



Comparative Phytochemical Profiling and Cytotoxic Activity of *Ranunculus polyanthemus* L. and *Ranunculus oxyspermus* M.B. Extracts on HepG2 Hepatocellular Carcinoma and L6 Myoblasts

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Abstract

This study presents a comparative phytochemical and cytotoxic evaluation of ethanol extracts from *Ranunculus polyanthemus* L. and *Ranunculus oxyspermus* M.B., two medicinally relevant species native to Central Asia. UPLC–Q-TOF–MS/MS analysis identified more than 80 metabolites representing major classes such as flavonoids, alkaloids, phenolic acids, coumarins, and fatty acid derivatives. Cytotoxic activity was assessed using the CCK-8 assay against HepG2 hepatocellular carcinoma cells and L6 myoblasts across the concentration range of 10–160 µg/mL. The selection of this range was based on preliminary solubility tests and previous reports of *Ranunculus* ethanol extracts showing moderate cytotoxic effects within comparable concentrations. Results demonstrated a dose-dependent but non-selective decrease in cell viability, with selectivity index (SI) values below 1 for both extracts. The low SI suggests that the cytotoxicity is not tumor-specific and may be associated with the presence of protoanemonin-like lactones typical of the Ranunculaceae family. These findings highlight that ethanol extracts may not capture the full spectrum of anticancer constituents, and future studies should employ less polar or mixed solvents to improve extraction efficiency.

Keywords: Cytotoxicity; *R. polyanthemus*; *R. oxyspermus*; HepG2; L6; Chemical composition; Ranuncosid; Biologically active substances.

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1. Introduction

Ranunculus L. is a genus of flowering in the Ranunculaceae family.^[1] The genus *Ranunculus* comprises around 600 species. These plants are widely distributed throughout the northern hemisphere, as well as in southern temperate regions and the tropics, where they are generally found at higher altitudes.^[2-4] In addition, scientific studies have demonstrated that extracts of *Ranunculus* plants have antioxidant, anti-inflammatory, antimutagenic, antimalarial, antibacterial, antitumour, cardioprotective and wound-healing properties.^[5] Over the past ten years, the pharmacological activity and chemical composition of *Ranunculus* have been the subject of extensive scientific research. Furthermore, studies have shown that *Ranunculus* extracts possess a variety of beneficial properties,

including antioxidant, anti-inflammatory, antimutagenic, antimalarial, antibacterial, antitumour, cardioprotective and wound-healing effects. Over the past decade, the pharmacological activity and chemical composition of *Ranunculus* have been the focus of extensive scientific research.^[6]

Over the centuries, natural compounds have served as a foundation for the development of drugs and biologically active substances. Many of these compounds exhibit significant potential as chemopreventive agents and anticancer therapeutics. In traditional medicine, medicinal plants have long been employed for the treatment of infectious diseases, inflammation, rheumatism, and various dermatological conditions.^[7] Contemporary research increasingly focuses on bioactive substances of natural origin to identify novel pharmacological strategies for managing malignant, viral, and bacterial diseases. In this context, particular attention is directed towards flora traditionally used in medicine as a promising source of new therapeutic agents.^[8]

Plants of the genus *Ranunculus*, particularly *R.*

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polyanthemus and *R. oxyspermus*, are of considerable interest due to their rich content of secondary metabolites, including flavonoids, saponins, and phenolic compounds, which may confer diverse biological activities.^[9] Despite their widespread ethnomedicinal use, the pharmacological profiles of many species remain insufficiently characterized, especially with regard to cytotoxic and antimicrobial activities. Given the increasing adverse effects associated with conventional chemotherapeutic agents, the identification of novel bioactive compounds from plant sources is of growing importance. Scientific validation of the traditional applications of species such as *R. polyanthemus* and *R. oxyspermus* underscores the relevance of screening studies aimed at isolating and characterizing their chemical constituents.^[10,11]

The search for new anticancer agents derived from natural products has emerged as a critical area of research. In this context, plants occupy a central role in the development of complex traditional medicinal systems, particularly those with a long history of use in cancer treatment. Historical references indicate that bioactive plant-derived substances possess substantial therapeutic efficacy.^[12]

Several species of the genus *Ranunculus* have been traditionally used to treat a range of conditions, including jaundice, edema, malaria, asthma, pain syndromes, gout, rheumatism, inflammatory skin disorders, cancer, and hypertension. Experimental studies suggest that extracts from this genus exhibit antioxidant, anti-inflammatory, antimutagenic, antimalarial, antibacterial, antitumour, cardioprotective, and wound-healing properties.^[13] Various pharmacological activities, including anti-inflammatory, analgesic, antimicrobial, antiparasitic, and antitumour effects, have been reported across members of the buttercup family. Moreover, derivatives of compounds isolated from this family, such as cimifugazide, helleberrhigenin, hydrastine, and thymoquinone, have been applied in the treatment of cancer, cardiovascular diseases, and inflammatory disorders.^[14]

Ranunculus polyanthemus L. - perennial herbaceous plant 30-80 cm tall. The species is characterised by a thin, cord-like, fringed rhizome and a shortened, underdeveloped root system. The stem is erect, branching, with a scalloped surface, bearing numerous flowers. Flowers are golden-yellow, 2-3 cm in diameter. Inhabits dry meadow areas, steppe depressions, river and lake banks, areas with close groundwater, as well as pine forests, at the edges of thickets, along roads and in foothills. The distribution area covers Ustyurt, Tobol-Esil Plain, Caspian Sea, Betpakdala, Ulytau, Teriskei Alatau, Eastern Melkogorye, Karkara, Altai, Tarbagatai, Dzungarian Alatau, Kungei-Ile Alatau and Western Tien Shan. This plant is toxic

(Fig. 1).

Ranunculus oxyspermus M.B. - perennial herbaceous plant, 20-36 cm tall, with thickened tuberous roots. The upper part of the stem is strongly branched, bearing numerous flowers. The root leaves are petiolate, oval-shaped, with a cuneate or rounded base, less often almost heart-shaped; leaf lobes are emarginate-tuposubdentate. Flowers are golden-yellow, 2-3 cm in diameter. The species is found in dry meadows, old fallow lands, sometimes in arable land and along roadsides, and often appears as a weed. It is widespread in the regions of Ustyurt, Tobol-Esil Plain, Karatau and Western Tien-Shan. It has medicinal properties (Fig. 1).^[15]

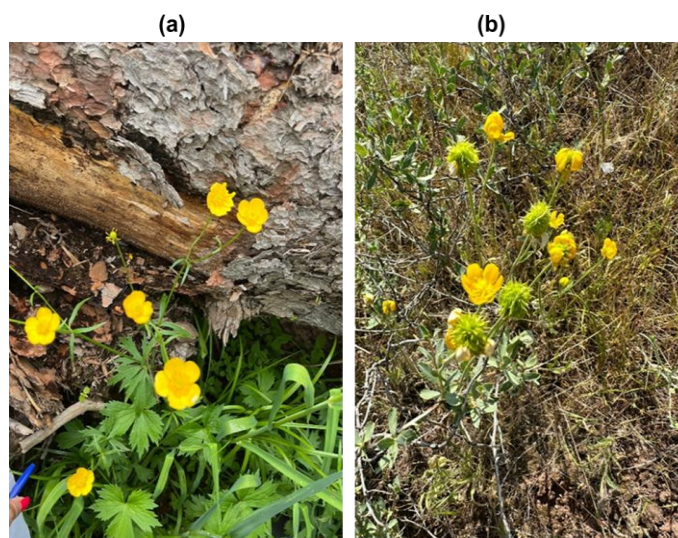


Fig. 1: (a) *Ranunculus polyanthemus* L. (b) *Ranunculus oxyspermus* M.B.

Members of the genus *Ranunculus* (e.g., *R. oxyspermus*, *R. polyanthemus*) are known to produce a diverse array of secondary metabolites, including flavonoids, saponins, and phenolic compounds, which exhibit considerable biological potential.^[16] The chemical diversity of these taxa suggests a broad but largely unexplored pharmacological capacity. Despite their long-standing ethnomedicinal use, the pharmacological profiles of many *Ranunculus* species remain insufficiently characterized, particularly concerning their cytotoxic and antimicrobial properties, mechanisms of action, and the specific active constituents responsible for biological effects.

The increasing need for alternative therapeutic strategies with fewer adverse effects than conventional chemotherapy, as well as the growing problem of resistance among pathogenic microorganisms, underscores the importance of exploring new biologically active phytoconstituents. Comprehensive investigation of such plants aligns with current trends in natural product-based drug discovery. Therefore, systematic screening and detailed chemical analyses of underexplored species such as *R. oxyspermus* and *R. polyanthemus* are essential to scientifically substantiate their traditional applications^[17-19] and to identify bioactive compounds with therapeutic relevance.

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Medicinal plants that have been used for centuries in traditional healing systems for the treatment of cancer, infections, and inflammatory disorders represent an invaluable source for the identification of novel pharmacologically active agents of natural origin. Consequently, an integrated assessment of the bioproductivity and pharmacological potential of *Ranunculus* species constitutes an important and timely research objective with significant applied value.^[20]

Cell viability, defined as the proportion of living, metabolically active cells within a population, serves as a fundamental indicator of cellular health. Quantitative assessment of cell viability plays a pivotal role in in vitro research, particularly in toxicological and pharmacological studies. Such assays are crucial for evaluating the effects of chemical or biological agents on cultured cells, enabling the determination of cytotoxic thresholds and dose–response relationships. The pharmaceutical industry extensively employs cell viability assays to assess the safety and efficacy of novel compounds. Similarly, biomedical researchers rely on these techniques to screen and characterize new therapeutic candidates, especially in the development of anticancer drugs.^[21]

2. Materials and methods

2.1 Plant material and identification. Aerial parts of *Ranunculus polyanthemus* L. and *R. oxyspermus* M.B. were collected during the flowering stage from natural populations in the Almaty region (Kazakhstan) in June 2023. Voucher specimens (No. 2546-O – *R. oxyspermus*, No. 2546-P – *R. polyanthemus*) are deposited in the Main Botanical Garden. Plant identification was verified by a senior botanist of the institute.

2.2 Preparation of ethanol extracts. Air-dried and powdered plant material (10 g) was extracted with 70 % ethanol (100 mL) by maceration at room temperature for 48 h, followed by filtration and concentration under reduced pressure at 40 °C. The extracts were lyophilized to dryness and stored at –20 °C until use. Extract yields were expressed as % w/w relative to dry material.^[22]

The obtained samples were designated as follows:

RPF-EE – *R. polyanthemus* flower ethanolic extract,

RPSL-EE – *R. polyanthemus* stem and leaf ethanolic extract,

RPR-EE – *R. polyanthemus* root ethanolic extract,

ROF-EE – *R. oxyspermus* flower ethanolic extract,

ROSL-EE – *R. oxyspermus* stem and leaf ethanolic extract,

ROR-EE – *R. oxyspermus* root ethanolic extract.

2.3 UPLC-Q-TOF-MS/MS Analyses. The compounds in RPF, RPSL, RPR, ROF, ROSL and ROR were analyzed by ultra performance liquid chromatography-quadrupole-time of flight-mass spectrometry (UPLC-Q-TOF-MS) technique, and the detailed analysis was performed as follows. Chromatographic separation was carried out on an ACQUITY UPLC HSS T3 column (100 mm×2.1 mm, 1.8µm, Waters). The column temperature and flow rate applied for the column were 40 °C, 0.4 mL/min respectively. The mobile phase utilized for the column consisted of solvent A (0.1 % formic acid, distilled water) and solvent B (0.1% formic acid, acetonitrile). The gradient elution program was set as follows: 0–1 min, 2%–5% B; 1–7 min, 5%–20% B; 7–9 min, 20% B; 9–12.5 min, 20%–28% B; 12.5–18 min, 28%–40% B; 18–22 min, 40%–98% B; 22–25 min, 98% B; 25–30 min, 98%–2% B. The injection volume was 2 µL. the mass spectrometry was completed out on aquadrupole orthogonal acceleration time-of-flight tandem mass spectrometer (Waters Q-TOF

Premier™), with the nebulizer gas set to high-purity nitrogen at 500 °C and a flow rate of 1000 L/h. the flow rate of the cone gas was adjusted to 150 L/h, the source temperature to 150 °C, the capillary voltage to 205 kV, and the cone TOF data was taken between m/z 50 and 1200. Positive ion ([M+H]⁺=556.2771) and negative ion ([M-H][–]=554.2615) modes were monitored using leucine enkephalin at a flow rate of 10 L/min as the locking mass through a locking spray interface to acquire precise masses. Waters Masslynx 1.4.1 software was utilized to handle data collection and processing.^[23]

Metabolites were tentatively identified by comparison with available databases (Metlin, HMDB, and MassBank) and literature data. Compounds were classified into major phytochemical groups for comparative analysis (Table 1). The complete list of identified compounds is presented in [Supporting Information file Table S1](#).

2.4 Cell culture and cytotoxicity assay. HepG2 (human hepatocellular carcinoma) and L6 (rat skeletal myoblast) cell lines were obtained from the Cell Bank of the Institute of Cytology (Russia). Cells were cultured in DMEM supplemented with 10 % FBS, 1 % penicillin–streptomycin, at 37 °C in 5 % CO₂. Cell viability was determined using the CCK-8 assay. Cells (1 × 10⁴ cells/well) were seeded in 96-well

Table 1: Summary of major classes of compounds identified in ethanol extracts of *R. polyanthemus* and *R. oxyspermus*.

Compound class	Representative compounds	<i>R. polyanthemus</i>	<i>R. oxyspermus</i>
Flavonoids	Quercetin, Kaempferol, Rutin, Isoquercitrin	+++	++
Phenolic acids	Caffeic acid, Ferulic acid, p-Coumaric acid	++	+
Alkaloids	Berberine, Columbamine, Palmatine	+	+++
Coumarins	Umbelliferone, Scopoletin	+	++
Terpenoids	β-Sitosterol, Lupeol	++	+
Fatty acids	Linoleic acid, Palmitic acid	++	++

Note: +: detected in trace amounts; ++: moderate; +++: abundant.

plates and treated with ethanol extracts at concentrations of 10, 20, 40, 80, and 160 µg/mL for 24 h. Absorbance was measured at 450 nm using a microplate reader (BioTek Synergy HTX). The control group received vehicle (0.1 % ethanol).^[23]

The selection of the concentration range (10–160 µg/mL) was based on solubility limits and previously reported bioactivity levels for *Ranunculus* extracts. Each experiment was performed in triplicate and repeated independently three times.

2.5 Statistical analysis. Data were analyzed using GraphPad Prism 9.0. Results are presented as mean ± SD (n = 3). Statistical significance was determined using one-way ANOVA followed by Tukey's post hoc test, with $p < 0.05$ considered significant. The selectivity index (SI) was calculated as $IC_{50} (L6)/IC_{50} (HepG2)$.^[25]

3. Results and discussion

The genus *Ranunculus* (buttercups) comprises many species of herbaceous plant found throughout the temperate zones of Europe, Asia and North America. Buttercups are characterised by the diversity of their chemical composition, which includes flavonoids, glycosides, alkaloids, coumarins and fatty acids.^[26] These compounds determine the plants' pharmacological activity, which is manifested in antioxidant, anti-inflammatory, antibacterial, hepatoprotective, neuroprotective and other biological effects. Due to their diverse biochemical composition, plants in the genus *Ranunculus* are a promising source of new biologically active substances for use in phytopreparations and the development of modern drugs.^[27]

UPLC–Q-TOF–MS/MS analysis of the ethanol extracts from *R. polyanthemus* and *R. oxyspermus* revealed a diverse metabolite profile. A total of 84 compounds were tentatively identified, including flavonoids, alkaloids, phenolic acids, coumarins, terpenoids, and fatty acid derivatives. These findings indicate that both species possess a complex secondary metabolite composition typical of the Ranunculaceae family.

While the two extracts shared several core metabolites (e.g., quercetin, kaempferol, caffeic acid, ferulic acid), differences in compound abundance were notable. *R. polyanthemus* was particularly rich in flavonoid glycosides such as rutin and isoquercitrin, which are known antioxidants and cytoprotective agents. In contrast, *R. oxyspermus* contained a higher proportion of alkaloid-type constituents (e.g., berberine, columbamine) and coumarin derivatives (e.g., scopoletin), compounds often associated with stronger cytotoxic or antimicrobial activity.

These chemical differences suggest potential variations in biological behavior between the species. The major compound classes and representative examples are summarized in [Table 1](#), while detailed chromatographic data are available in [Supporting Information file Table S1](#).^[28–101]

UPLC–Q-TOF–MS/MS profiling of ethanol extracts from *Ranunculus polyanthemus* and *R. oxyspermus* revealed a complex chemical composition comprising 87 individual metabolites distributed across different anatomical parts of the plants. Marked interspecific and organ-specific variations

were observed in the accumulation of bioactive constituents.

In *R. polyanthemus*, the flowers contained the highest diversity of metabolites (58 compounds, 66.7% of the total), highlighting their role as the primary site of biosynthesis and accumulation of bioactive substances. The roots contained 33 compounds (37.9%), and the stems contained 42 compounds (48.3%), reflecting moderate metabolic activity in vegetative tissues. In contrast, *R. oxyspermus* displayed a more balanced distribution, with 50 compounds (57.7%) in flowers, 47 (54.0%) in roots, and 47 (54.0%) in stems. This even allocation of metabolites across plant organs suggests a more uniform biosynthetic potential. The predominance of compounds in the aerial parts may be linked to protective mechanisms in reproductive organs, which are frequently exposed to environmental stressors such as UV radiation and pathogens.

A detailed examination of the 87 identified compounds demonstrated a wide spectrum of biological activities characteristic of each structural class ([Table S1](#)). The most prevalent pharmacological properties were antioxidant, anti-inflammatory, neuroprotective, and antitumor effects. Among the detected metabolites, 57 compounds (65%) exhibited *antioxidant activity*, mainly flavonoids and phenolic acids such as quercetin 3-O-rutinoside-7-O-glucoside, rutin, orientin, isoquercitrin, vitexin, isovitexin, luteolin, tricrin, kayaflavone, ferulic and vanillic acids, scopolin, and scoparone.

Thirty-one compounds (41%) were associated with *anti-inflammatory activity*, including rutin, scopoletin, orientin, vitexin, tricrin, luteolin, and coumaric acid, many of which overlap with antioxidant compounds, suggesting synergistic effects.

Neuroprotective potential was identified in 13 compounds (15%), notably vitexin, isovitexin, orientin, scopoletin, scopolin, daucosterol, β-amyryn, and isoquercitrin. These metabolites have been reported to modulate oxidative stress and neurotransmission, indicating their potential in managing neurodegenerative conditions.

Antitumor activity was attributed to 12 compounds (14%), including columbamine, stigmast-4-ene-3,6-dione, kayaflavone, β-amyryn, ranunculoside B, ternatin A, tricrin, and 7,3'-dihydroxy-5'-methoxyisoflavone, which are known to inhibit tumor cell proliferation and induce apoptosis.

In addition, *antimicrobial activity* was observed in 23 compounds (27%), particularly among phenolic and coumarin derivatives (scopoletin, scoparone, vanillic acid, coumaric acid, esculin, ranunculoside B, and 1,4,6-trihydroxy-9,10-anthraquinone).

Hepatoprotective activity was reported for five compounds (6%), including scopoletin, scoparone, and stigmast-4-ene-3,6-dione, while antituberculosis potential (3.2%) was identified in ternatoside B and D.

Hypolipidemic effects were associated with columbamine and stigmast-4-ene-3,6-dione, and cardioprotective activity was found in 10 compounds (11%), mainly glycosides and

flavonoids such as luteolin and vitexin.

Overall, the majority of identified compounds demonstrated multitarget biological activities, reflecting the integrative pharmacological potential of these plants. The combination of antioxidant, anti-inflammatory, and neuroprotective effects highlights the relevance of *R. polyanthemus* and *R. oxyspermus* as valuable sources of multifunctional phytochemicals for further pharmacological and biotechnological exploration.

With the rapid progress and technological advancement of the pharmaceutical and biotechnological industries, there is an increasing need to evaluate the toxicity and safety of medicinal plants. Although traditional toxicity testing often relies on in vivo animal experiments, modern research increasingly emphasizes in vitro cellular assays as reliable, ethical, and cost-effective alternatives for preliminary screening.

Cytotoxicity assay. Among these, the WST-8 assay has become one of the most widely used methods for assessing cytotoxicity due to its high sensitivity, reproducibility, and operational simplicity compared to other tetrazolium-based tests. The WST-8 method employs a water-soluble tetrazolium salt, chemically known as 2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium sodium salt, which is reduced by cellular dehydrogenases in metabolically active cells.

This reduction leads to the formation of a water-soluble formazan dye, whose intensity of color directly correlates with the number of viable cells. The amount of formazan produced is quantified by measuring optical density (OD) at 450 nm

using a microplate reader. Thus, the WST-8 assay provides a sensitive and quantitative measure of cell viability and metabolic activity, making it a robust tool for evaluating the cytotoxic potential of plant extracts and natural compounds.

The cytotoxic activity of ethanolic extracts obtained from different anatomical parts of *R. polyanthemus* and *R. oxyspermus* was evaluated using the CCK-8 assay on the L6 cell line. Extracts from flowers (F), stems (S), and roots (R) were tested within the concentration range of 10–160 µg/mL, which was chosen based on preliminary solubility screening and literature data on similar phytochemical extracts. Concentrations below 10 µg/mL did not produce measurable biological responses, whereas higher concentrations (>160 µg/mL) caused solubility instability and interference with optical readings. Therefore, this range ensured reliable dose–response evaluation without non-specific cytotoxic effects.

The results of cell viability (%) after treatment with extracts of *R. polyanthemus* (RPF-EE, RPSL-EE, RPR-EE) and *R. oxyspermus* (ROF-EE, ROSL-EE, ROR-EE) are presented in Fig. 2a–2f. The two species demonstrated differences in their effect on L6 cell viability compared to the negative control (NC). The most pronounced decrease in viability was observed with root extracts (RPR-EE and ROR-EE), where the values were significantly lower than in the control group (****, $p < 0.0001$, Fig. 2). This indicates that the roots contain compounds with the strongest cytotoxic activity. In contrast, extracts from flowers and stems of both species did not cause a substantial reduction in viability, and their values remained close to the control.

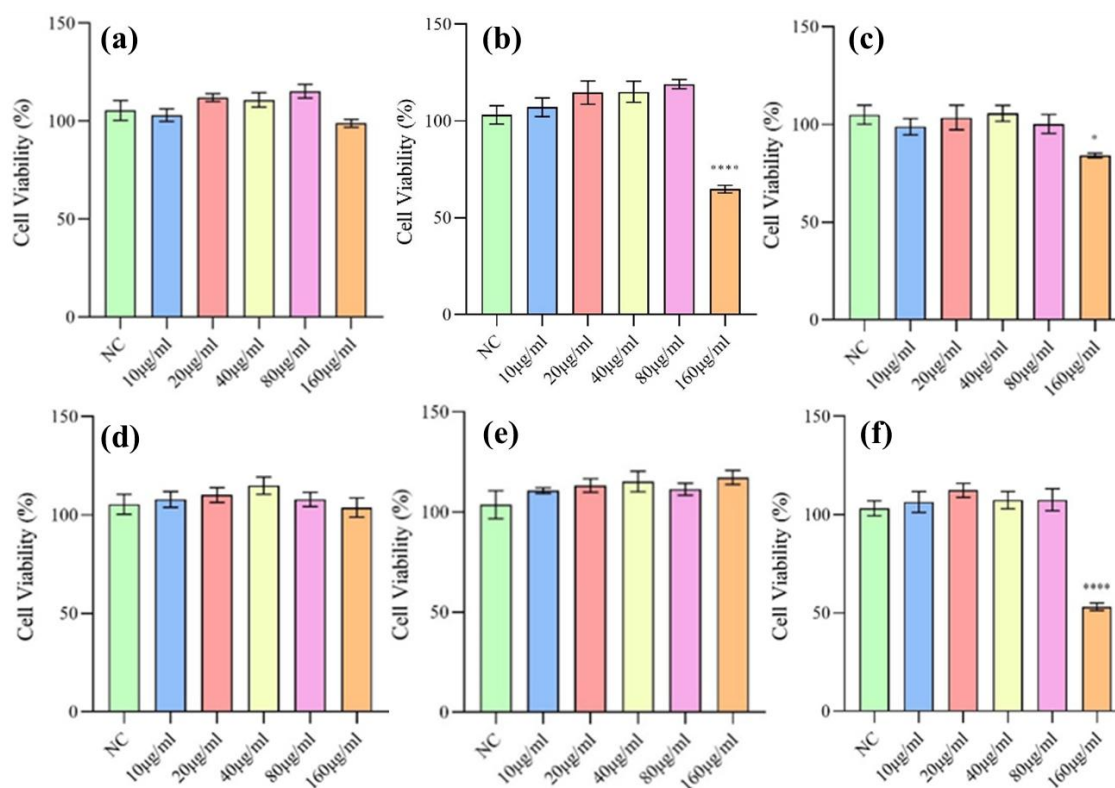


Fig. 2: Proliferation of RPF-EE (a), RPSL-EE (b), RPR-EE (c), ROF-EE (d), ROSL-EE (e) and ROR-EE (f) treated L6 cells was tested by the CCK-8 assay (n=3). * $P < 0.1$, **** $P < 0.0001$, compared with NC group.

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These findings suggest that the root extracts of *R. polyanthemus* and *R. oxyspermus* possess the highest cytotoxic potential against L6 cells, likely due to a greater

accumulation of biologically active alkaloids, lactones, and coumarin-type metabolites in underground tissues.

The cytotoxic potential of ethanolic extracts of *R. polyanthemus* (RPF-EE, RPSL-EE, RPR-EE) and *R. oxyspermus* (ROF-EE, ROSL-EE, ROR-EE) was further evaluated against the human hepatocellular carcinoma cell line HepG2 using the CCK-8 assay. Across the tested concentration range (10–160 µg/mL), all extracts demonstrated minimal or no cytotoxic effects (Fig. 3a–3f).

Cell viability remained above 90% in nearly all treatments, indicating the absence of significant toxic influence. The most stable results were observed for RPF-EE, RPSL-EE, and ROF-EE, where viability values were statistically indistinguishable from the negative control (NC). A slight, non-significant reduction in viability (to approximately 90%) was recorded for RPR-EE and ROR-EE at higher concentrations (80 and 160 µg/mL); however, this decrease did not reach statistical significance ($p > 0.05$).

The limited cytotoxicity observed against HepG2 cells may be attributed to several factors. First, ethanol, as a relatively polar solvent, predominantly extracts hydrophilic metabolites such as flavonoids and phenolic acids, which generally exhibit antioxidant rather than cytotoxic behavior. Second, HepG2 cells are known for their intrinsic resistance to xenobiotics due to elevated expression of detoxifying enzymes and efflux transporters. Consequently, compounds extracted under these conditions are less likely to exert pronounced cytotoxic effects on this cell line.

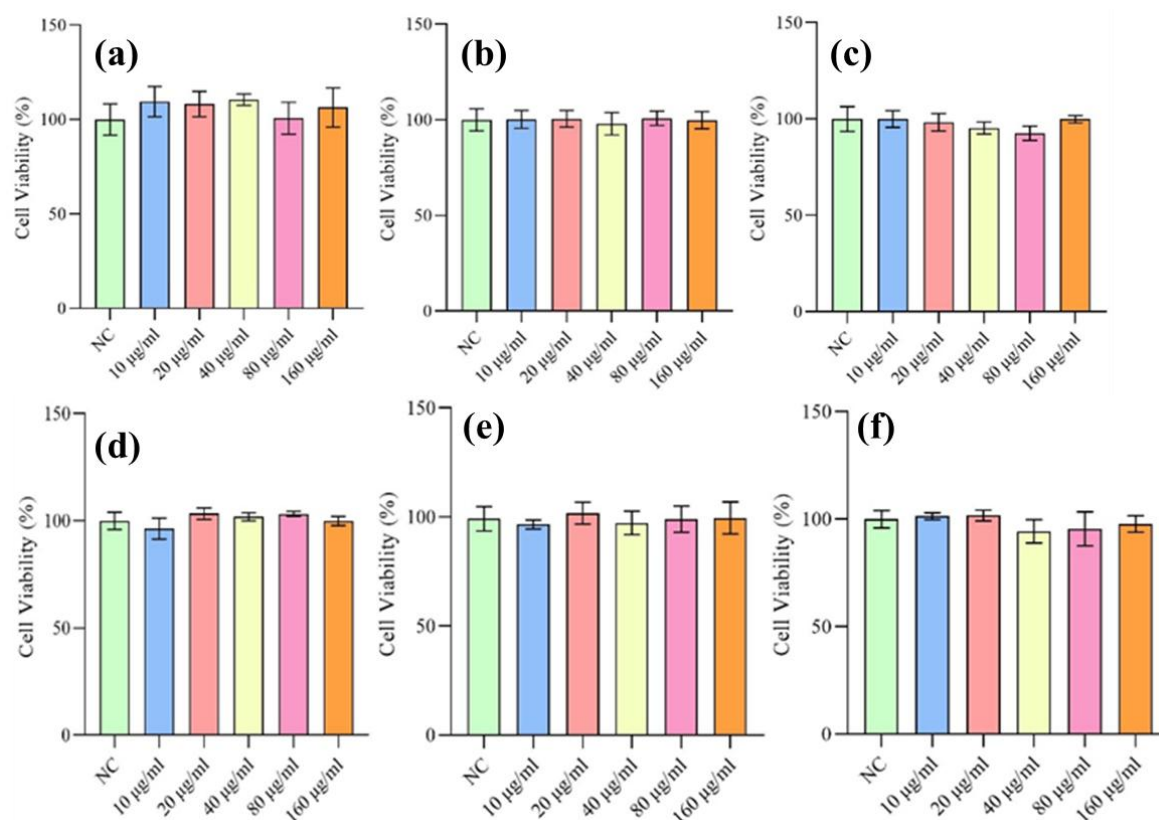


Fig. 3: Proliferation of RPF-EE (a), RPSL-EE (b), RPR-EE (c), ROF-EE (d), ROSL-EE (e) and ROR-EE (f) treated HepG2 cells was tested by the CCK-8 assay (n=3), compared with NC group.

Table 2: IC₅₀ and SI values of the *R. polyanthemus*, *R. oxyspermus* extract in different cell lines.

Extract	IC ₅₀ (L6) [μg/mL]	IC ₅₀ (HepG2) [μg/mL]	SI (L6/HepG2)
RPF-EE	>160	>160	>1
RPSL-EE	>160	>160	>1
RPR-EE	>160	>160	>1
ROF-EE	>160	>160	>1
ROSL-EE	>160	>160	>1
ROR-EE	152	>160	<0.9

Overall, these findings indicate that ethanolic extracts of *R. polyanthemus* and *R. oxyspermus* show low toxicity and lack of selective anticancer activity under the tested conditions, suggesting the need for further fractionation using less polar solvents to identify potential cytotoxic constituents.

All tested ethanolic extracts of *R. polyanthemus* (RPF-EE, RPSL-EE, RPR-EE) and *R. oxyspermus* (ROF-EE, ROSL-EE, ROR-EE) exhibited no significant cytotoxicity against either normal L6 myoblasts or HepG2 hepatocellular carcinoma cells at concentrations up to 160 μg/mL (IC₅₀ > 160 μg/mL). The *R. oxyspermus* root extract (ROR-EE) demonstrated moderate toxicity toward L6 cells (IC₅₀ = 152 μg/mL), yet failed to reach 50% inhibition of HepG2 growth even at the maximum tested concentration (IC₅₀ > 160 μg/mL), resulting in a selectivity index (SI) < 0.95 (Table 2).

These findings indicate the absence of pronounced selective cytotoxicity of *Ranunculus* extracts toward HepG2 cells. The limited activity of ROR-EE against normal myoblasts suggests the presence of non-specific cytotoxic constituents, necessitating caution when evaluating its therapeutic potential. Several factors may explain this observation: (i) the relatively low abundance of cytotoxic compounds in the plant tissues tested; (ii) intrinsic resistance of the HepG2 cell line to phytochemicals; and (iii) methodological constraints, as the tested concentration range (up to 160 μg/mL) might not fully reflect the dose–response potential of the extracts.

The observed toxicity of root extracts toward L6 cells is consistent with the accumulation of protoanemonin-type metabolites in the roots of *Ranunculaceae* species. Protoanemonin is a volatile, irritant compound whose concentration varies with species and developmental stage, typically peaking during flowering. Although it rapidly polymerizes to the non-toxic anemonin, protoanemonin is known to cause mucosal irritation, gastrointestinal discomfort, and dermal blistering at high or repeated doses. These properties explain the traditional yet risky use of buttercup preparations in folk medicine for the treatment of abscesses, skin inflammations, and muscular pain.^[102–104]

The lack of cytotoxic effect on HepG2 cells contrasts with some earlier reports describing anticancer potential in *Ranunculus* species. This discrepancy may be due to (i) extraction limitations, since ethanol primarily extracts polar compounds and may not solubilize non-polar cytotoxic constituents such as diterpenoid alkaloids or lactones; (ii) a sub-therapeutic concentration ceiling of 160 μg/mL for

HepG2; and (iii) metabolic inactivation of extract components by the HepG2 cell line.

Importantly, the inverse selectivity (SI < 1 for ROR-EE) raises safety concerns, as normal cells appear more sensitive than cancer cells. In contrast, effective chemotherapeutic agents typically demonstrate SI > 2, indicating preferential toxicity toward tumor cells.

In summary, while ethanolic extracts of *R. polyanthemus* and *R. oxyspermus* display measurable cytotoxic effects only at high concentrations, their activity is non-selective and likely attributable to general phytochemical constituents such as protoanemonin derivatives. These findings highlight a key methodological consideration: ethanol may not extract the full spectrum of potential anticancer compounds. Future studies should therefore employ non-polar or mixed solvent systems (e.g., ethyl acetate, chloroform, or hydroalcoholic mixtures) and fractionation-based approaches to isolate and identify the truly active cytotoxic components.

4. Conclusion and perspectives

This study provides a comparative analysis of the phytochemical profiles and cytotoxic activities of *Ranunculus polyanthemus* and *R. oxyspermus* from Central Asia. UPLC–Q-TOF–MS/MS profiling revealed 87 metabolites representing flavonoids, alkaloids, phenolic acids, coumarins, triterpenoids, and fatty acid derivatives, confirming the chemical diversity and pharmacological promise of these species. Despite this richness, ethanolic extracts showed low and non-selective cytotoxicity against both L6 myoblasts and HepG2 hepatocellular carcinoma cells, with IC₅₀ values exceeding 160 μg/mL. The *R. oxyspermus* root extract exhibited only moderate toxicity to L6 cells but had no significant effect on HepG2 viability (SI < 1), suggesting general rather than tumor-selective toxicity. The weak cytotoxic response likely reflects the polarity of ethanol, which limits extraction of non-polar cytotoxic constituents such as diterpenoid alkaloids and lactones.

Overall, the results emphasize the chemical richness but limited tumor selectivity of ethanol extracts of *Ranunculus* species. Future work should employ fractionation and extraction with less polar or mixed solvents to recover a broader range of active metabolites. Integrating bioassay-guided isolation with molecular docking and omics-based analyses could elucidate mechanisms and molecular targets. Expanding studies to other bioactivities—anti-inflammatory, antioxidant, or antimicrobial—will help define the broader

therapeutic potential of these Central Asian *Ranunculus* species.

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Conflict of Interest

There is no conflict of interest.

Supporting Information

Applicable.

CRediT Statement

Gulnaz Askerbay: Data curation, Data validation, Visualization, Supervision, Writing - Review and editing. **Ashirali Smanov:** Methodology, Visualization, Software, Review and editing. **Alibek Ydyrys:** Methodology, Conceptualization and Funding acquisition. **Xin Zhou Yang:** Methodology, Formal analysis and resources.

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