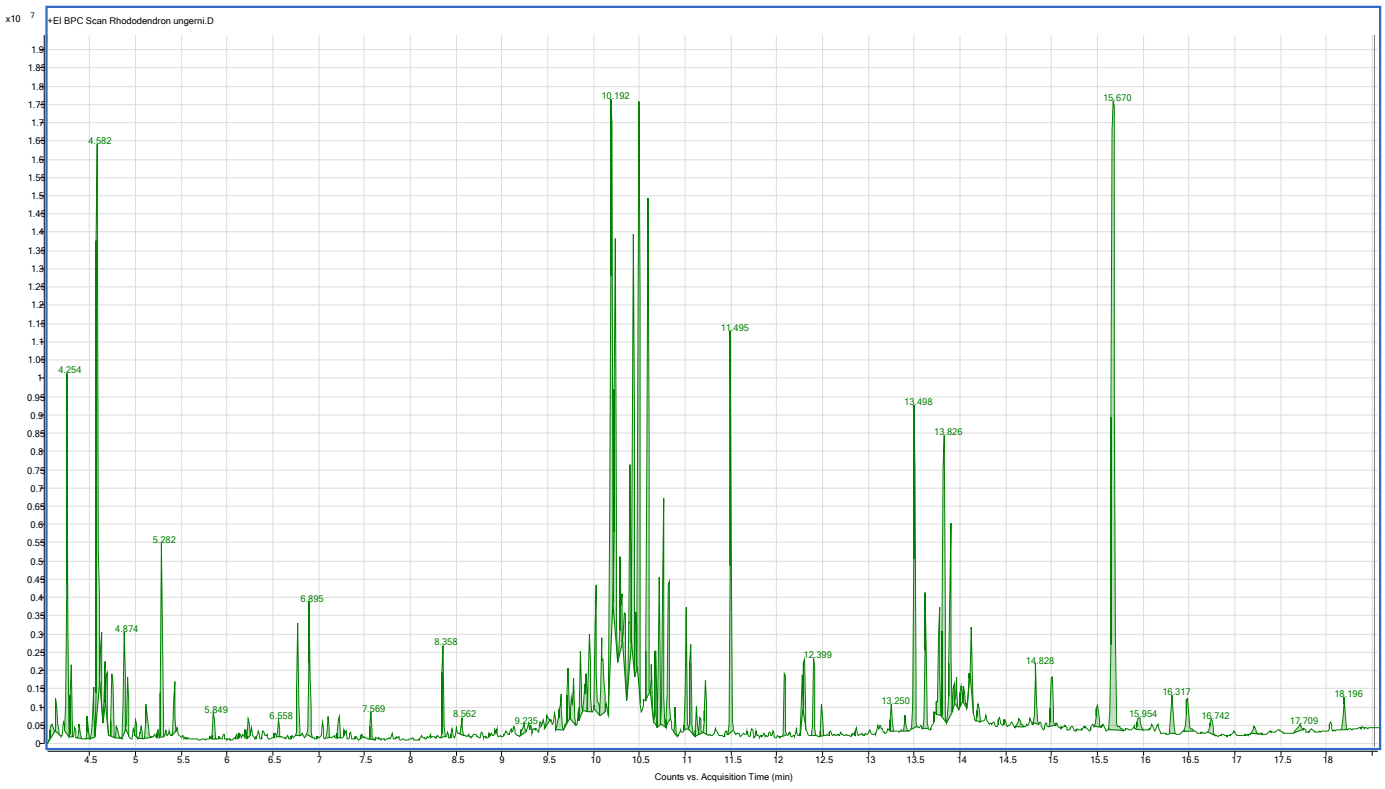

Figure 4. *GC–MS chromatogram of Rhododendron ungernii extract.*

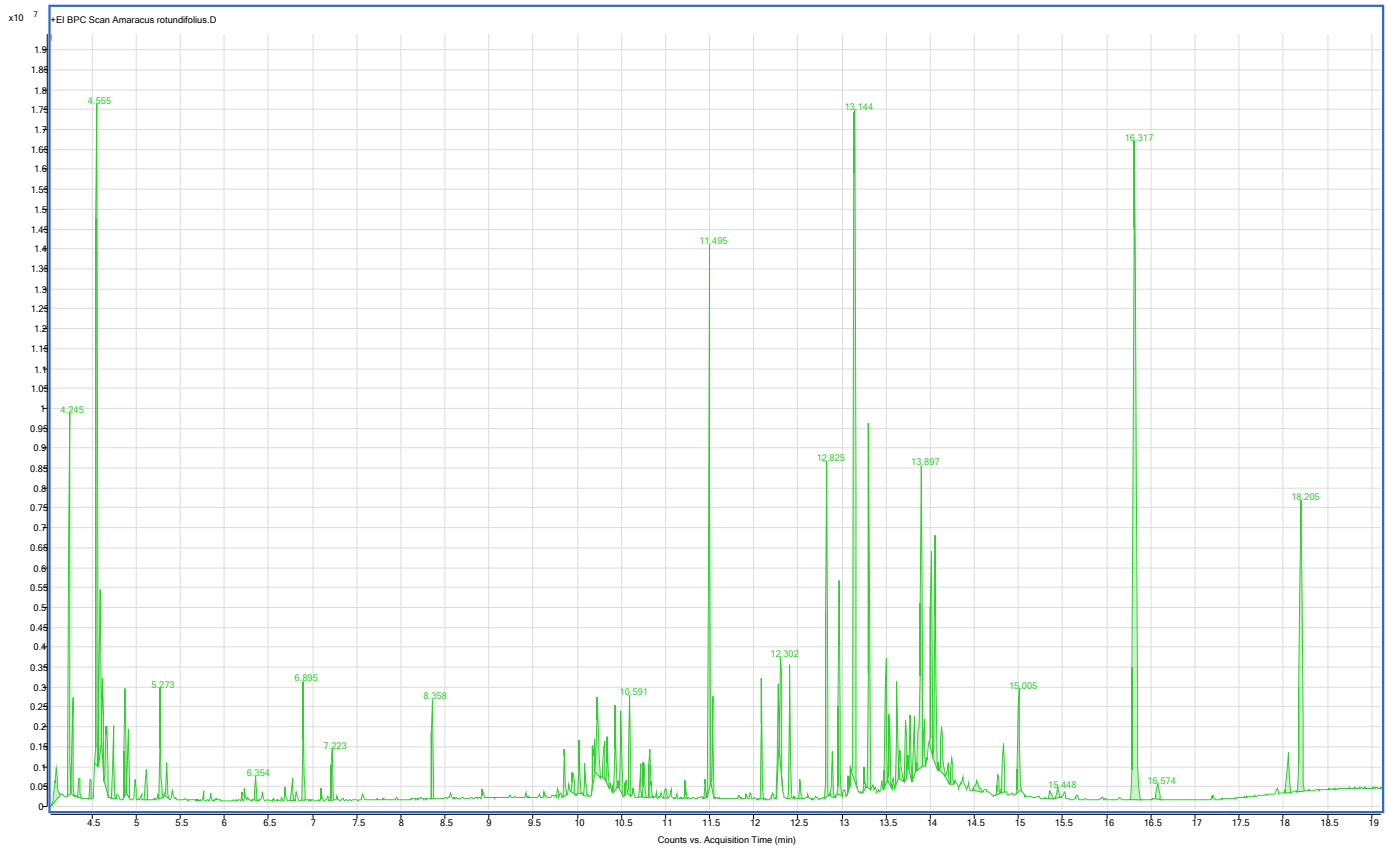

Figure 5. *GC–MS chromatogram of Amaracus rotundifolius extract.*

Table 1. *Characterization of the major dominant compounds Halfordin; β-Sitosterol TMS.*

Name	Formula	MW	Exact Mass	CAS	NIST	ID	Relative content (% Area)	RT (min)
Halfordin	C14H12O6	276	276.063389	18646-71-4	13349	218730	8.6	12.39
β-Sitosterol TMS	C32H58OSi	486	486.425694	2625-46-9	331677	101993	7.1	19.88

Table 2. *Characterization of Catechin (2R-E)-, 5TMS; Friedelan-3-ol; Malic acid 3TMS, Citric acid 4TMS.*

Name	Formula	MW	Exact Mass	CAS	NIST	ID	Relative content (% Ara)	RT
Malic acid 3TMS	$C_{13}H_{30}O_5Si_3$	350	350.140104	38166-11-9	332853	38880	9.5	8.35
Citric acid 4TMS	$C_{18}H_{40}O_7Si_4$	480	480.18511	14330-97-3	333874	191513	8.4	10.75
Catechine (2R-E)-5TMS	$C_{30}H_{54}O_6Si_5$	650	650.276672	89267-68-5	250062	40232	9.2	15.67
Friedelan-3-ol	$C_{30}H_{50}O$	426	426.386166	559-74-0	412502	8644	5.9	18.19

Table 3. *Characterization of Ledol.*

Name	Formula	MW	Exact Mass	CAS	NIST	ID	Relative content (% Area)	RT
Ledol	C15H26O	222	222.198365	577-27-5	249593	1803	7.8	9.35

Table 4. *Characterization of Friedo-18,19-secolup-19-ene, β-Sitosterol TMS, β-Amyrin TMS, α-Amyrin TMS.*

Name	Formula	MW	Exact mass	GAS	NIST	ID	Relative content (% Area)	RT
Friedo-18,19-secolup-19-ene, 3,10-epoxy-, (3β,10β)-	C30H50O	426	426.386166	35060-26-5	32298	33170	7.2	19.60
β-Sitosterol TMS	C32H58OSi	486	486.425694	2625-46-9	331677	101993	6.7	19.88
β-Amyrin TMS	C33H58OSi	498	498.425694	1721-67-1	374765	171601	8.3	20.22
α-Amyrin TMS	C33H58OSi	498	498.425694	1721-67-2	374766	171566	7.9	20.68

Table 5. *Characterization of trans-β-Terpineol, Isopimaric acid TMS, Dehydroabietic acid TMS, Abietic acid TMS.*

Name	Formula	MW	Exact mass	GAS	NIST	ID	Relative content (% Area)	RT
trans-β-Terpineol	C10H18O	154	154.135765	7299-40-3	140974	2508	5.4	8.35
Isopimaric acid TMS	C23H38O2Si	374	374.264107	21414-47-1	90665	39747	7.3	12.97
Dehydroabietic acid TMS	C23H36O2Si	372	372.248457	21414-49-3	79558	180083	8.7	19.26
Abietic acid TMS	C32H58OSi	486	486.425694	2625-46-9	331677	101993	7.9	19.88

Table 6. *Determination of anti-fungal activity by agar well diffusion method.*

Type of fungal strain	*extraction method*	*Astragalus sommieri*	*Quercus petraea subsp. dshorochensis*	*Rhododendr. smirnowii*	*Rhododendr. ungernii*	*Amaracus rotundifolius*
Colletotrichum gloeosporoides	aqueous extract 1:1	22.5 ± 2	17 ± 2	18.5 ± 2.0	24.2 ± 2.2	15.8 ± 1.5
	aqueous extract 1:2	22.0 ± 2	16.5 ± 2	18.0 ± 2.1	24.0 ± 2.0	15.2 ± 1.5
	aqueous extract 1:4	21.8 ± 1,5	15.8 ± 1.5	17.8 ± 1.9	23.8 ± 1.5	14.8 ± 2.3
	40% ethanol extract 1:4	21.5 ± 2	17 ± 2	19.0 ± 1.5	23.7 ± 1.7	16.2 ± 2.2
	40% ethanol extract 1:6	21.9 ± 2	16.9 ± 2	18.7 ± 2,0	22.8 ± 1.6	15.8 ± 1.3
Alternaria alternata	aqueous extract 1:1	21.5 ± 1,7	15.8 ± 2	18.4 ± 2,0	22.4 ± 1.5	15.7 ± 1.3
	aqueous extract 1:2	20.8 ± 2	15.5 ± 2	17.8 ± 1,5	22.3 ± 1.6	15.3 ± 1.3
	aqueous extract 1:4	19.3 ± 1,5	15.0 ± 1,5	17.3 ± 2,0	20.9 ± 1.3	14.8 ± 1,5
	40% ethanol extract 1:4	21.3 ± 2	16.0 ± 2	18.8 ± 2,0	22.7 ± 1.5	14.9 ± 1.5
	40% ethanol extract 1:6	20.7 ± 2	15.7 ± 2	18.2 ± 1,8	21.8 ± 1.5	14.0 ± 1.5
Fusarium solani	aqueous extract 1:1	20.8 ± 2	15.8 ± 1,7	17.8 ± 1,8	22. 9 ± 1.2	15.7 ± 1.8
	aqueous extract 1:2	19.5 ± 2	15.2 ± 1,6	17.5 ± 1.5	22.7 ± 1.2	15.2 ± 1.5
	aqueous extract 1:4	19.0 ± 1,5	14.5 ± 2,0	16.4 ± 1,3	21.2 ± 1.1	14.5 ± 2
	40% ethanol extract 1:4	20. 2 ± 1,5	17.8 ± 2,1	17.0 ± 1,5	23.5 ± 1.5	14.9 ± 1.3
	40% ethanol extract 1:6	19.8 ± 1,5	17.3 ± 2,0	16.5 ± 1,5	22.9 ± 1.5	14.0 ± 1.8

Table 7. *Evaluation of the cytotoxic activity of methanolic extracts of endemic plants of Adjara–Lazeti in an in vitro assay.*

Cell line / Sample	A-549	WS-1
Astragalus sommieri Freyn.	55 ± 3 μg/ml	72 ± 3 μg/ml
Quercus petraea subsp. Dshorochensis c. Koch.	>150 μg/ml	>150 μg/ml
Amaracus rotundifolius (Boiss.)Briq.	>120 μg/ml	>125 μg/ml
Rhododendron smirnovii Trautv.	30 ± 1 μg/ml	8.5 ± 0.8 μg/ml
Rhododendron ungernii Trautv.	12.4 ± 0.5 μg/ml	120 ± 31 μg/ml
Etoposide	1.6 ± 0.2 μM	40 2 μM

ledol, while triterpenoid compounds comprised lupeol, α-amyrin, β-amyrin derivatives, friedelan-3-ol, friedelin, and β-Sitosterol. Polyphenolic compounds identified included flavonoids and catechins, along with various steroidal compounds and others (Figure 4 and Table 4).

During the GC-MS analysis of *Amaracus rotundifolius*, 55 compounds were identified. These include carbon, various organic and fatty acids, and phenolic acids such as malic, hydroxybenzoic, shikimic, vanillic, protocatechuic, syringic, coumaric, ferulic, and linolenic acids. Pentanol derivatives were also detected. Among sugars, deoxyribose, ribofuranose, galactofuranose, and erythrofuranose were found. Benzimidazole derivatives, lignans, and styrene derivatives were identified as well. Triterpenoid compounds included lupeol, α-amyrin, and β-amyrin derivatives, while steroidal compounds comprised stigmasterol and α-sitosterol (Figure 5 and Table 5).

Fungicidal Activity of Leaf Extracts of the Studied Specimens:

Quantitative data on the fungicidal activity, obtained using the methodology described above, are presented in the table 5. The table indicates the phytopathogenic strains used, the extraction method (aqueous and ethanolic extracts at different concentrations), and the inhibition zones statistically calculated based on three replicates.

Aqueous and 40% ethanolic leaf extracts were used for antifungal assays, as described in the extraction protocol.

Screening of aqueous and ethanolic extracts revealed that leaf extracts of *Rhododendron ungernii* and *Astragalus sommieri* exhibit pronounced fungicidal activity. A significantly strong antimicrobial effect was also observed in the leaf extracts of *Rhododendron smirnowii*.

Cytotoxic Activity of Leaf Extracts of the Studied Species:

As shown in table 6, the study evaluated the cytotoxic activity of methanolic extracts from five endemic plants of Adjara-Lazeti on human lung carcinoma cells (A-549) and normal skin fibroblasts (WS-1). Etoposide was used as a positive control for comparison.

Extracts of *Quercus petraea* subsp. *dshorochensis* and *Amaracus rotundifolius* did not exhibit significant activity

within the tested concentration range (IC_{50} > 120–150 μg/ml). The methanolic extract of *Astragalus sommieri* showed moderate cytotoxicity against A-549 cells (IC_{50} = 55 ± 3 μg/ml), while the IC_{50} for WS-1 cells was slightly higher (72 ± 3 μg/ml), indicating weak selectivity.

The extract of *Rhododendron smirnowii* exhibited the opposite trend: its activity against normal fibroblasts (IC_{50} = 8.5 ± 0.8 μg/ml) was significantly higher than against cancer cells (IC_{50} = 30 ± 1 μg/ml), indicating undesirable cytotoxicity toward normal cells.

The most pronounced and selective activity was observed for the extract of *Rhododendron ungernii*. The extract showed a low IC_{50} against A-549 cells (12.4 ± 0.5 μg/ml), while the IC_{50} for WS-1 cells was 120 ± 31 μg/ml. The calculated selectivity index (SI = 9.68) was substantially higher than for the other samples, indicating preferential inhibition of cancer cells. Etoposide, used as a control, exhibited IC_{50} values of 1.6 ± 0.2 μM for A-549 cells and 40.2 μM for WS-1 cells, consistent with its expected high efficacy and selectivity.

Discussion.

During the experimental study, the use of different extraction solvents was dictated by the specific objectives of the study. Methanol was selected for GC–MS profiling and cytotoxicity assays due to its efficiency in extracting phenolic compounds, flavonoids, and triterpenoids associated with anticancer activity. In contrast, aqueous and 40% ethanolic extracts were used for antifungal screening, as these solvents are commonly applied in antimicrobial assays and better reflect potential practical applications.

Based on the study of biologically active compounds in the investigated species, the following analysis can be made:

GC-MS analysis of *Astragalus sommieri*, an endemic species, revealed compounds that may possess biological activity, particularly antimicrobial effects. These include: phenolic acids such as benzoic acid, known for antibacterial and antifungal activity; polyphenolic compounds such as knidimin and archangelicin, which exhibit antibacterial activity against certain bacteria; flavonoids such as quercetin and kaempferol, showing strong antimicrobial activity against bacteria and fungi; triterpenoids including lupeol, α-amyrin, and ursolic acid, often demonstrating antibacterial and antifungal properties; and sterols such as β-sitosterol, campesterol, and stigmasterol, theoretically showing minor antimicrobial effects.

Compounds with cytotoxic and antitumor potential include polyphenols (knidimin, archangelicin), which have shown cytotoxic effects in various cancer cell lines; flavonoids (quercetin, kaempferol), known for antitumor and cytotoxic activities, inhibiting cell cycle progression and inducing apoptosis; triterpenoids (lupeol, α-amyrin, ursolic acid), exhibiting strong antitumor effects, particularly lupeol and ursolic acid; and sterols, where β-sitosterol occasionally shows pro-apoptotic/cytotoxic properties [11-30].

GC-MS analysis of *Quercus petraea* subsp. *dshorochensis* revealed the presence of benzylquinol, a compound with potent antimicrobial activity, particularly against bacteria and fungi. Antitumor effects were associated with phenolic acids such as cumaroilquinone and pentacyclic triterpenoids like friedelin, known for antitumor and antimicrobial properties, especially in leukemia and skin cancer models. Polyphenolic compounds including catechin and epigallocatechin (EGC/EGCG) were also detected, exhibiting strong antioxidant, antimicrobial, and antitumor effects, promoting apoptosis in cancer cells and inhibiting replication of various bacteria and viruses [11-30].

GC-MS analysis of *Rhododendron smirnowii* identified biologically active compounds potentially characterized by antimicrobial and cytotoxic properties. These include phenolic compounds such as benzoic acid, with strong antimicrobial effects and reported antitumor activity; benzodihydropyridines, some of which exhibit antitumor and antimicrobial activity; the steroidal compound α-sitosterol, known for antimicrobial activity against certain bacteria and fungi, with occasional in vitro antitumor effects; and sesquiterpenes such as ledol, which show anti-inflammatory, antimicrobial, and antitumor profiles and sometimes cytotoxic effects in cancer cell lines[11-30].

GC-MS analysis of *Rhododendron ungernii* revealed phenolic compounds with remarkable antimicrobial and antitumor properties, including protocatechuic acid (strong antioxidant, antimicrobial, and cytotoxic effects, stimulates apoptosis in cancer cells) and gallic acid (potent natural antimicrobial and antitumor phenol, active against ESBL-producing bacteria and multidrug-resistant strains, induces apoptosis, increases ROS, and suppresses proliferation in cancer cells). Fatty acids, such as α-linolenic acid (ω-3), exhibited antitumor potential by inhibiting metastasis, modulating membrane lipid composition, and moderate antimicrobial activity. Sesquiterpenes such as ledol, triterpenoids including lupeol (strong antitumor effect via inhibition of PI3K/AKT and Wnt/β-catenin pathways, anti-inflammatory and antimicrobial activity), α- and β-amyrin (anti-inflammatory, antimicrobial, and cytotoxic effects, induces apoptosis in cancer cells), friedelin (antimicrobial and moderate antitumor activity), friedelanol (cytotoxic and antioxidant properties), and ursolic acid (strong natural antimicrobial, antiviral, and antitumor triterpene, induces apoptosis, inhibits NF-κB pathways, suppresses angiogenesis) were also detected. Polyphenols such as flavonoids (quercetin, luteolin, apigenin, kaempferol, etc.) demonstrated strong antimicrobial and antitumor effects through multiple mechanisms, including ROS regulation, inhibition of mTOR and PI3K/AKT pathways, and suppression of angiogenesis. Catechins, including EGCG-type, exhibited pronounced cytotoxic and antimetastatic effects. Steroidal compounds, including phytosterols (β-sitosterol, etc.), showed moderate antitumor and antimicrobial activity [11-30].

GC-MS analysis of *Amaracus rotundifolius* identified biologically active compounds with documented antimicrobial and antitumor activity: potent compounds include lupeol, α- and β-amyrin, β-sitosterol, stigmasterol, protocatechuic acid, syringic acid, coumaric acid, ferulic acid, and vanillic acid; compounds with moderate antimicrobial and antitumor activity include linolenic acid, benzimidazole derivatives, lignans, and shikimic acid [11-30].

Screening of aqueous and ethanolic leaf extracts at various concentrations against phytopathogenic and human-pathogenic fungi revealed pronounced fungicidal activity in *Rhododendron ungernii* and *Astragalus sommieri* leaf extracts. A significant

antimicrobial effect was also observed in *Rhododendron smirnowii* extracts. Other species warrant further study.

Cytotoxicity studies showed that *Rhododendron ungernii* exhibited the most pronounced and selective activity, indicating its potential as a candidate for selective anticancer activity. *Astragalus sommieri* displayed moderate potential, whereas *Rhododendron smirnowii* may exert toxic effects on normal cells. The other tested species were less active under the experimental conditions.

A comparative analysis of the GC–MS profiles provides insight into the observed differences in biological activity.

Rhododendron ungernii exhibited a higher relative abundance of triterpenoids (ursolic acid, lupeol, α- and β-amyrin derivatives) and phenolic acids (gallic and protocatechuic acids), compounds widely associated with selective anticancer activity and apoptosis induction in cancer cells. Importantly, the abundance of highly toxic or non-selective constituents was comparatively lower, which may explain the high selectivity index (SI = 9.68) observed for this species.

In contrast, *Rhododendron smirnovii* was characterized by a notable presence of sesquiterpenes such as ledol. While ledol exhibits antimicrobial and anti-inflammatory activity, it has also been associated with nonspecific cytotoxic effects, which may account for the higher toxicity observed toward normal fibroblasts (WS-1 cells).

Conclusion.

As a result of our study, it was determined that the endemic species: *Astragalus sommieri*, *Quercus petraea* subsp. *Dshorochensis*, *Amaracus rotundifolius*, *Rhododendron smirnovii*, and *Rhododendron ungernii* contain a diverse spectrum of bioactive compounds, as revealed by GC–MS analyses, including phenolic acids, flavonoids, terpenoids, sterols, and others. The extract of *Rhododendron ungernii* exhibited the highest selective cytotoxicity against A-549 cells (IC_{50} = 12.4 ± 0.5 μg/mL; SI = 9.68), while strong antifungal activity was observed in the extracts of *R. ungernii*, *Astragalus sommieri*, and *R. smirnovii*. These findings indicate the potential of these endemic species as sources of selective anticancer and antimicrobial agents and highlight the need for further in-depth investigation.

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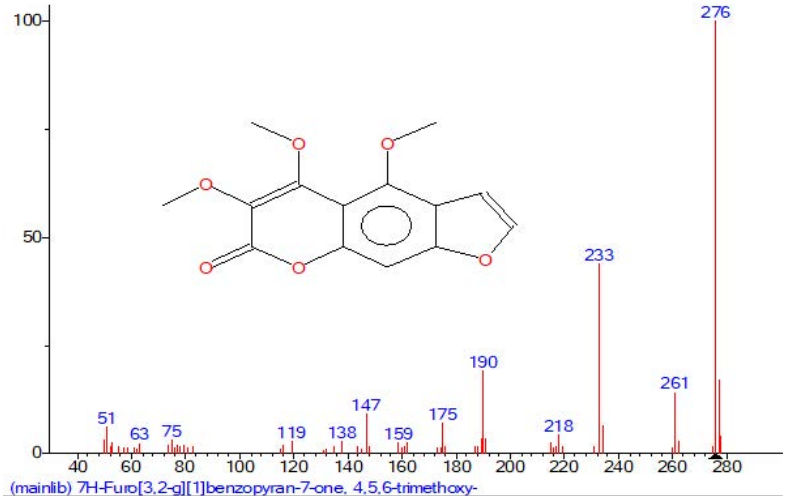

Figure S1. *Mass spectrum of Halfordin*

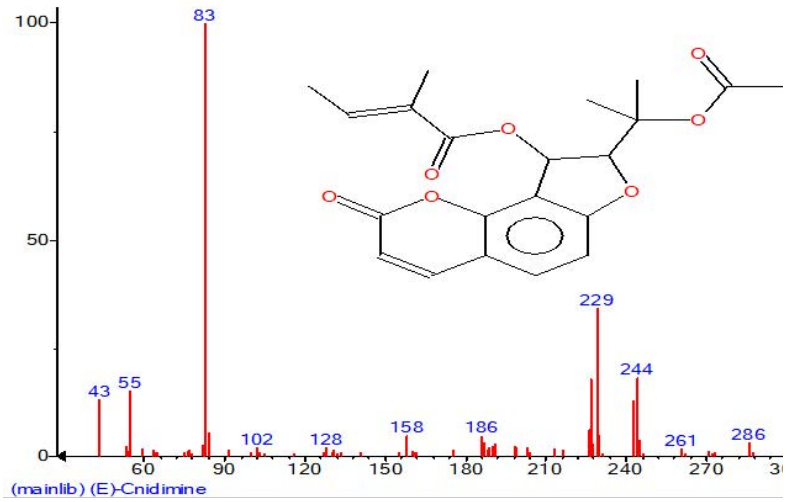

Figure S2. *Mass spectrum of* ***E-Knidimine***

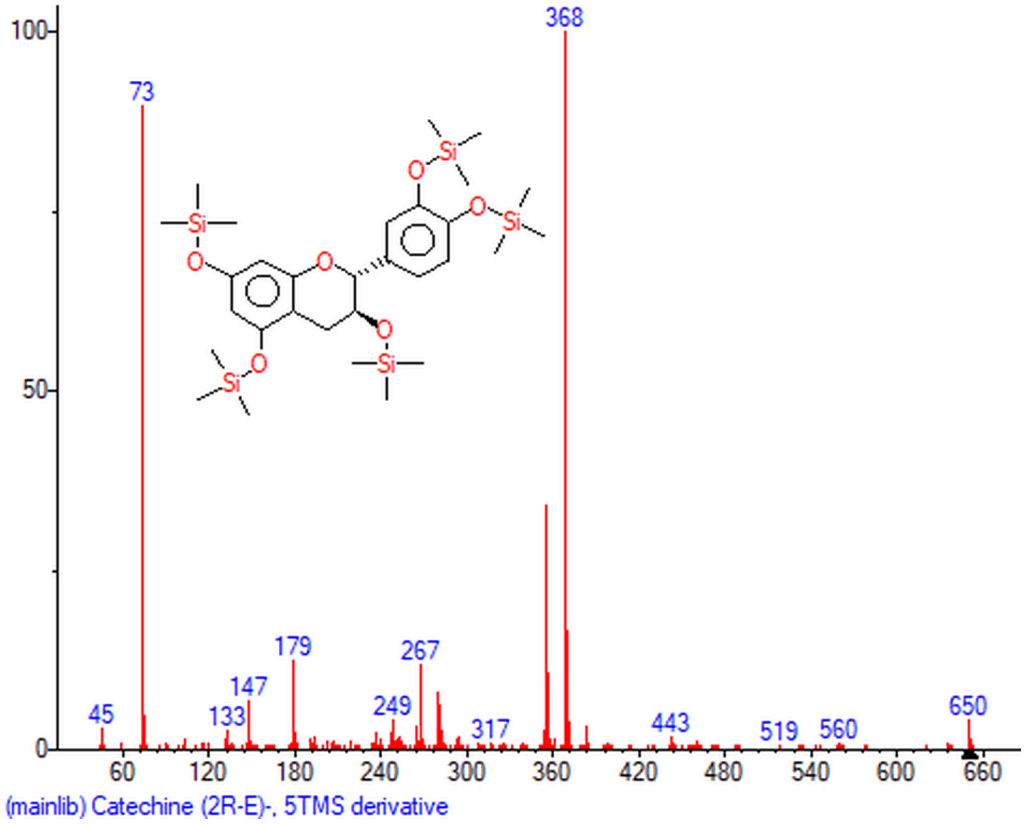

Figure S3. *Mass spectrum of Catechin (2R-E)-, 5TMS*

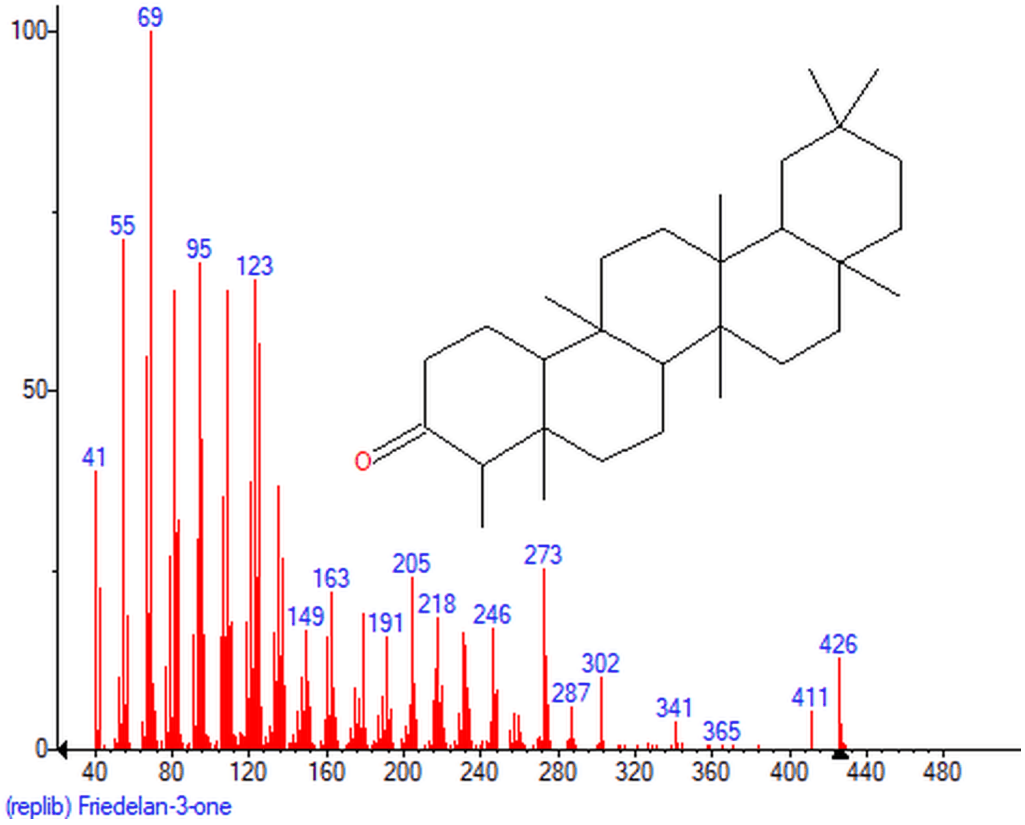

Figure S4. *Mass spectrum of Friedelan-3-ol*

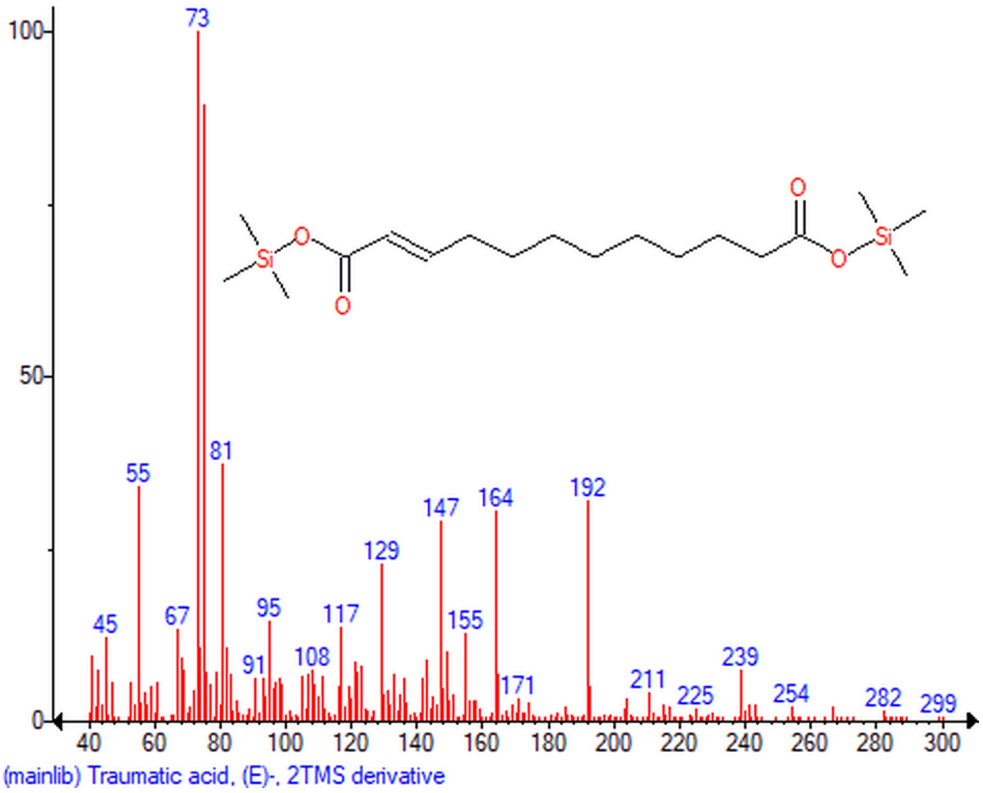

Figure S5. *Mass spectrum of Traumatic acid, (E)-, 2TMS*

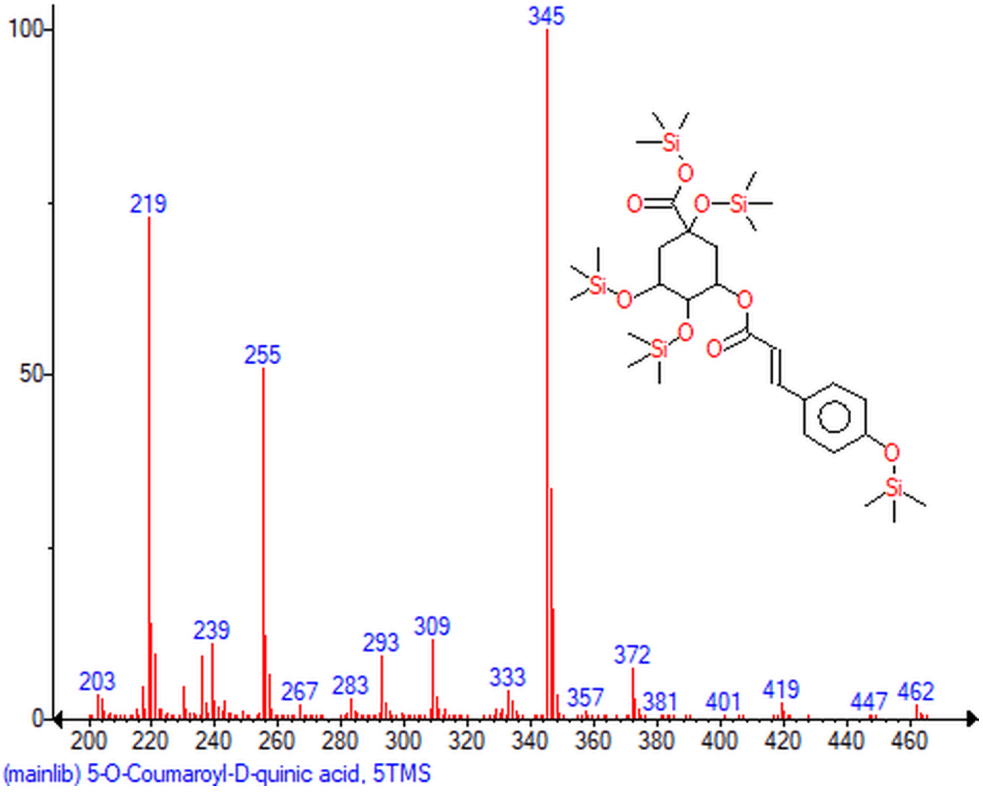

Figure S6. *Mass spectrum of 5-O-Coumaroyl-D-quinic acid, 5TMS*

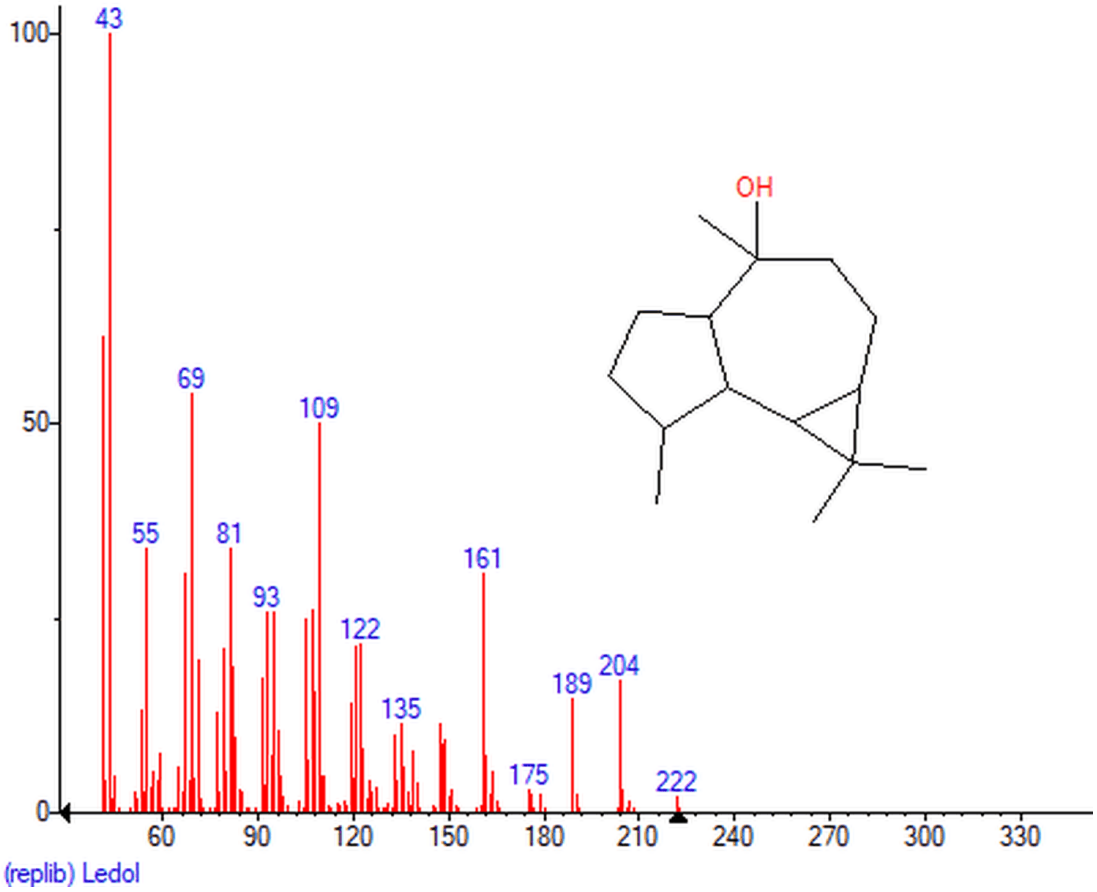

Figure S7. *Mass spectrum of Ledol*

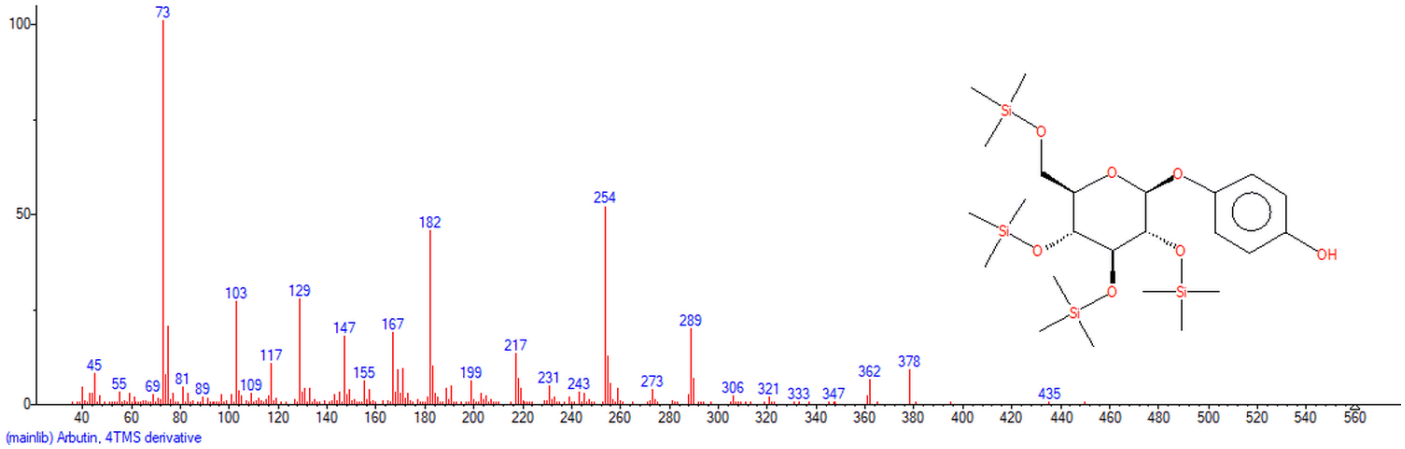

Figure S8. *Mass spectrum of Arbutin, 4TMS*

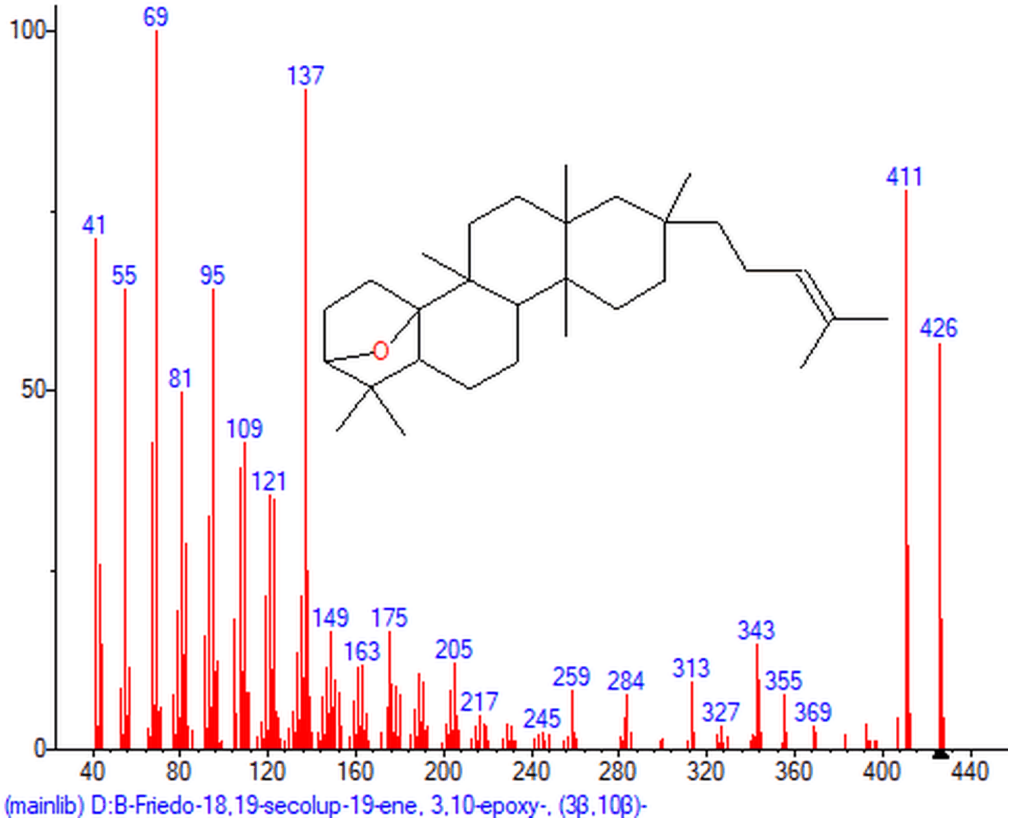

Figure S9. *Mass spectrum of Friedo-18,19-secolup-19-ene, 3,10-epoxy-, (3β,10β)*

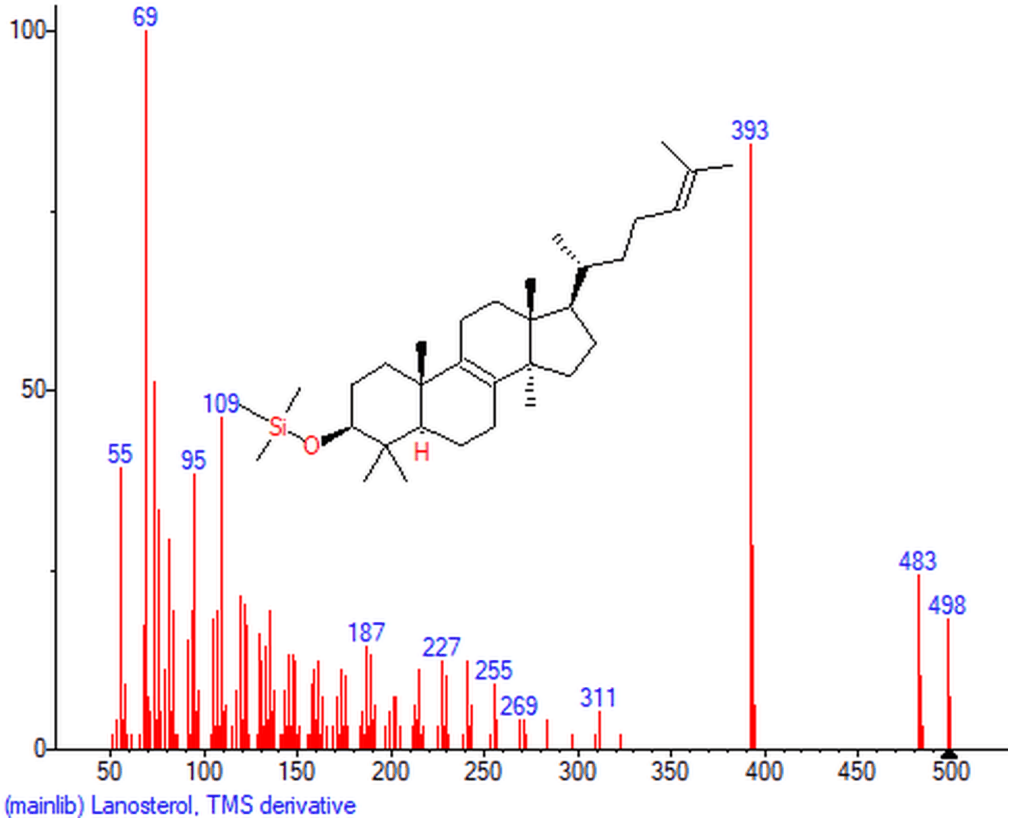

Figure S10. *Mass spectrum of Lanosterol TMS*

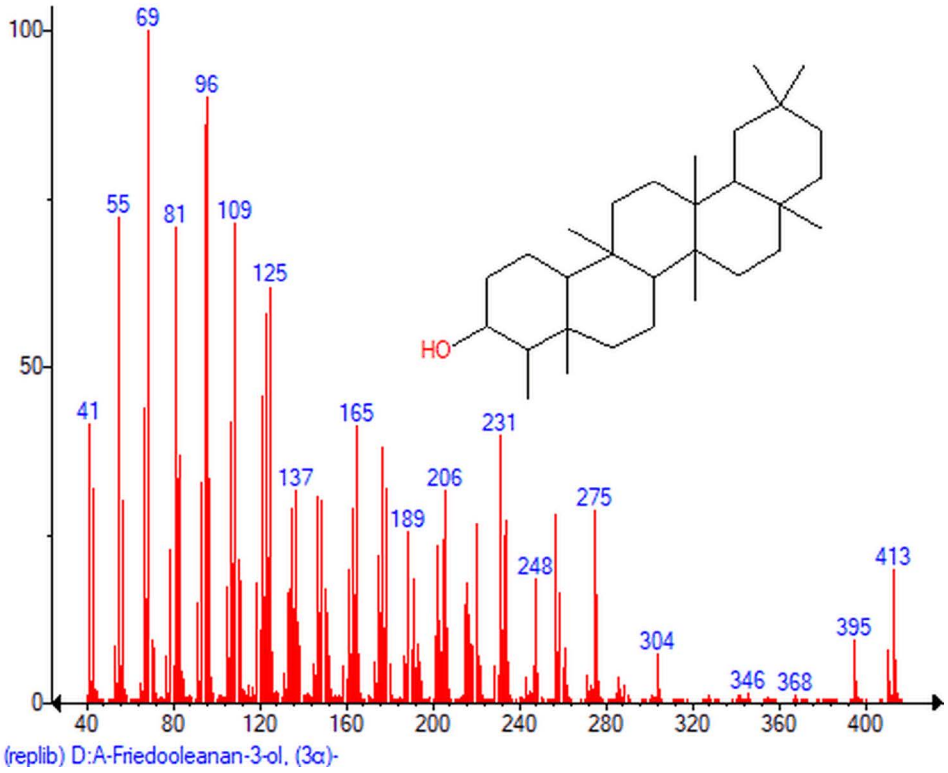

Figure S11. *Mass spectrum of Friedolan-3-ol, (3α)*

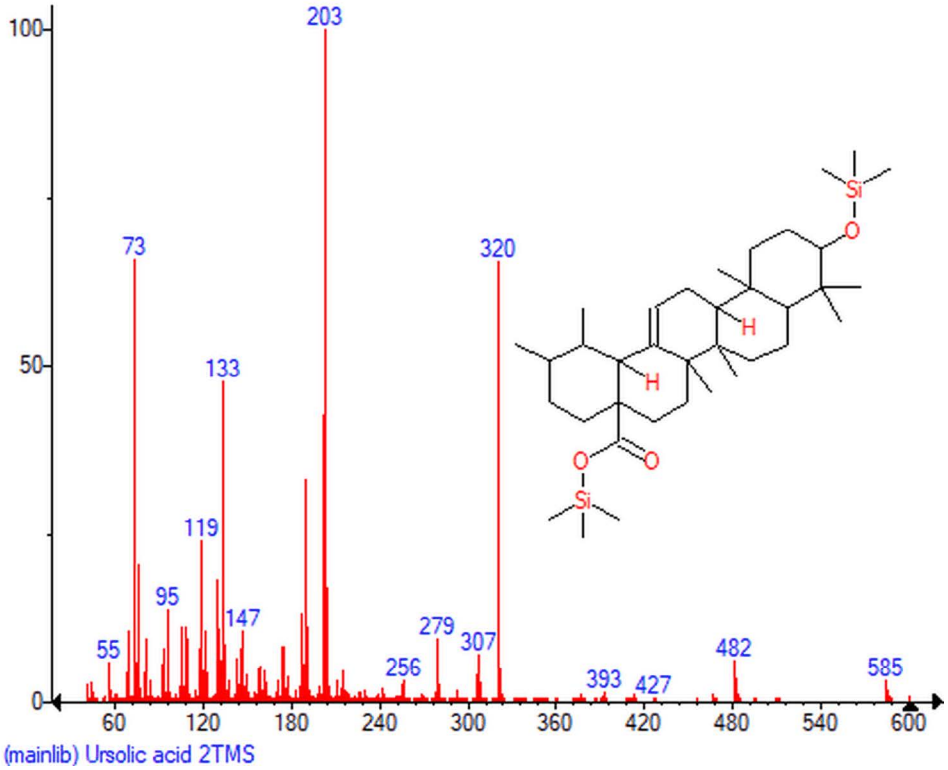

Figure S12. *Mass spectrum of Ursolic acid, 2TMS*

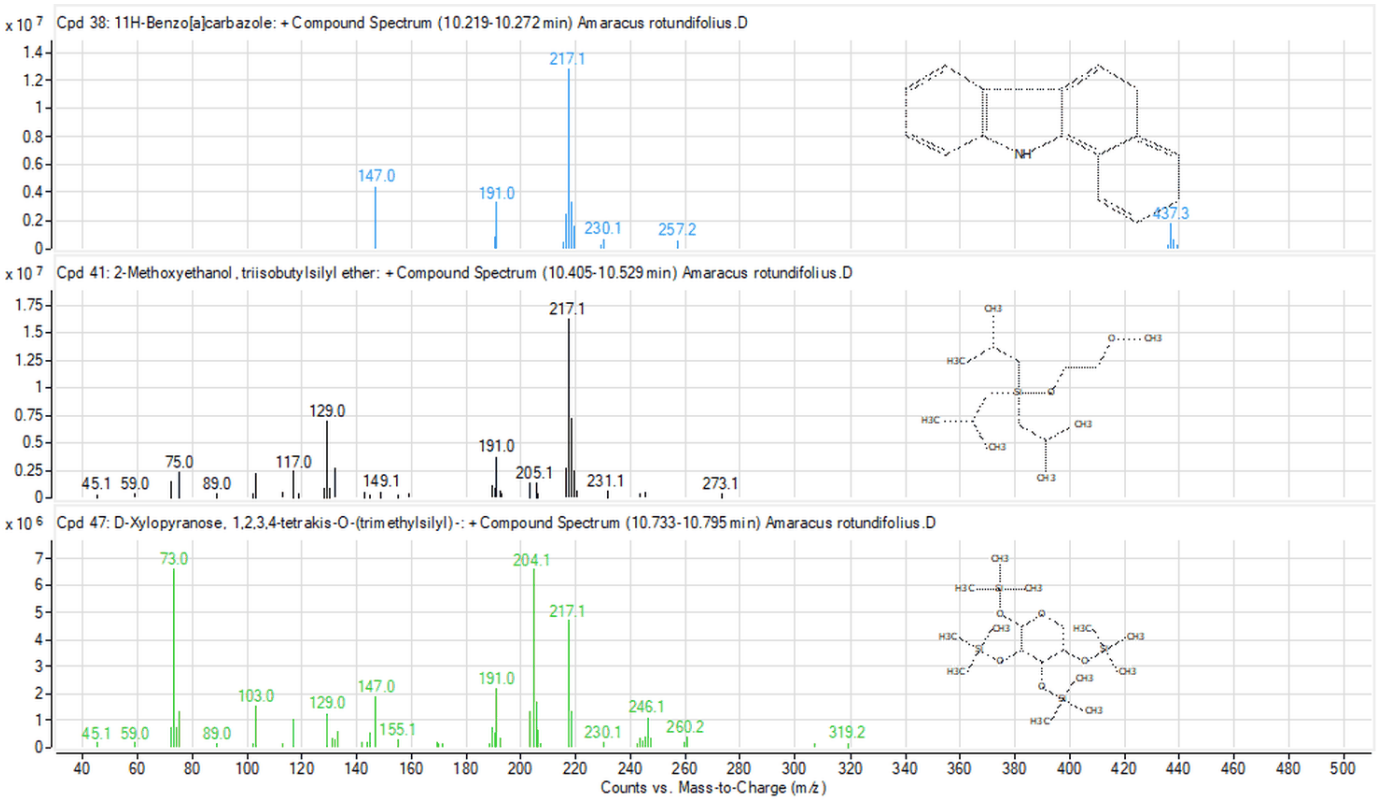

Figure S13. *Mass spectra of selected compounds from the leaf extract of Amaracus rotundifolius*