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LC-MS Analysis of Secondary Metabolites of *Asphodelus aestivus* Brot. (Asphodelaceae) grown wild in Jordan

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ABSTRACT

The phytochemical composition of *Asphodelus aestivus* Brot., a plant with therapeutic properties in traditional medicine, remains largely unexplored, particularly in the specific environmental conditions of Jordan. This study utilized advanced LC-MS techniques to comprehensively analyze the secondary metabolites of a plant species endemic to Jordan. The development of the LC-MS method involved optimizing parameters such as solvent composition, gradient elution, and ionization techniques to achieve comprehensive metabolite profiling. The method was validated to ensure accurate, precise, sensitive, and specific identification and quantification of the compounds. Our analysis identified seven distinct compounds, including both familiar molecules and more complex anthrones and glycosides. This finding emphasizes the wide range of chemical compounds found in the plant and highlights the distinct chemical variations influenced by regional environmental factors. These findings contribute to our understanding of *Asphodelus aestivus* Brot. and highlight the potential therapeutic uses of its distinct phytochemical composition. This research makes a significant contribution to the field of plant-based natural products by combining modern analytical methods with traditional medicinal knowledge to investigate the complex phytochemical composition of *Asphodelus aestivus*.

Keywords: *Asphodelus aestivus* Brot; Phytochemical profile; Jordanian cultivation; LC-MS analysis; Secondary metabolites; Traditional medicinal plant.

1- INTRODUCTION

Asphodelus aestivus Brot., a member of the Asphodelaceae family [1], plays a vital role in both the plant kingdom and traditional medicine. Asphodelus aestivus Brot. has been traditionally used in different cultures for its significant anti-inflammatory, antioxidant, and antimicrobial properties [2-5]. The therapeutic applications of this plant highlight its potential contribution to the development of pharmacological products derived from natural sources. Previous studies have predominantly examined Asphodelus aestivus Brot. utilizing conventional extraction techniques, which have often restricted the investigation to a specific set of metabolites [6]. Moreover, many studies have focused on samples cultivated in particular geographic areas, as illustrated in Figure 1 based on [7, 8], compared with our study in Jordan.

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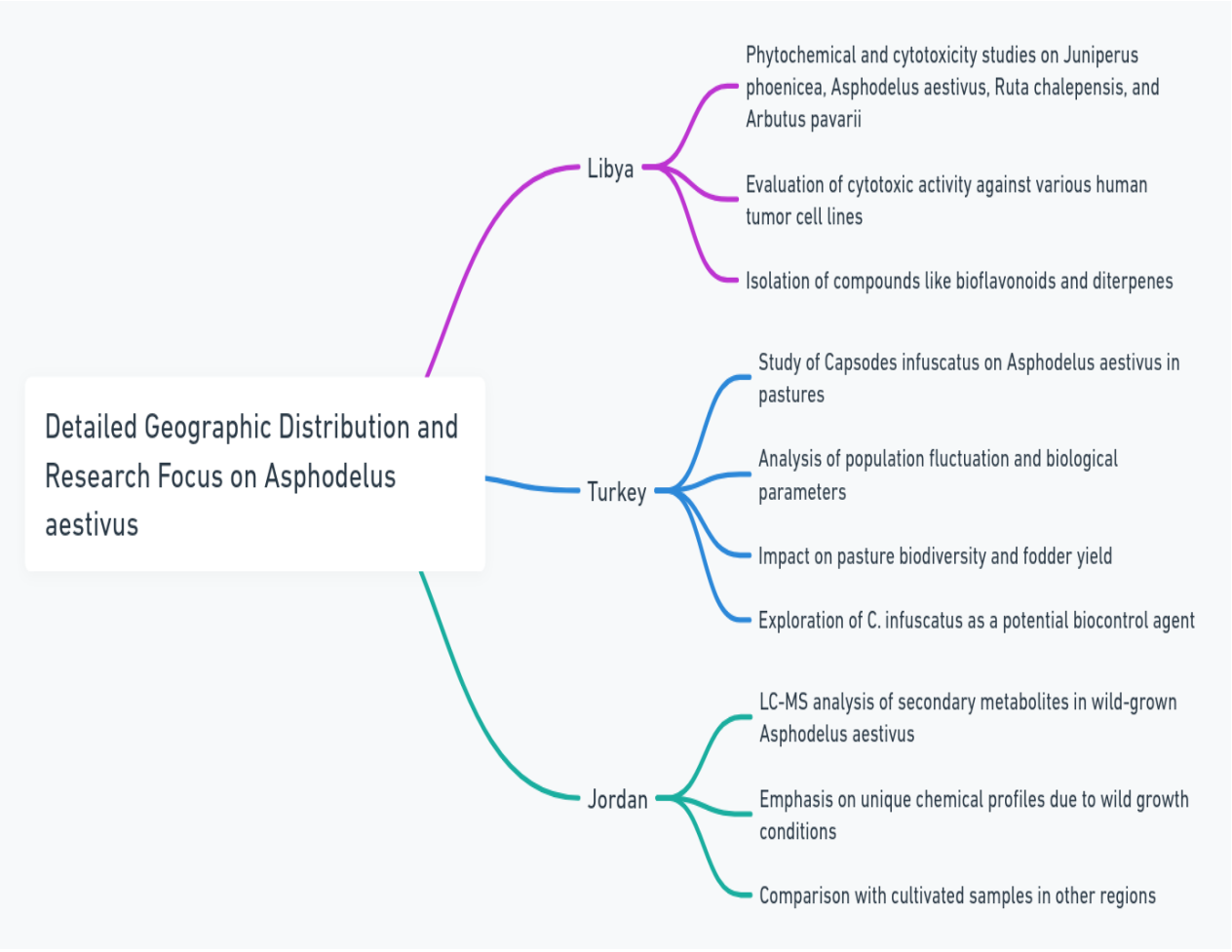

Figure 1: Geographic Distribution and Research Focus of Asphodelus aestivus. The diagram depicts the research approaches employed in Libya, Turkey, and Jordan, particularly focusing on the current utilization of LC-MS analysis for studying wild-grown specimens in Jordan.

This approach neglects the significant impact of environmental factors on the phytochemical composition of the plant. The ecological conditions in Jordan, specifically its diverse environment, have the potential to greatly influence the secondary metabolite profile of plants. However, this aspect has not been extensively studied in the existing literature. Our study suggests the utilization of advanced LC-MS techniques [9, 10], taking into account these factors, as illustrated in Figure 2.

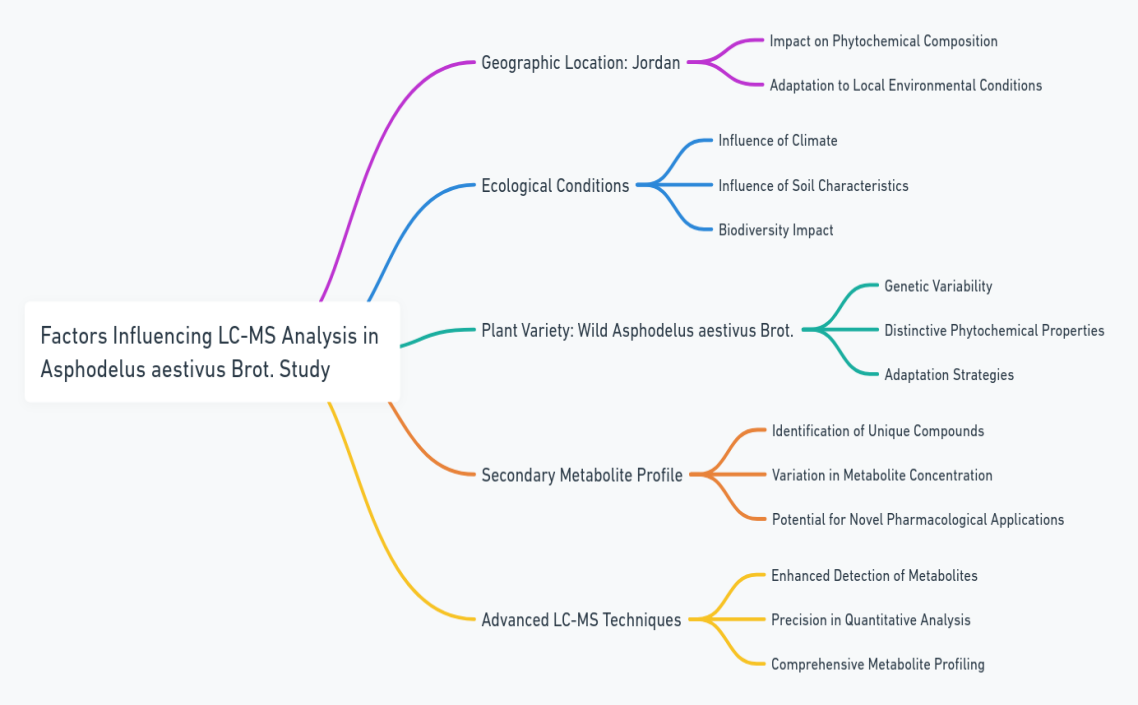

Figure 2 Factors in LC-MS Analysis of Asphodelus aestivus Brot.: This mind map highlights the key elements that impact LC-MS studies of Asphodelus aestivus Brot., focusing on geographic, ecological, and plant-specific factors. Additionally, it emphasizes the importance of advanced analytical techniques in identifying distinct pharmacological compounds found in the native Asphodelus aestivus Brot. of Jordan.

This approach allows for a thorough analysis of the secondary metabolites in *Asphodelus aestivus* Brot., specifically focusing on wild varieties cultivated in Jordan. The objective is to offer a comprehensive analysis of the phytochemical composition specifically suited to the unique ecological context. This perspective is expected to reveal a wider range of secondary metabolites, potentially uncovering unique compounds specific to the Jordanian environment. The findings present potential for pharmacological applications by utilizing the diverse range of phytochemicals in the region. The main objective of this study is to analyze and categorize the secondary metabolites of *Asphodelus aestivus* Brot. discovered in Jordan using advanced LC-MS techniques. Our objectives are to enhance the existing literature in the fields of phytochemistry and pharmacology, with a particular focus on plant-based natural products, and to bridge the gap between traditional herbal knowledge and modern scientific investigation, thereby making a significant contribution to ongoing research in natural product discovery.

2- MATERIALS AND METHODS

2.1- Materials

Experimental Standards: Chrysophenol, aloe-emodin, and frangulin B, sourced from Sigma-Aldrich. Chemicals, Solvents, and Reagents: All of ACS analytical grade, procured from Sharluo, Spain.

2.2- Plant Collection and Classification

Location and Time of Collection: Rhizomes of Asphodelus aestivus collected from Rujm Al-Shoof area, 15 km northeast of Amman, in March 2019.

Taxonomic Verification: Conducted by Dr. Mohammad Gharaibeh, Faculty of Agriculture, Jordan University of Science and Technology. Comparison with voucher specimens at the faculty's herbarium.

Voucher Specimen: Deposited in the Department of Medicinal Chemistry and Pharmacognosy, Faculty of Pharmacy, Jordan University of Science and Technology (ID No.: Phar 09-4).

2.3- General Experimental Procedures:

Column Chromatography (CC): Conducted using silica gel (63-200 µm, Merck, Germany).

Thin Layer Chromatography (TLC): Utilized pre-coated Kiese gel 60 F254 plates (0.2 mm, Merck, Germany). Detection through Ce(SO4)2 / H2SO4 treatment.

Preparative TLC: Employed Silica gel GF254 (20×20 cm, 1 mm thickness).

2.4- Extraction and Purification:

Initial Processing: Dried rhizomes ground and extracted using 70% methanol, followed by 50% methanol.

Defatting and Concentration: Crude extracts defatted with petroleum ether; solvent removal under reduced pressure at 60°C.

Yield and Sub-fractionation: Yield of 15.4% (185g); column chromatography for sub-fractionation into 7 fractions (F1-F7) based on TLC behavior as shown in (Table 1) and illustrated in Figure 3.

Table 1 Summary of Extraction and Identification of Compounds

Fraction	Mass (mg)	Compound	Mass of Compound (mg)	Yield (%)	Method of Isolation	Method of Confirmation
1	15	Chrysophanol (Compound 1)	6	40	n-hexane/EtOAc elution	co-TLC, LC-MS
1	15	Aloe-emodin (Compound 2)	2	13.3	n-hexane/EtOAc elution	co-TLC, LC-MS
2	35	Chlorogenic Acid (Compound 3)	2	8.7	Preparative TLC (CHCl3: MeOH, 90:10, v/v)	co-TLC, LC-MS
3	23	Frangulin B (Compound 4)	1.2	5.2	Preparative TLC (CHCl3: MeOH, 90:10, v/v)	co-TLC, LC-MS
4	18	10-(Chrysophanol-7'-yl)-10-hydroxychrysophanol-9-anthrone (Compound 5)	3.2	17.8	Preparative TLC (CHCl3: MeOH, 95:5, v/v)	LC-MS
5 & 6 (Pooled)	38	Ramosin (Subfraction 1)	7	18.4	Sephadex LH-20 Column (Methanol)	LC-MS
5 & 6 (Pooled)	38	Aspheloside B (Subfraction 2)	9.2	24.2	Sephadex LH-20 Column (Methanol)	LC-MS

Note: Isolation methods such as n-hexane/EtOAc elution and Sephadex LH-20 column chromatography were used for fraction separation. Compounds were confirmed using co-thin layer chromatography (co-TLC) with standards and liquid chromatography-mass spectrometry (LC-MS) analysis.

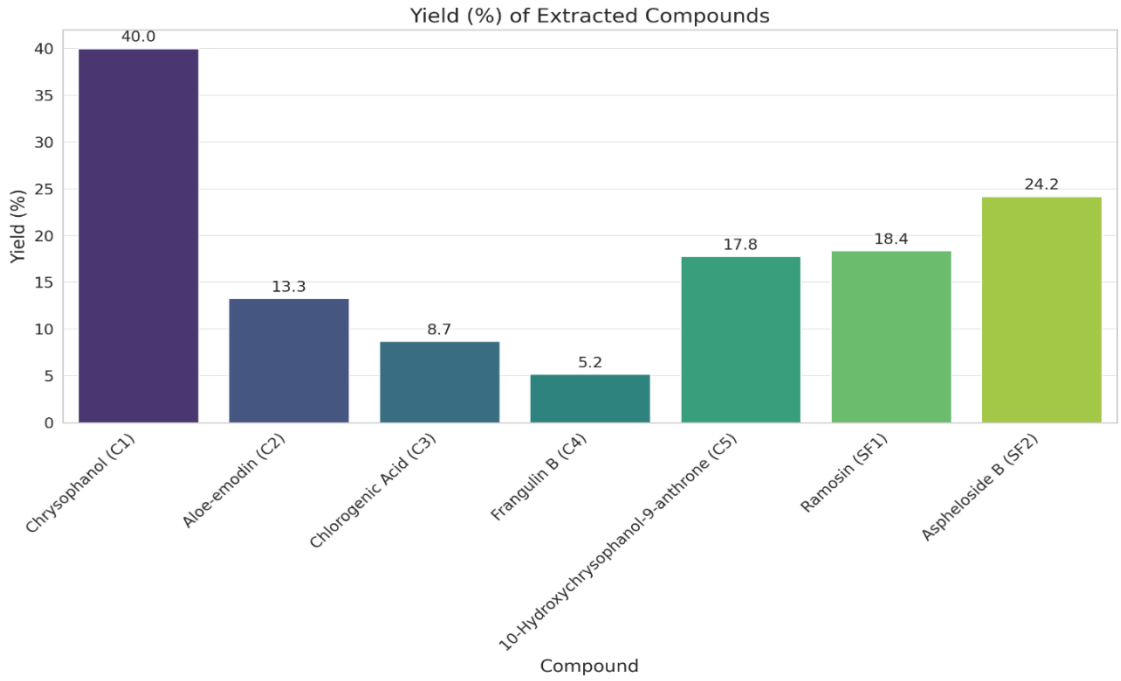

Figure 3 Figure investigates the comparative yield percentages of extracted compounds (C1 to SF2) across different fractions. The bar chart illustrates the efficiency of different isolation methods and their impact on yield outcomes.

2.5- Sample Preparation for LC-MS:

Solubilization: The phytochemical constituents present in the final extract were dissolved to obtain a uniform concentration of 10.0 mg/100 ml in anhydrous methanol. The solvent was chosen based on its ideal compatibility with the organic compounds and minimal impact on the efficiency of ionization in mass spectrometry.

Dilution Optimization: The methanolic solution was diluted deliberately to adjust the analyte's concentrations to match the LC-MS detector's dynamic range. This was done to improve the signal-to-noise ratio and ensure reliable analytical performance.

Microfiltration: A 0.45 µm PTFE syringe filter was used to achieve sterility and eliminate sub-micron particulate contaminants. This step is crucial for preserving the integrity of the analytical column and improving the accuracy of the mass spectrometric analysis.

2.6- LC-MS Analysis:

Instrumentation: Phenomenex Gemini C18 column (250 mm x 4.6 mm i.d.; 5 µm particle size).

Conditions: Negative ion mode, full scan spectra (m/z 50-900), and MS/MS fragmentation on selected ions. Nebulization and drying with high-purity nitrogen at specified temperatures and flow rates.

Data Analysis: Utilizing Analyst 1,6.3 software (Germany-Darmstadt).

The mobile phase

Solution A: Water mixed with 0.005M ammonium acetate and 0.1% acetic acid. Solution B: Acetonitrile mixed with 0.005M ammonium acetate and 0.1% acetic acid. The mobile phase for the LC-MS analysis consisted of a combination of Solutions A and B. The gradient elution was performed at a constant flow rate of 1.0 µL/min as shown in (Table 2).

Table 2: Gradient Elution Steps

Step	Total Time(min)	Flow Rate(µl/min)	A (%)	B (%)
0	0.00	1000	90.0	10.0
1	5.00	1000	90.0	10.0
2	15.00	1000	10.0	90.0
3	20.00	1000	10.0	90.0
4	25.00	1000	90.0	10.0
5	30.00	1000	90.0	10.0

Note: The table above provides a detailed overview of the gradient elution protocol used in the LC-MS analysis. The sequence of steps outlines the changes in the concentration of Solutions A and B over time, essential for the separation and identification of compounds.

3- RESULTS AND DISCUSSION

We have successfully identified seven distinct compounds in the extract of *Asphodelus aestivus* using LC-MS analysis. The compounds mentioned in Table 3 encompass various molecules, including Chrysophanol, Aloe-emodin, Chlorogenic acid, Frangulin B, as well as complex anthrones and glycosides such as 10-(chrysophanol-7'-yl)-10-hydroxychrysophanol-9-anthrone, Aspheloside B, and Ramosin.

Table 3: Mass Spectrometry Results for Compounds Extracted from Asphodelus aestivus

Compound	Formula	MS Peak	Molecular Ion Peak	Major Fragments	Retention Time, (min)
Chrysophanol (1)	C15H10O4	m/z 252.7	m/z 253 (C15H10O4+)	m/z 253 (C15H9O4.), m/z 239 (C14H7O4.)	17.85
Aloe-emodin (2)	C15H10O5	m/z 269	m/z 270 (C15H10O5+)	m/z 253 (C15H9O4.), m/z 239 (C14H7O4.)	15.15
Chlorogenic acid (3)	C16H18O9	m/z 354.7	m/z 355 (C16H19O9+)	m/z 191 (C7H11O6.), m/z 163 (C9H7O3.)	12.84
Frangulin B (4)	C20H18O9	m/z 401	m/z 402 (C20H19O9+)	m/z 253 (C15H9O4.), m/z 149 (C5H9O5.)	10.35
10-(chrysophanol-7'-yl)-10-hydroxychrysophanol-9-anthrone (5)	C30H20O8	m/z 505	m/z 506 (C30H21O8+)	m/z 238.06 (C15H10O32.), m/z 253.05 (C15H9O4.)	20.45
Aspheloside B (6)	C35H27O11	m/z 639	m/z 640 (C35H28O11+)	m/z 507 (C30H19O8.)	16.27
Ramosin (7)	C36H31O12	m/z 671	m/z 672 (C36H32O12+)	m/z 492, m/z 255	14.7

Note: This table summarizes the mass spectrometry results for seven key compounds identified in Asphodelus aestivus. The data includes the molecular formula, mass-to-charge ratio (MS Peak), molecular ion peak, major fragment ions, and retention times, providing insights into the molecular structures and fragmentation patterns of each compound.

DISCUSSION

Analysis of Molecular Ion Peaks:

The molecular ion peaks played a crucial role in the identification of the compounds present in *Asphodelus aestivus*. Chrysophanol (1) and Aloe-emodin (2) exhibited molecular ion peaks at m/z 253 and m/z 270, respectively, confirming their respective structures. Chlorogenic acid (3) and Frangulin B (4) were analyzed using mass spectrometry, providing significant information about their molecular structures. Chlorogenic acid exhibited a peak at m/z 355, while Frangulin B showed a peak at m/z 402. These findings offered valuable insights into the configurations of these compounds, as illustrated in Figure 4.

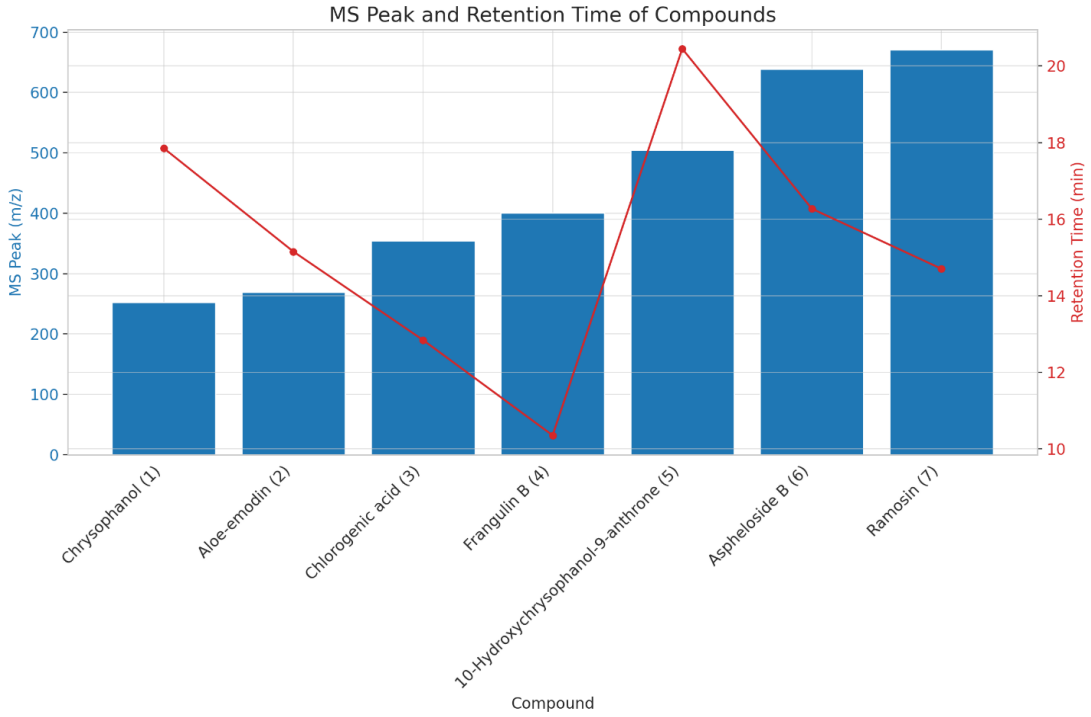

Figure 4 Correlation between Mass Spectrometry Peaks (m/z) and Retention Times (min) for Specific Compounds. The graph illustrates a dual-axis representation, where blue bars represent MS Peaks and a red line represents Retention Times. This format compares mass-to-charge ratios and chromatographic retention characteristics of compounds, specifically Chrysophanol (1) to Ramosin (7).

Insights from Advanced Compound Analysis:

The identification of compounds in our study relied heavily on the presence of distinct molecular ion peaks. Compound 5, known as 10-(chrysophanol-7'-yl)-10-hydroxychrysophanol-9-anthrone, exhibited a prominent peak at m/z 506. Compound 6, also referred to as Aspheloside B, displayed a significant [M+H]+ ion at m/z 640, with the molecular formula C30H20O8. The peak at m/z 640 observed for Aspheloside B is consistent with prior studies [11-16] and played a crucial role in confirming its molecular composition. The cleavage of the C10–C6′ bond in Aspheloside B resulted in significant fragments at m/z 238.06 and 253.05, providing valuable insights into its intricate molecular structure. These results highlight the complex molecular nature of Aspheloside B and underscore the effectiveness of mass spectrometry in revealing detailed compound characteristics.

Furthermore, Ramosin (7) exhibited a notable peak at

m/z 672, offering valuable insights into its potential biological functionalities. The mass spectrometry analysis of Ramosin revealed a significant peak at m/z 655 (C36H32O12+), indicative of a hydroxyl (OH) group removal from the carbon atom at position 10'. Additional fragmentation produced a notable ion at m/z 492, suggesting phenolic glycosides with included glucose units [17, 18]. The presence of substantial mass units at m/z 255 suggests the likely presence of a chrysophenol (1) component in the compound [17-19].

Overall, the molecular ion peaks and fragmentation patterns observed in our analysis facilitated the identification of these compounds, providing insights into their molecular structures and potential bioactivities.

Interpretation of Fragmentation Patterns:

Chrysophanol (1) and Aloe-emodin (2) exhibited fragmentation patterns consistent with the absence of hydroxyl and carbonyl functional groups. Frangulin B (4) displayed fragmentation, resulting in the production of chrysophanol and 3-(D-Apio-β-D-furanosyloxy) compounds, indicating involvement in an intricate biosynthetic pathway. Moreover, Aspheloside B (6) exhibited a notable reduction in mass units, specifically from m/z 253 to 162, suggesting the elimination of a phenolic group. This fragmentation pattern is a distinctive characteristic of this compound. The fragmentation pattern of Ramosin (7) elucidates its intricate structure and potential biosynthetic pathways by identifying specific ions formed and their mass-to-charge ratios.

Pharmacological Implications:

The discovery of distinct compounds in *Asphodelus aestivus*, particularly those exclusive to Jordan, presents promising opportunities for pharmacological use. The compound 10-(chrysophanol-7'-yl)-10-hydroxychrysophanol-9-anthrone (5) exhibits an intricate structure with potential interactions with distinct biological pathways. Due to its distinctive molecular structure, *Asphodelus aestivus* shows potential as a candidate for anti-inflammatory or antioxidant properties, aligning with its traditional uses. Further exploration of these potential activities through detailed bioassays and in-vivo studies is recommended. These studies may provide valuable insights into the therapeutic mechanisms of these compounds, potentially leading to the development of novel pharmacological agents.

Environmental Influences on Phytochemical Profile:

Our research indicates that Jordan's unique environmental factors, including soil composition, climate, and local biodiversity, significantly influence the phytochemical composition of *Asphodelus aestivus*. The observed differences in compound profiles between Jordanian samples and those from other regions suggest that the distinctive environmental conditions in Jordan may stimulate the production of specific secondary metabolites, including recently discovered compounds. This aligns with the concept of 'phytochemical plasticity'. Further research is necessary to fully understand the precise impact of environmental factors on the biosynthesis of these compounds. This knowledge could have profound implications for developing sustainable harvesting and cultivation practices.

CONCLUSION

The therapeutic uses of *Asphodelus aestivus* Brot. have been extensively documented. However, the phytochemical composition of this plant, specifically when cultivated in Jordan's unique environment, has not yet been studied. Using advanced liquid chromatography-mass spectrometry (LC-MS) techniques, we have successfully identified seven distinct compounds. This discovery sheds light on the diverse chemical composition of the plant and the intricate pathways involved in its biosynthesis. Our research expands the current knowledge of *Asphodelus aestivus* Brot. and highlights its unexplored possibilities in region-specific investigations. This study

combines modern analytical methods with traditional knowledge to pave the way for future investigations into plant-based natural products. It also provides opportunities for potential therapeutic advancements.

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Conflicts of interest

The authors have stated that there is no conflict of interest associated with the publication.

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تحليل الكروماتوغرافيا السائلة المزدوجة مع الطيف الكتلي للمركبات الاستقلابية الثانوية لنبات أسفوديلوس أيستيفوس بروت (فصيلة الأسفوديلية) النامي بريًا في الأردن

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ملخص

أُظهرت الدراسات أن نبات أسفوديلوس أيستيفوس بروت يُستخدم على نطاق واسع في الطب التقليدي لخصائصه العلاجية. ومع ذلك، لا يزال هناك نقص في المعرفة حول المكونات الكيميائية النباتية لهذا النبات، خاصة عند نموه في الأردن. في هذه الدراسة، تم استخدام تقنيات الكروماتوغرافيا السائلة المزدوجة مع الطيف الكتلي المتقدمة لدراسة المركبات الاستقلابية الثانوية لهذا النوع من النباتات النامية بريًا في الأردن. كشفت نتائجنا عن وجود سبعة مركبات مختلفة، تشمل مركبات معروفة وأنثرونات وجليكوسيدات معقدة، مما يُبرز التنوع الكيميائي للنبات. تُسهم هذه النتائج في فهمنا لأسفوديلوس أيستيفوس بروت وتُظهر التباين الكيميائي بحسب المناطق، بالإضافة إلى التطبيقات العلاجية المحتملة. وتعتبر هذه الأبحاث إضافة قيمة للمنتجات الطبيعية النباتية من خلال دمج الطرق التحليلية المعاصرة مع المعرفة التقليدية.

الكلمات الدالة: أسفوديلوس أيستيفوس بروت؛ المكونات الكيميائية النباتية؛ زراعة الأردن؛ تحليل كروماتوغرافيا مزدوجة؛ مركبات استقلابية ثانوية؛ نبات طبي تقليدي.

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