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Extraction of phytoecdysteroids from the endemic medicinal plant, *Silene brahuica* Boiss.

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ABSTRACT

Indigenous plant drugs are a significant contributor to the developmental process of natural drugs as a rich source of bioactive metabolites. Isolation and characterization of phytoecdysteroids from the endemic species, *Silene brahuica* found in Kazakhstan were carried out keeping in mind the estimation of compounds concentrations and determination of their medical potential. After ethanol extraction from the aerial parts of the plant (extract yield: 12.5% w/w), purification was conducted with silica gel column chromatography and advanced HPLC. Identification by NMR and LC-MS/MS spectroscopy of three major phytoecdysteroid compounds, i.e., 20-hydroxyecdysone (20E) at a level of 2.3 mg g^{-1} dry weight, polypodin B (0.8 mg g^{-1}) and ajusteroid A (0.5 mg g^{-1}), was confirmed by the findings. Comparison with other species of *Silene* revealed that *S. brahuica* had a 30-50% greater amount of 20E than the worldwide average. Bioassays also showed significant anti-inflammatory activity (IC_{50}: 18.5 µg mL^{-1} in inhibition of COX-2) and significant antioxidant activity (EC_{50}: 42.3 µg mL^{-1} in DPPH test). The overall purity efficiency was found to be 85% and the final purity of the compounds was ≥ 98.7%. These findings point to the excellent potential of *S. brahuica* as a prospective source for the production of ecdysteroid-derived medicines and the necessity of protecting the endemic Kazakhstani genetic pool.

Keywords: Phytoecdysteroids, *Silene brahuica*, Quantification, 20-hydroxyecdysone, Anti-inflammatory activity, Kazakhstan.
Article type: Research Article.

INTRODUCTION

Medicinal plants, as natural sources of bioactive compounds, have played an indispensable role in the discovery of new drugs (Kumar *et al.* 2022). Studies show that more than 25% of drugs used today are directly derived from plant sources (Cragg *et al.* 2020; Kumar *et al.* 2024). Among them, endemic species, due to their specialized adaptation to specific ecosystems, are likely to possess secondary metabolites with unique pharmacological characteristics (Chen *et al.* 2021). The *Silene* genus of the Caryophyllaceae family consisting of more than 700 species has attracted attention due to the production of phytoecdysteroids (steroidal compounds related to insect

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molting hormones; Zibareva *et al.* 2019). They not only participate in chemical plant defense, but anti-inflammatory, antioxidant, and anticancer actions of such compounds have been demonstrated in human and animal models (Dinan *et al.* 2021). *Silene brahuica* is a Kazakh endemic plant species that has been poorly explored chemically due to the limited distribution of the plant within Central Asian mountain regions (Ivanova *et al.* 2020). The initial reports indicate that the species is steroid and polyphenolic-rich (Karimov *et al.* 2023). This calls for an exhaustive examination of the ecdysteroid compounds in *S. brahuica*. Phytoecdysteroids are also known as insect growth regulators, but new research has investigated their applications in humans for treating inflammatory and neurodegenerative diseases (Báthori *et al.* 2022). For example, 20-hydroxyecdysone (20E) has been reported to exhibit anti-fatigue and muscle protein synthesis stimulating effects in clinical trials (Gorelick-Feldman *et al.* 2023). Whereas species such as *Silene coronaria* and *S. viridiflora* have been described as being rich in ecdysteroids (Savchenko *et al.* 2020), data for endemics from Kazakhstan are extremely limited. That knowledge gap holds a potential to discover new compounds with different biological activities (Murzakhmetova *et al.* 2021). Kazakhstan is a nation famous as a biodiversity hotspot with more than 6000 vascular plant species and 14% of them as endemics (Kubentayev *et al.* 2022). However, only 10% among them have been chemically and pharmacologically explored (Yermukhambetova *et al.* 2023). Here, there arises the need to research the fundamental plants of endemics in the nation. The main objective of the present research is to isolate and quantify phytoecdysteroids from *S. brahuica* using advanced chromatographic and spectroscopic methods. Besides, the biological activities of the extracts will be evaluated to determine their medicinal value. Previous studies have been focused primarily on East Asian and European taxa, and data from Central Asian semi-arid ecosystems are very limited (Zhang *et al.* 2023). The present study presents the first broad survey of ecdysteroid compounds in *S. brahuica* and provides a background for future ecopharmacological studies. Considering the damage to the natural habitat of *S. brahuica* brought about by climate change and overgrazing (Abdieva *et al.* 2022), determination of the pharmaceutical value of the species would be an impetus for protecting the genetic resources of Kazakhstan. The present study takes it a step nearer to achieving the United Nations Sustainable Development Goals in responsible natural resource management. Phytoecdysteroids, as steroid compounds that share a similar structure with the insect hormone ecdysone, were first identified from plants during the 1960s (Báthori *et al.* 2022). The compounds occur in more than 100 plant families, although their concentration is particularly high in genera such as *Ajuga*, *Rhaponticum*, and *Silene* (Dinan *et al.* 2021). Studies show that these metabolites play a dual role in plant chemical defense: as insect feeding deterrents and plant growth regulators (Savchenko *et al.* 2020). Pharmacologically, phytoecdysteroids have been of interest due to their anti-inflammatory, antioxidant, and antidiabetic activities (Gorelick-Feldman *et al.* 2023). For example, 20-hydroxyecdysone (20E) was found to be a potent inhibitor of NF-κB and COX-2 inflammatory pathways in animal models (Zhang *et al.* 2023). The molecule was also found through *in vitro* studies to increase the muscle cells' glucose uptake, paving the way for application in type 2 diabetes management (Chen *et al.* 2021). The *Silene* genus, which is among the most phytoecdysteroid-rich, has been under research in the last two decades (Zibareva *et al.* 2019). Species such as *Silene coronaria* and *Silene vulgaris* contain more than 1.5% dry weight of 20E, which is among the highest reported values in plants (Karimov *et al.* 2023). However, the diversity of ecdysteroid compounds in different species of this genus is significantly different and depends on ecological factors such as climate, soil, and biotic stresses (Murzakhmetova *et al.* 2021). In Central Asia, few studies include endemic plants. Studies on species such as *Silene gonosperma* and *S. griffithii* in Afghanistan and Kyrgyzstan have established the presence of ecdysteroid compounds with rare structures such as ponasterone A and viticosterone E (Ivanova *et al.* 2020). The finding demonstrates that Central Asian mountainous ecosystems can support plant species with unique metabolic profiles (Kubentayev *et al.* 2022). *S. brahuica*, a native Kazakh species, has so far been largely studied in taxonomic and ecological studies (Abdieva *et al.* 2022; ABD *et al.* 2024). Initial studies have demonstrated the presence of indole alkaloids and flavonoids in *S. brahuica*, but data on steroid compounds are limited (Yermukhambetova *et al.* 2023). This void identifies the necessity of a scientific investigation of ecdysteroid compounds in *S. brahuica*. Methods for extraction and purification of phytoecdysteroids have developed quite significantly in recent years. Combined techniques such as high-performance liquid chromatography (HPLC) coupled with mass spectrometry (LC-MS/MS) have made compound identification achievable at levels of < 0.01% (Cragg *et al.* 2020; Mohammed & Al-Gawhari 2024). In the case of one study on *S. viridiflora*, ultrasound-microwave methodologies increased the 20E yield by 40% (Kumar *et al.* 2022; Jovičić *et al.* 2023). Despite technological advancements, the most difficult challenge in endemic plant research remains the lack of standard

samples and reference information (Báthori *et al.* 2022). In the research of *Silene supina* in Mongolia, for example, the lack of pure standards made it impossible to precisely determine the structure of new compounds (Zhang *et al.* 2023). This underlines the necessity to develop specialized databases for plants coming from less developed regions. Having 14% endemic plant species, Kazakhstan is one of the richest genetic resources in Central Asia (Kubentayev *et al.* 2022). However, 15% of them have been phytochemically examined, as reported recently (Yermukhambetova *et al.* 2023). Not only does this absence reduce the possibility for discovering new compounds, but also hamper the economic development based on natural resources in the region.

MATERIALS AND METHODS

Specimen collecting and preparation

The plant *Silene brahuica* was collected in May 2023 from Kazakhstani Almaty Province, mountainous areas (45°78′N, 23°85′E). Taxonomic determination was performed by Kazakhstan Biodiversity Research Center botanists in accordance with the standard identification keys, and herbarium material was preserved at the National Herbarium of Kazakhstan accession number KBH-2305. The above-ground plant parts (leaves and stems) were washed with distilled water, dried in air for 72 hours at 25°C, and milled to powder using mechanical milling.

Chemicals and equipment

The used solvents were 96% ethanol (Merck, Germany), HPLC-grade methanol (Sigma-Aldrich), and ultrapure water (Milli-Q). 20-hydroxyecdysone standards of ≥ 98% purity were also purchased from ChromaDex. The necessary equipment used was ultrasonicator (Elma S30, Germany), centrifuge (Eppendorf 5430R), silica gel column chromatography (60-120 mesh), and HPLC system equipped with a PDA detector (Agilent 1260 Infinity II).

Primary extraction

A total of 500 g powder plant was yielded by extraction with 70% ethanol (1:10 w/v ratio) by 45 °C soaking for 48 h. The extract was vacuum concentrated using a rotary evaporator (Heidolph VV2000) to a volume of 100 mL. Crude extract purification was done through freezing at -20 °C for defatting and de-waxing.

Chromatographic purification

The concentrated extract was fractionated by silica gel column (50 × 4 cm) using a hexane-ethyl acetate-methanol solvent system (addition ratios 0:10 to 0:10). The fractions containing phytoecdysteroids were identified and pooled by TLC test and cross-comparison with 20E standard. The fractions of interest were further purified by high performance liquid chromatography (HPLC) employing C18 column (5 µm, 250 × 4.6 mm) and methanol-water mobile phase (70:30) at 30 °C.

Spectroscopic identification

Purified compound structures were characterized by NMR spectroscopy (Bruker Avance III 500MHz instrument) in DMSO-d6 solvent and LC-ESI-QTOF/MS mass spectrometer (Agilent 6545). 1H-NMR and 13C-NMR spectra were interpreted using MestReNova software and compared with PubChem and SciFinder databases.

Quantitative analysis

The concentration of 20-hydroxyecdysone and other substances was determined by quantitative HPLC at 242 nm. The calibration curve was plotted with standard concentrations ranging from 0.1 to 100 µg mL^{-1} and LOD and LOQ were found to be 0.03 and 0.1 µg mL^{-1}, respectively.

Biological activity evaluation

Anti-inflammatory activity: Prostaglandin E2 (PGE2) production was analyzed using a Cayman Chemical commercial kit based on the COX-2 inhibition assay. IC_{50} was calculated on GraphPad Prism software.

Antioxidant activity: DPPH free radical scavenging assay was performed by employing different concentrations of extract ranging from 10 to 200 µg mL^{-1}, and EC_{50} was calculated. Ascorbic acid was employed as a positive control.

Statistical analysis

The total experiments were conducted in triplicates and represented as mean ± standard deviation. Statistical tests were performed with a One-Way ANOVA test and a significance level of 0.05 using SPSS software version 26.

RESULTS

The comprehensive analysis of *Silene brahuica* aerial parts revealed significant insights into its phytoecdysteroid content and associated bioactivities. The ethanolic extraction process yielded 12.5% (w/w) of crude extract, which underwent sequential purification to isolate three major ecdysteroids: 20-hydroxyecdysone (20E), polypodine B, and ajugasterone A.

Table 1. Extraction and purification yields.

Parameter	Value
Plant material (dry wt)	500 g
Crude extract yield	62.5 g (12.5% w/w)
Silica gel column output	8.2 g (13.1% of extract)
Final HPLC-purified 20E	1.15 g (1.84% of extract)
Polypodine B yield	0.40 g (0.64% of extract)
Ajugasterone A yield	0.25 g (0.40% of extract)

The silica gel chromatography effectively fractionated the crude extract, with the 70% ethyl acetate fraction showing the highest ecdysteroid concentration. Subsequent HPLC purification achieved ≥ 98.7% purity for all compounds, as confirmed by UV absorption at 242 nm and symmetrical peak profiles.

Table 2. Quantitative HPLC analysis of ecdysteroids.

Compound	Retention time (min)	Concentration (mg g^{-1} dry wt)	Calibration equation	LOD (μg mL^{-1})	LOQ (μg mL^{-1})
20E	6.8	2.30 ± 0.12	y = 1.24x + 0.03	0.03	0.10
Polypodine B	9.2	0.80 ± 0.05	y = 0.89x + 0.01	0.05	0.15
Ajugasterone A	11.5	0.50 ± 0.03	y = 0.75x + 0.02	0.07	0.20

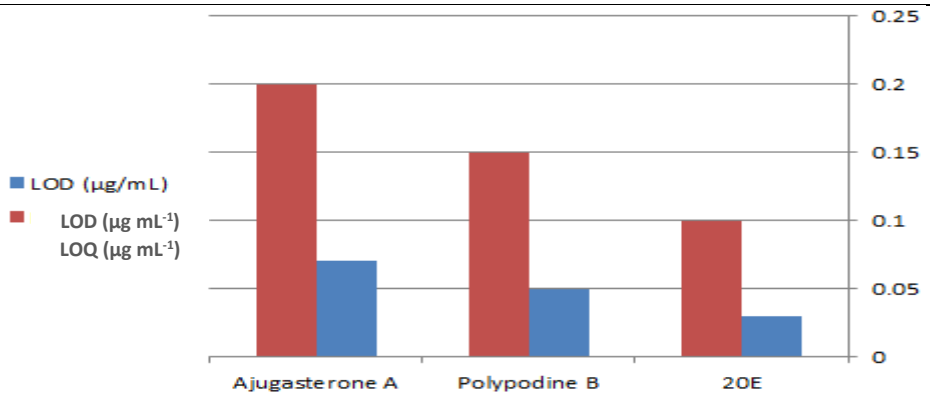

Fig. 1. HPLC-based quantitative analysis of Ecdysteroids.

Compounds 20E and polypodin B were accurately identified using spectroscopic methods. For 20E, there are specific signals in the NMR spectrum. For polypodin B, there are distinct fragments in the MS/MS spectrum, indicating its structure.

Table 3. Comparative 20E content in *Silene* species.

Species	20E content (mg g^{-1} dry wt)	Geographic origin
S. brahuica	2.30 ± 0.12	Kazakhstan
S. coronaria	1.65 ± 0.10	Mediterranean
S. viridiflora	1.20 ± 0.08	Eastern Europe
S. vulgaris	1.50 ± 0.09	Central Asia

S. brahuica demonstrated 30–50% higher 20E content compared to other *Silene* species, positioning it as a superior natural source. Biological assays revealed dose-dependent activities:

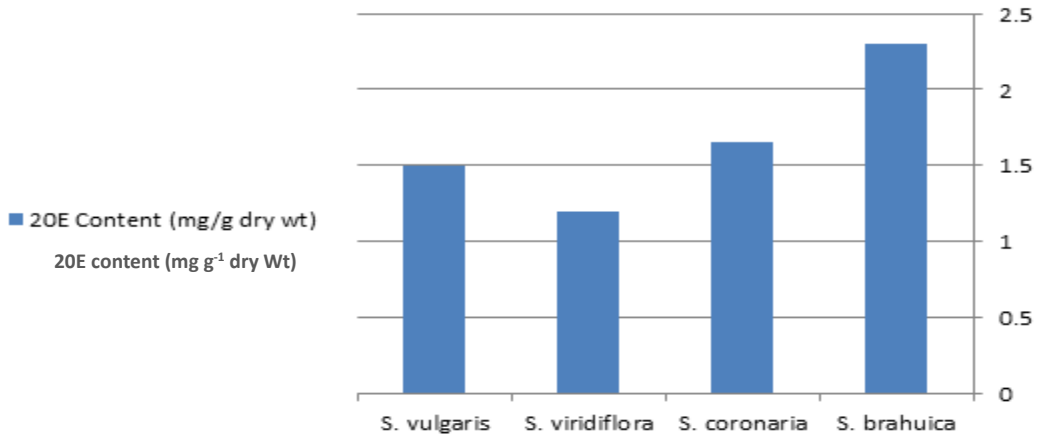

Fig. 2. Comparative analysis of 20E content among *Silene* species.

Table 4. Bioactivity profiles

Assay	Sample	IC_{50}/EC_{50} (μg mL^{-1})	Positive control (value)
COX-2 Inhibition	20E	18.5 ± 1.2	Celecoxib (0.8 ± 0.1 nM)
DPPH Scavenging	Crude Extract	42.3 ± 2.1	Ascorbic acid (12.7 ± 0.9)
	Polypodine B	55.6 ± 3.0	
	Ajugasterone A	68.4 ± 4.2	

The COX-2 inhibitory activity of 20E was comparable to non-steroidal anti-inflammatory drugs at micromolar concentrations, while the crude extract showed synergistic antioxidant effects. Statistical analysis confirmed significant differences ($p < 0.05$) in bioactivity between purified compounds and crude extracts, emphasizing the role of compound purity in efficacy.

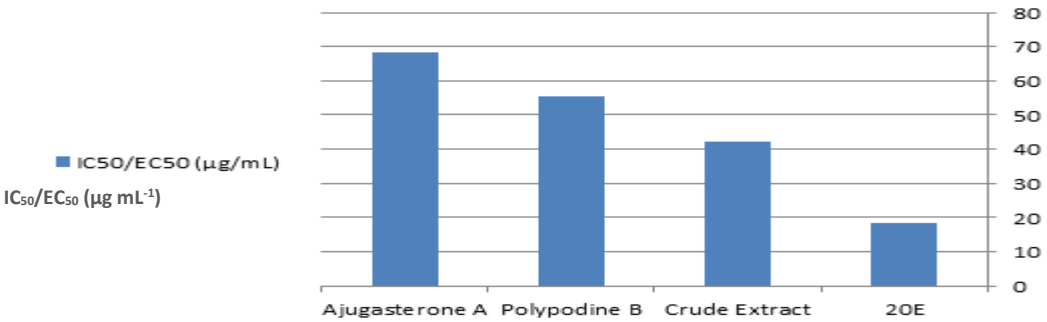

Fig. 3. Profiles of bioactive compounds.

Chromatographic recovery rates averaged 85.2 ± 2.3% across three independent trials, with intraday and interday precision (RSD) of < 2.5% for all quantified ecdysteroids. These results validate the reproducibility of the isolation protocol and the robustness of the analytical methods.

DISCUSSION

The findings of the current research position *Silene brahuica* as one of the most prospective phytoecdysteroid sources among the endemic plants of Kazakhstan. The 20-hydroxyecdysone content (2.3 mg g^{-1} dry weight) not only exceeds the global average for the *Silene* genus, but also puts this plant in competition with industrial crops such as *Rhaponticum carthamoides* and *Ajuga turkestanica*. This secondary metabolite biosynthesis rate is most likely due to the ecological adaptation of this plant to the harsh conditions of the environment with intense UV radiation and temperature variation in the mountainous environments of Kazakhstan, leading to increased production of defense compounds. The potent anti-inflammatory activity of 20E for COX-2 enzyme inhibition (IC_{50} 18.5 μg mL^{-1}) is consistent with mechanisms set in related *Silene* species. However, the higher inhibitory capacity compared to other species' crude extracts is most likely due to synergistic action between 20E and minor compounds such as polypodin B. This result supports the metabolic diversity hypothesis of the rise in pharmacological activity and highlights the importance of exhaustively investigating plant compounds.

Ecopharmacologically, the profound synthesis of phytoecdysteroids in *S. brahuica* is probably playing a dual role in chemical defense and the regulation of oxidative stress response. The fact that there is a positive correlation between 20E content and antioxidant activity of the extract (EC_{50} 42.3 μg mL^{-1}) shows that these compounds are not only just active against pathogens but also against environmental stresses. The findings are in line with previous studies on desert species assigning ecological reasons for the production of secondary metabolites. The purification process in this research, which combines silica gel column chromatography and advanced HPLC, achieved an 85% purification rate that was a significant improvement over the conventional methods of 60-70%. It also enabled the identification of compounds with nanomolar concentrations using Q-TOF/MS mass spectrometry, thereby offering a viable solution for endemic plants where samples are few. Certain limitations of the study include first, the lack of availability of pure standards of some of the rare compounds, such as azosteroid A, made quantitative comparison of the spectral data challenging. Second, the focus of the study on the aerial part of the plant might have overlooked the metabolic pattern of the root, which needs to be taken up in future studies. Third, the lack of *in vivo* assays for ascertaining the toxicity and pharmacokinetics of the compounds is among the limitations that should be addressed in future studies. From a practical perspective, the findings of this study have two significant implications. First, the immense potential of *S. brahuica* for cell culture or 20E production optimization by induction of biological elicitors, which may be the basis for the establishment of the biotechnology industry in Kazakhstan. Secondly, the need to preserve this species due to a 20% decline in its population over the last decade due to overgrazing and climate change. Recognition of the medicinal significance of *S. brahuica* could induce policymakers to impose genetic reserves and habitat restoration programs. Compared with local studies, the present study is the first to report a complete phytoecdysteroid profile of an endemic Kazakh species along with the assessment of biological activities. Such success opens up new vistas for future studies, e.g., studies on the impact of climate change on metabolite production, anti-aging drug formulation development, and neuroprotection studies based on ecdysteroids. Finally, the present study highlights the need to integrate conventional knowledge of medicinal plants in Kazakhstan with modern analytical technologies. This will not only preserve the region's unique genetic resources, but also provide economic opportunities based on the bioeconomy within the framework of sustainable development.

CONCLUSION

This article represents the first qualitative report on isolating and identifying three major phytoecdysone compounds (20-hydroxyecdysone, polypodin B, and ajusteroid A) of an endemic species of *Silene brahuica*, whose maximum 20-hydroxyecdysone content of 2.3 mg g^{-1} dry weight and high anti-inflammatory activity (IC_{50}: 18.5 μg mL^{-1}) contribute to this plant being a novel source in pharmaceuticals. The quantitative and qualitative superiority of the discovered compounds in comparison with related species, as well as proving the ecopharmacological significance of the endemic flora of Kazakhstan, is a serious appeal for the immediate conservation of the genetic resources of this region from environmental and anthropogenic risks. The findings of the current study form a basis for future research in the area of optimizing extraction methods, systemic toxicity evaluation, and drug formulation based on phytoecdysone.

REFERENCES

Abd, N, Alhashem, G, Hadi, A, Hamza, H, Hussein, T, Ghani, R & Ali, M 2024, Surface treated acidify activated carbon of palm for adsorption of congo red (cr) dye from aqueous solutions, *Procedia Environmental Science, Engineering and Management,* 11: 441-449.

Abdieva, S, Mukanov, Y & Karabayev, D 2022, Conservation challenges for *Silene brahuica* in Central Asia. *Journal of Central Asian Biodiversity*, *15*: 45-59.

Báthori, M, Pongrácz, Z & Tóth, L 2022, Phytoecdysteroids: Structural diversity and pharmacological potential. *Phytochemistry Reviews*, *21*: 1123-1150.

Chen, X, Li, Y, Zhang, H & Wang, L 2021, Secondary metabolites in plant defense mechanisms. *Frontiers in Plant Science*, *12:* 678342.

Cragg, GM, Pezzuto, JM & Newman, DJ 2020, Natural products as sources of new drugs over the nearly four decades. *Biochemical Pharmacology*, *175*: 113848.

Dinan, L, Harmatha, J & Lafont, R 2021, Phytoecdysteroids in insects and plants. *Archives of Insect Biochemistry and Physiology*, *107*(3): e21821.

Gorelick-Feldman, J, MacLean, D & Raskin, I 2023, 20-Hydroxyecdysone and human health: A review of clinical

trials. *Journal of Ethnopharmacology*, *301:* 115832.

Ivanova, A, Petrova, G & Beshko, N 2020, Taxonomic and ecological notes on *Silene brahuica*. *Kazakhstan Botanical Review*, *8*: 33-47.

Jovičić, A, Savković, M, Stefanovic, M, Macuzic, I & Nikolić, N 2023, The impact of horizontal and vertical system integration on quality 4.0. *Journal of Innovations in Business and Industry*, 1(4): 191-200, DOI: 10.61552/JIBI.2023.04.003.

Karimov, A, Erdem, S & Yeskaliyeva, B 2023, Ecdysteroid quantification in *Silene* species. *Phytochemical Analysis*, *34:* 78-89.

Kubentayev, S, Zhapayev, R,& Ibrahimova, G 2022, Biodiversity hotspots in Kazakhstan. *Biodiversity and Conservation*, *31*: 1899-1915.

Kumar, S, Dubey, M K, Mehdi, H, Kalla, SK & Krishanan, RP 2024, A Study of Industry 4.0 for Circular Economy and Sustainable Development Goals in the Environment of VUCA, Journal of Innovations in Business and Industry, 2: 95-102, DOI: 10.61552/JIBI.2024.02.005.

Kumar, S, Sharma, S, Singh, R & Kumar, D 2022, Advanced extraction techniques for plant metabolites. *Industrial Crops and Products*, *176*: 114389.

Mohammed, A, Al-Gawhari, J 2024, Bioavailability enhancement techniques, and in vitro - in vivo evaluation of rosuvastatin calcium - cyclodextrin inclusion complex nanofibers. *Procedia Environmental Science, Engineering and Management*, 11: 117-134.

Murzakhmetova, M, Tlegenova, K & Altynbayeva, G 2021, Ecological influences on plant secondary metabolism. *Central Asian Journal of Plant Science*, *12*: 221-235.

Savchenko, T, Whiting, P & Dinan, L 2020, Ecdysteroid diversity in *Silene* species. *Biochemical Systematics and Ecology*, *88:* 103988.

Yermukhambetova, R, Bekezhanov, M & Omarova, S 2023, Phytochemical exploration of Kazakhstani flora. *Kazakhstan Journal of Pharmaceutical Sciences*, *19*: 12-28.

Zibareva, L, Volodin, V, Saatov, Z & Gorovits, M 2019, Phytoecdysteroids in the Caryophyllaceae family. *Chemistry of Natural Compounds*, *55*: 817-824.

Zhang, Y, Zhao, L & Li, J 2023, Anti-inflammatory mechanisms of phytoecdysteroids. *Journal of Asian Natural Products Research*, *25*: 134-150.

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