

Polyphenolic Profile and Multimodal Biological Activity of *Ajuga turkestanica* Extract: From In vitro Antioxidant Potential to In vivo Antihypoxic Protection

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Natural adaptogens are increasingly evaluated for their ability to mitigate environmental stress, yet the non-ecdysteroid components of *Ajuga turkestanica* remain poorly characterized. The present study aimed to characterize the polyphenolic profile of *Ajuga turkestanica* and to evaluate its antioxidant, antihypoxic, and antimicrobial activities using integrated phytochemical and biological approaches. The ethanolic extract was systematically examined for total phenolics, flavonoids, and phenolic acids using spectrophotometric methods. Quantitative assessment demonstrated a total phenolic content of 10.03 ± 1.68 mg GAE/g, accompanied by flavonoid levels of 0.46 ± 0.02 mg QE/g and total phenolic acids amounting to 0.96 ± 0.12 mg CAE/g. In vitro antioxidant assays demonstrated robust radical scavenging activity, with an IC₅₀ for DPPH at 0.42 mg/mL and significant hydroxyl radical inhibition (IC₅₀ = 0.04 mg/mL). In vivo studies demonstrated that the extract significantly enhanced survival under hypoxic conditions in outbred white mice and reduced alloxan-induced oxidative stress in outbred white rats. Hemic hypoxia models showed the strongest response to the extract; a 120 mg/kg dose extended survival by 38.7% (19.7 ± 1.0 min vs 14.2 ± 1.1 min in control, $p = 0.01$). Furthermore, the extract exhibited broad-spectrum antimicrobial activity, particularly against *Candida* species, with inhibition zones up to 10.33 ± 0.58 mm for *C. glabrata* and *C. krusei*. Acute toxicity tests confirmed a high safety profile (LD₅₀ > 10,000 mg/kg), classifying the extract as a low-toxicity substance. These observations indicate the potential applicability of *A. turkestanica* extract as a natural adjuvant in conditions involving acute oxygen deprivation, such as high-altitude exposure or ischemic events.

Keywords: *Ajuga turkestanica*; Antihypoxic protection; Antimicrobial activity; Antioxidant activity; Hemic hypoxia; Oxidative stress; Polyphenols.

A major focus of modern pharmacology is the search for natural compounds that help the body adapt to extreme environmental stress.¹ Cellular hypoxia and subsequent oxidative stress act as

primary triggers for diverse pathologies.² Despite the availability of synthetic adaptogens such as bromantane (ladasten) and phenylpiracetam, frequent side effects including insomnia, increased

psychomotor agitation, and rapid tolerance development often limit their suitability for prolonged administration. This underscores the growing demand for plant-based alternatives with multi-target protective profiles. *Ajuga turkestanica* (Regel) Briq., a perennial herb native to Central Asia, has attracted considerable interest within the scientific community, largely attributable to its richness in phytoecdysteroid compounds, notably 20-hydroxyecdysone, which are known for their anabolic and adaptogenic properties.³ However, research has largely overlooked the non-ecdysteroid components of *A. turkestanica*, leaving its polyphenolic profile and potential contribution to systemic defense insufficiently explored. Phenolic compounds, including flavonoids and hydroxycinnamic acids, are recognized as potent antioxidants capable of neutralizing reactive oxygen species (ROS) and modulating endogenous defense systems.^t Recent molecular studies further indicate that disruption of redox homeostasis under hypoxic conditions activates HIF-dependent signaling pathways, leading to mitochondrial dysfunction and inflammatory cascades.^u

Recent metabolomic investigations have provided deeper insight into the chemical complexity of *A. turkestanica*. Advanced analytical approaches such as UHPLC-MS and NMR profiling have revealed that, in addition to phytoecdysteroids, this species contains diverse classes of secondary metabolites, including phenylpropanoids, iridoids, diterpenoids, and flavonoid derivatives.^v These findings suggest that the biological activity of *A. turkestanica* may reflect a multi-component phytochemical system rather than the action of ecdysteroids alone. Furthermore, emerging evidence indicates that plant extracts frequently exert their pharmacological effects through synergistic interactions among multiple metabolite classes.^w

In vitro antioxidant assays remain widely used for screening phytochemical activity; however, increasing evidence suggests that *in vitro* antioxidant capacity does not necessarily predict *in vivo* biological efficacy, emphasizing the need for integrated experimental models.^x In the context of *A. turkestanica*, the potential synergistic interaction between these polyphenols and its established adaptogenic components may contribute to the extract's overall cytoprotective

effects through complementary mechanisms. Despite the documented uses of *Ajuga* species in traditional medicine for treating inflammation and exhaustion³, there is a lack of comprehensive data linking the specific quantitative profile of its polyphenols with systemic physiological resilience. Specifically, the bridge between *in vitro* antioxidant capacity and *in vivo* survival under acute hypoxic and oxidative challenges has not been fully established for the *A. turkestanica* extract. As a result, the use of *A. turkestanica* as a standardized multi-target agent for antioxidant and antihypoxic therapy requires further validation. In this study, we investigated a detailed phytochemical characterization of the *A. turkestanica* extract, focusing on total polyphenols, flavonoids, and phenolic acids with an evaluation of its multimodal biological efficacy. Furthermore, we evaluate the biological efficacy of the extract through a dual approach: assessing its radical-scavenging activity *in vitro* and its protective influence on mice models subjected to acute hypoxia and H₂O₂-induced oxidative stress. This work expands the therapeutic scope of *A. turkestanica*, suggesting its potential beyond its well-known anabolic ecdysteroids.

MATERIALS AND METHODS

Animal Ethics

All experimental procedures involving animals were evaluated and authorized by the Institutional Animal Care and Use Committee prior to the initiation of the study. Animals were housed in the institutional vivarium under controlled environmental conditions, including a temperature of 22 ± 2 °C and relative humidity of 55–65%, with free access to standard laboratory feed and drinking water. All animal-related procedures were carried out in strict compliance with the European Directive 2010/63/EU on the protection of animals used for scientific research. Formal ethical approval was granted by the Animal Ethics Committee of the Institute of Bioorganic Chemistry, Academy of Sciences of the Republic of Uzbekistan (Protocol No. 133/1a/h).

Plant Material and Extract Preparation

The dry powdered extract of *Ajuga turkestanica* (Regel) Briq. was obtained from Bioton LLC (Tashkent, Uzbekistan). The raw plant material was harvested from wild-

growing populations in the Baysun mountains (Surkhandarya region, Uzbekistan) during the flowering period in July 2025. The aerial parts were dried and subjected to extraction using 70% ethanol as the primary solvent. The resulting solution was filtered, concentrated under reduced pressure, and spray-dried to obtain a standardized fine powder.

Standardization and Chemical Profile

The content of turkesterone (5%) and 20-hydroxyecdysone (10%) was verified by the manufacturer (Bioton LLC, Tashkent, Uzbekistan) by means of an HPLC-based analytical method coupled with ultraviolet detection, in accordance with their internal quality control procedures. The authors relied on this specification for standardization purposes, in accordance with common practice for commercially supplied botanical extracts when independent analytical validation is not feasible.

Additional phytochemical screening and quantitative analyses were performed spectrophotometrically in the authors' laboratory: Total phenolic content (TPC) was assessed using the Folin–Ciocalteu reagent following the protocol originally proposed by Singleton and Rossi, with the results calculated and reported as gallic acid equivalents (GAE).⁹ Total flavonoid content (TFC) was evaluated by means of the aluminum chloride colorimetric method in accordance with the procedure described.¹⁰ The data were expressed as quercetin equivalents (QE). Total phenolic acid content (TPAC) was determined employing Arnow's reagent composed of 10% NaNO₂ and 10% Na₂MoO₄.¹¹ Absorbance values were recorded at 490 nm, and the results were expressed as caffeic acid equivalents (CAE).

In vitro Antioxidant Assays

The total antioxidant capacity (TAC) of the *A. turkestanica* extract was determined using the phosphomolybdenum method.¹² Results were calculated as trolox equivalent antioxidant capacity (TEAC). DPPH radical scavenging activity was evaluated employing the stable 2,2-diphenyl-1-picrylhydrazyl radical.¹³ Superoxide anion (O₂^{•−}) scavenging activity was assessed using the riboflavin–light–nitroblue tetrazolium (NBT) assay. Hydroxyl radical (•OH) scavenging capacity was examined based on the deoxyribose degradation method. Hydrogen peroxide (H₂O₂) scavenging activity was quantified by monitoring

the reduction in absorbance at 240 nm. Ferric reducing antioxidant power (FRAP) and iron-chelating activity were determined according to the previously described method, with the results expressed as gallic acid equivalents (GAE).¹⁴

In vivo Inhibition of Oxidative Stress

Oxidative stress was experimentally induced by administration of alloxan (Sigma-Aldrich) in accordance with a previously described protocol.¹⁹ Alloxan was freshly prepared in physiological saline immediately prior to intraperitoneal (i.p.) injection. White outbred male rats were randomly assigned into five experimental groups (n = 8 per group). The intact group comprised healthy animals that received no treatment. Animals in the control group were subjected to oxidative injury via a single i.p. injection of alloxan at a dose of 120 mg/kg body weight.

Rats in the experimental groups were treated orally with *A. turkestanica* extract at a daily dose of 75 mg/kg for 14 consecutive days. This dosage regimen was selected to ensure a therapeutically relevant intake of phytoecdysteroids, corresponding to approximately 3.75 mg/kg of turkesterone, which has been reported to provide subchronic metabolic protection during a 14-day treatment period, based on earlier pharmacological investigations of *Ajuga* species.^{2u, 3p} On day 24 of the experimental timeline, all animals were euthanized by decapitation under light anesthesia. Subsequently, the brain, heart, kidneys, small intestine, pancreas, testes, and liver were rapidly excised and processed for biochemical analyses.

Malondialdehyde (MDA) Analysis

Malondialdehyde (MDA) levels in tissue homogenates were determined following the procedure described by Heath and Packer.¹⁵ Briefly, 1 mL of tissue supernatant was combined with 4 mL of 20% trichloroacetic acid (TCA) containing 0.5% thiobarbituric acid (TBA). The reaction mixture was incubated at 95 °C for 30 min. After cooling to room temperature, samples were centrifuged at 10,000 × g for 10 min. The absorbance of the resulting MDA–TBA adduct was recorded spectrophotometrically at 532 nm, and the data were expressed as nmol per mg of protein.

Evaluation of Antihypoxic Activity

The antihypoxic potential of the *Ajuga turkestanica* extract was evaluated using three

experimental models: hemic, acute normobaric, and circulatory hypoxia.^{19, 20} Hemic hypoxia was induced by a subcutaneous (s.c.) injection of sodium nitrite (NaNO_2) at a dose of 350 mg/kg.²¹ Acute normobaric hypoxia was modeled by placing mice individually into a hermetically sealed chamber (100 mL).²² Circulatory hypoxia was experimentally induced by intraperitoneal injection of sodium fluoride (NaF) at a dose of 150 mg/kg, as previously described.²³ The study was performed using 400 outbred white male mice weighing 20 ± 2.0 g. Animals in the control group received distilled water (0.2 mL), whereas mice in the experimental groups were orally administered *A. turkestanica* at doses of 30, 60, or 120 mg/kg, 1 h prior to hypoxia induction. The antihypoxic efficacy of the extract was assessed based on a statistically significant prolongation of survival time in treated animals relative to the control group.

Antimicrobial Activity

Antimicrobial activity was evaluated using the disc diffusion assay in accordance with a previously reported method.^{24, 25} A total of eleven reference microorganisms were included in the study.

- Yeast: *Candida albicans* (CCM 8186), *C. glabrata* (CCM 8270), *C. krusei* (CCM 8271), *C. parapsylosis* (CCM 8260), and *C. tropicalis* (CCM 8223).
- Gram-negative bacteria: *Salmonella enterica* subsp. *enterica* (CCM 3807), *Yersinia enterocolitica* (CCM 5671), and *Escherichia coli* (CCM 3988).
- Gram-positive bacteria: *Staphylococcus aureus* subsp. *aureus* (CCM 2461), *Listeria monocytogenes* (CCM 4699), and *Enterococcus faecalis* (CCM 4224).

All microbial strains were obtained from the Czech Collection of Microorganisms (CCM). Prior to testing, bacterial and yeast cultures were propagated in nutrient broth (Imuna, Slovakia) at 37 °C for 24 h. Aliquots of 0.1 mL microbial suspension, adjusted to a density of 10^8 CFU/mL, were evenly spread onto Mueller–Hinton agar plates (MHA; Oxoid, Basingstoke, United Kingdom) in accordance with CLSI recommendations.²⁶ Sterile paper discs (6 mm diameter) were loaded with 15 μL of the test solution (crude extract at 1 mg/mL) and placed on the inoculated agar surfaces. Plates were maintained at 4 °C for 2 h to allow compound

diffusion, followed by aerobic incubation at 37 °C for 24 h.

After incubation, inhibition zone diameters were measured in millimetres. All assays were performed in triplicate. Discs containing 10 μL of distilled water served as the negative control, while gentamicin (10 $\mu\text{g}/\text{disc}$; Oxoid, Basingstoke, United Kingdom) was used as the positive control.

Statistical Analysis

All quantitative results are presented as the mean $\pm$ standard error of the mean (SEM) derived from a minimum of three independent experiments. Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test, with statistical significance defined at $p < 0.05$.

RESULTS

Phytochemical screening of the *A. turkestanica* extract (Table 1) revealed a total phenolic content (TPC) of 10.03 ± 1.68 mg GAE/g. While this value is slightly lower than the levels reported for European species like *A. genevensis*, it confirms a significant polyphenolic contribution to the extract's matrix.²⁷ The low flavonoid (0.46 ± 0.02 mg QE/g) and phenolic acids (0.96 ± 0.12 mg CAE/g) content indicates that the antioxidant potential of this Central Asian species may be driven more by its specific phenolic acid profile and ecdysteroids rather than a high flavonoid density.

Antioxidant and Radical Scavenging Activity

The extract demonstrated pronounced antioxidant potential, as reflected by its strong scavenging capacity toward hydroxyl radicals ($\text{IC}_{50} = 0.04$ mg/mL) and hydrogen peroxide ($\text{IC}_{50} = 1.10$ mg/mL). The total antioxidant capacity (TAC) was recorded at 800.00 ± 12.5 mg TEAC/g (Table 2). Of particular interest is the extract's high affinity for neutralizing hydroxyl radicals ($\text{IC}_{50} = 0.04 \pm 0.01$ mg/mL) and hydrogen peroxide ($\text{IC}_{50} = 1.10 \pm 0.08$ mg/mL), which are primary drivers of cellular oxidative damage. The DPPH radical scavenging potency ($\text{IC}_{50} = 0.42 \pm 0.03$ mg/mL) and the inhibition of lipid peroxidation ($\text{IC}_{50} = 5.51 \pm 0.45$ mg/mL for MDA) further validate the extract's role as a potent chain-breaking antioxidant.

Acute Toxicity

The *A. turkestanica* extract exhibited a favorable safety profile. Oral administration

at doses up to 10,000 mg/kg was not associated with any mortality among treated animals (0/6). Accordingly, the median lethal dose (LD₅₀) was estimated to exceed 10,000 mg/kg, indicating that the extract can be categorized as a substance of low acute toxicity in accordance with internationally accepted criteria.

Inhibition of Lipid Peroxidation in Organ Homogenates

The quantification of malondialdehyde (MDA) was used as a key indicator of the intensity of lipid peroxidation (LPO) across various physiological systems. As shown in Figure 1, the systemic administration of alloxan triggered a massive surge in MDA levels in the control group, confirming widespread oxidative stress. The most acute damage was registered in the small intestine (19.65 nmol/mg), kidneys (18.45 nmol/mg), and brain (18.22 nmol/mg). However, treatment with the *A. turkestanica* extract significantly reduced lipid peroxidation levels. The extract exerted a potent protective effect, bringing MDA concentrations in the brain and small intestine back to intact values (11.28 ± 1.10 and 11.86 ± 0.11 nmol/mg, respectively). This virtually complete restoration highlights the extract's high efficacy

in stabilizing lipid membranes against chemical pro-oxidants (Figure 1).

The ability of *A. turkestanica* to suppress MDA formation *in vivo* is a critical validation of its therapeutic potential. Alloxan facilitates tissue damage via the excessive production of oxygen-derived free radicals, which initiate a chain reaction of lipid peroxidation by stripping electrons from polyunsaturated fatty acids.^{18, 31}

The neuroprotective data is particularly noteworthy. Owing to its elevated oxygen demand and lipid-enriched composition, the brain is particularly susceptible to oxidative damage. The fact that our extract maintained MDA at levels nearly identical to the intact group (11.28 vs 10.94 nmol/mg) suggests that its bioactive constituents either successfully cross the blood-brain barrier or significantly upregulate the brain's internal antioxidant defenses. This observation is consistent with recent reports by Mamadalieva *et al.*³² and Das *et al.*³³, which emphasize the neuroprotective effects of phytoecdysteroids against oxidative damage in neural tissues.

We attribute this systemic protection to a “dual-layer” synergy between the identified polyphenols and ecdysteroids (turkesterone and

Table 1. Polyphenolic composition of the *A. turkestanica* extract, including total phenols, flavonoids, and phenolic acids

	Total phenols (mg GAE per g extract)	Total flavonoids (mg QE per g extract)	Total phenolic acids (mg CAE per g extract)
<i>A. turkestanica</i> L.	10.03 ± 1.68	0.46 ± 0.02	0.96 ± 0.12

Table 2. Antioxidant and radical scavenging parameters of *A. turkestanica* ethanolic extract

Parameters	Unit	<i>A. turkestanica</i> extract
Antioxidant Capacity		
Total Antioxidant Capacity (TAC)	mg TEAC/g	800.00±12.5
Ferric Reducing Power (FRAP)	mg GAE/g	0.05±0.01
Radical Scavenging Activity		
DPPH Radical	IC ₅₀ , mg/ml	0.42±0.03
Hydroxyl Radical (·OH)	IC ₅₀ , mg/ml	0.04±0.01
Hydrogen Peroxide (H ₂ O ₂)	IC ₅₀ , mg/ml	1.10±0.08
Superoxide Anion (O ₂ ^{·-})	IC ₅₀ , mg/ml	13.35±1.22
Lipid Peroxidation (MDA)	IC ₅₀ , mg/ml	5.51±0.45

20-hydroxyecdysone). While the polyphenolic fraction directly scavenges free radicals, interrupting the LPO chain reaction at its source, the ecdysteroids likely promote metabolic adaptation by enhancing the functional status of endogenous antioxidant enzymes, including superoxide dismutase (SOD) and catalase.^{4,34} This multi-target approach not only prevents acute membrane damage but also fosters long-term cellular resilience, making *A. turkestanica* a promising candidate for development as an adaptogenic agent.

Antihypoxic Efficacy

The protective influence of *A. turkestanica* extract was evaluated across three distinct models of acute oxygen deprivation to discern its systemic and metabolic effects (Figure 2).

Hemic Hypoxia

The most significant physiological resilience was observed in the hemic hypoxia model, which simulates impaired oxygen transport due to methemoglobin formation. Administration of the extract at a dose of 120 mg/kg extended the survival duration of mice by 38.7% (19.7 ± 1.0 min

compared to 14.2 ± 1.1 min in the control group; $p = 0.01$). Even at the minimum dose of 30 mg/kg, a statistically significant increase in survival (32.4%) was recorded ($p = 0.03$). Taken together, these findings indicate that the extract's polyphenolic constituents may maintain the structural integrity of erythrocyte membranes or hinder the oxidative conversion of hemoglobin, thereby preserving gas exchange efficiency under chemical stress.

Acute Normobaric Hypoxia

In the normobaric model, which induces “closed-space” oxygen depletion, the extract demonstrated a consistent, albeit less pronounced, protective effect. Doses of 60 and 120 mg/kg increased life expectancy to 21 ± 0.3 and 22 ± 0.6 minutes, respectively ($p < 0.01$ for both). A notable finding was the 20% total survival rate observed in all treated groups, regardless of the dosage, whereas no animals in the control group survived the challenge. This baseline protection suggests a fundamental shift in cellular metabolic adaptation rather than a simple dose-dependent response.

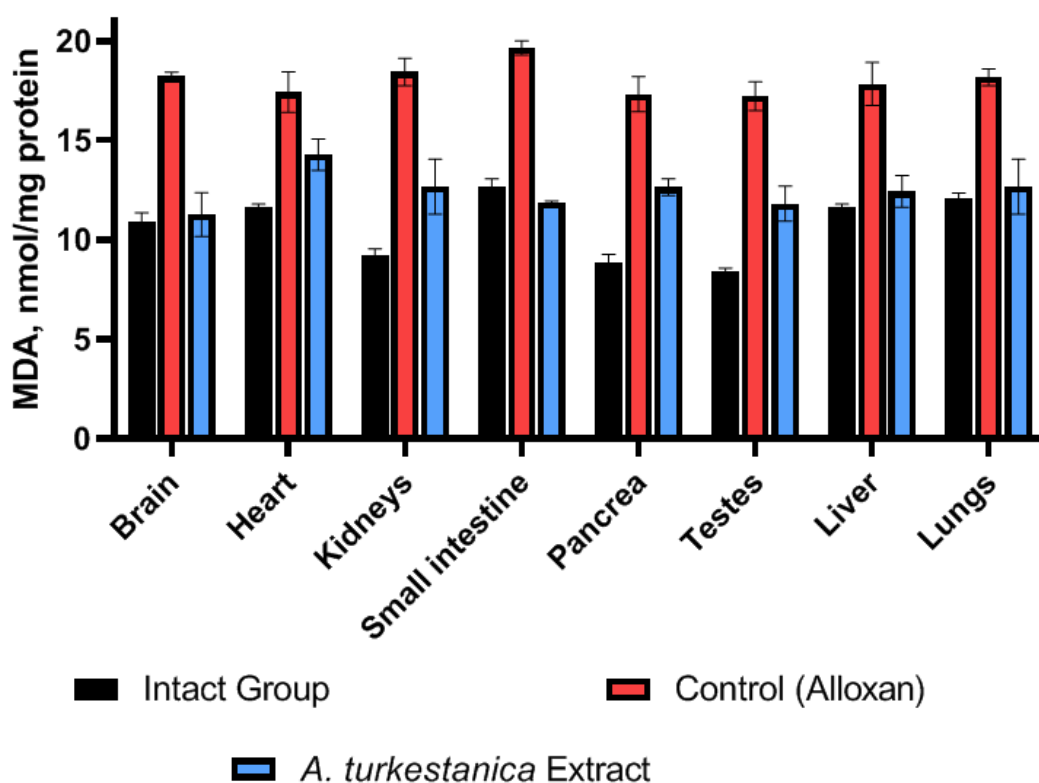


Fig. 1. Impact of *A. turkestanica* extract on MDA levels (nmol/mg protein) in organ homogenates.

Circulatory Hypoxia

Conversely, the circulatory hypoxia model, induced by NaF to simulate systemic ischemia, showed only marginal improvements.

The survival time increased by 9.6% at a dose of 60 mg/kg, but these changes failed to reach statistical significance. The lack of a robust response in this model implies that the extract's primary mechanism

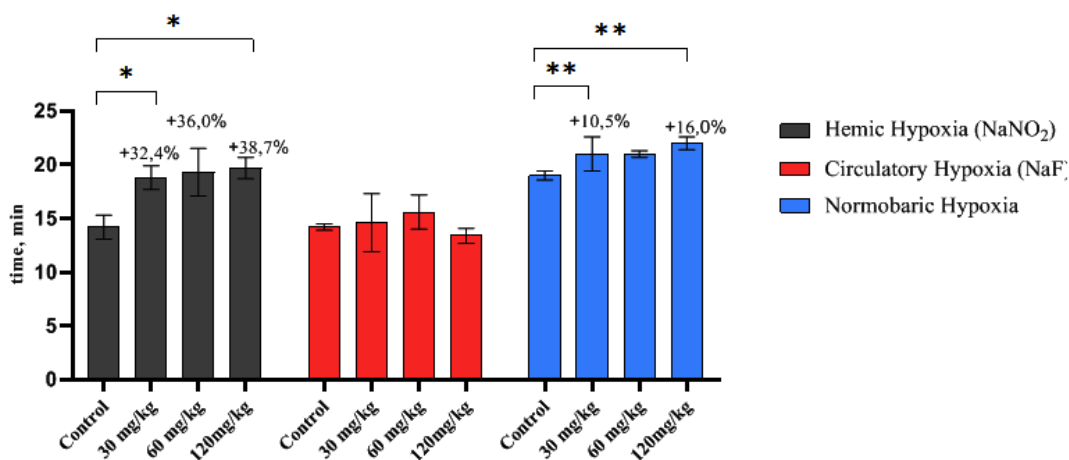


Fig. 2. Protective effect of *A. turkestanica* extract on survival time in various mouse models of hypoxia. Percentages above the bars represent the increase in survival time relative to the control group. Data are presented as mean $\pm$ SEM (n = 8 per group). Statistical significance was set at $p < 0.05$ (*) and $p < 0.01$ (**), relative to the corresponding control group.

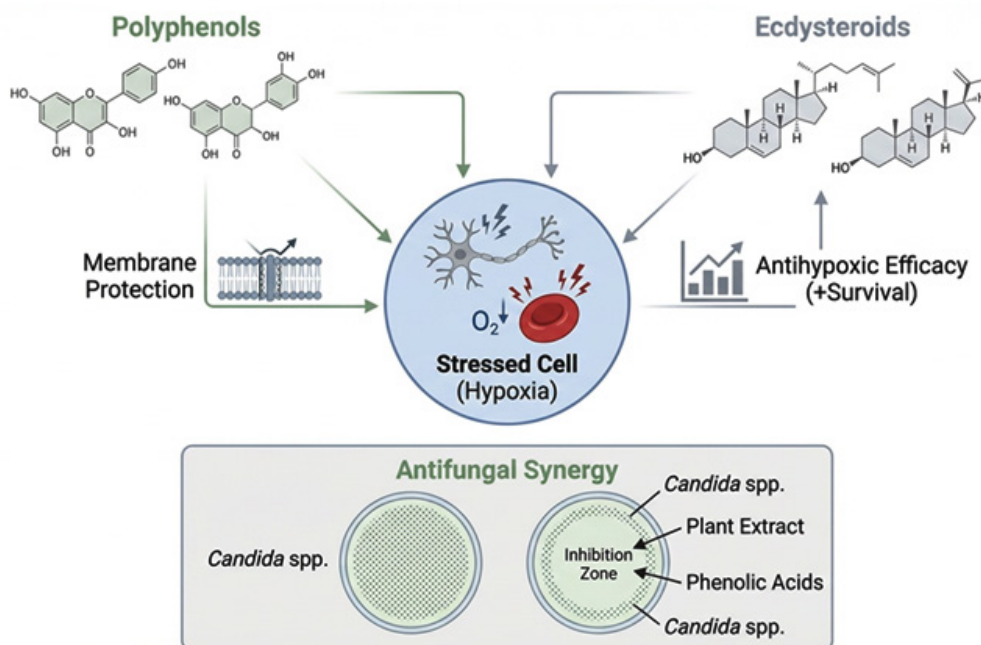


Fig. 3. Proposed mechanism of the multimodal biological activity of *A. turkestanica* extract. The figure illustrates the synergistic interaction between polyphenols (leading to LPO inhibition and MDA reduction) and ecdysteroids (enhancing cell survival and enzymatic defense), alongside the antifungal effect of phenolic acids via membrane destabilization.

Table 3. Antimicrobial activity of *A. turkestanica* extract (zone of inhibition in mm)

Microorganism	Zone of Inhibition (mm)
Yeast/Fungi	
<i>Candida glabrata</i>	10.33±0.58
<i>Candida krusei</i>	10.33±0.58
<i>Candida parapsilosis</i>	10.33±0.58
<i>Candida tropicalis</i>	6.33±0.58
<i>Candida albicans</i>	5.33±0.58
Gram-negative Bacteria	
<i>Salmonella enterica</i>	8.67±0.58
<i>Yersinia enterocolitica</i>	6.67±0.58
<i>Escherichia coli</i>	2.33±0.58
Gram-positive Bacteria	
<i>Listeria monocytogenes</i>	7.67±0.58
<i>Enterococcus faecalis</i>	6.67±0.58
<i>Staphylococcus aureus</i>	5.33±0.58

Data are presented as mean ± SEM (n = 3).

of action is likely metabolic and cytoprotective, targeting cellular oxidative pathways, rather than exerting a direct effect on systemic hemodynamics or heart rate regulation.

The pronounced antihypoxic effect observed, particularly in the hemic model, where survival time increased by 38.7%, underscores the metabolic versatility of the *A. turkestanica* extract. This protection is likely attributed to a synergistic interaction between its standardized ecdysteroids (turkesterone and 20-hydroxyecdysone) and the newly characterized polyphenolic fraction. While ecdysteroids are known to enhance protein synthesis and ATP production under stress conditions, the polyphenols (phenolic acids and flavonoids) provide a critical second line of defense by neutralizing reactive oxygen species (ROS) and stabilizing erythrocyte membranes against oxidative degradation.^{28, 29} Similar cytoprotective effects have been reported for other species of the genus, such as *A. genevensis* and *A. reptans*, which have also demonstrated significant antioxidant and cytoprotective properties linked to their polyphenolic profiles.²⁷

The discrepancy between the high efficacy in hemic hypoxia and the modest results in circulatory hypoxia is revealing. Hemic hypoxia involves chemical damage to hemoglobin and a reduction in blood oxygen-carrying capacity, where the extract's antioxidant potency (IC₅₀ for

hydroxyl radicals = 0.04 ± 0.01 mg/mL) plays a direct cytoprotective role. The validity of using these specific experimental models to assess such adaptogenic effects is well-supported in recent comparative studies of phyto-extracts.²² In contrast, the modest protective effect observed in the NaF-induced circulatory hypoxia model may be mechanistically explained by the distinct pathophysiology of this condition. Sodium fluoride (NaF) inhibits enolase, a key glycolytic enzyme, thereby blocking ATP production via anaerobic glycolysis.²³ Unlike hemic hypoxia, where oxidative damage to hemoglobin and erythrocyte membranes is central, circulatory hypoxia induced by NaF primarily disrupts cellular energy metabolism. The *A. turkestanica* extract, while rich in antioxidants and ecdysteroids, does not appear to directly modulate glycolytic flux or mitochondrial substrate-level phosphorylation. This metabolic limitation likely accounts for the lack of statistically significant survival extension in this model, underscoring that the extract's primary mode of action is cytoprotective rather than energetic (Figure 2).

This metabolic-centric mechanism aligns with the extract's high safety profile (LD₅₀ > 10,000 mg/kg), positioning it as a non-toxic candidate for long-term physiological resilience.

Antimicrobial Activity

The antimicrobial screening revealed that the ethanolic extract of *A. turkestanica* possesses a selective inhibitory effect, with a more pronounced impact on fungal pathogens compared to bacterial strains. The results of the disc diffusion assay are outlined in Table 3.

The extract displayed pronounced antifungal activity against multiple *Candida* species. The most prominent inhibitory effects were observed for *C. glabrata*, *C. krusei*, and *C. parapsilosis*, each exhibiting inhibition zones of 10.33 ± 0.58 mm. By comparison, *C. albicans* and *C. tropicalis* demonstrated moderate susceptibility, with inhibition zones measuring 5.33 ± 0.58 mm and 6.33 ± 0.58 mm, respectively.

With respect to antibacterial properties, the extract showed moderate activity against Gram-negative bacteria, with the highest inhibition recorded for *Salmonella enterica* (8.67 ± 0.58 mm), followed by *Yersinia enterocolitica* (6.67 ± 0.58 mm). Among Gram-positive microorganisms,

including *Listeria monocytogenes* and *Enterococcus faecalis*, inhibition zones ranged between 6.67 and 7.67 mm. The weakest antibacterial effect was detected against *Escherichia coli*, with an inhibition zone of 2.33 mm.

While *A. turkestanica* is traditionally prized for its ecdysteroid-driven anabolic effects, our findings, including its significant antifungal activity and antioxidant properties, suggest that its biological utility is far more multimodal. The potent antifungal activity against *C. glabrata* and *C. krusei* is of particular interest, as these species often show reduced sensitivity to conventional azole therapies. This antimicrobial action is likely the result of a coordinated defense system within the extract. The polyphenolic fraction (TPC: 10.03 mg GAE/g) contains phenolic acids that are known to destabilize microbial lipid bilayers. We hypothesize that these phenolics are likely to disrupt the microbial membrane, facilitating further damage, which then allows phytoecdysteroids to interfere with internal cellular processes. The pronounced sensitivity of *C. glabrata* to the *A. turkestanica* extract may reflect intrinsic vulnerabilities in its membrane defense systems. Unlike *C. albicans*, *C. glabrata* lacks robust efflux pump networks (e.g., CDR1/CDR2 overexpression is less common under basal conditions), making it more susceptible to membrane-disrupting phytochemicals such as phenolic acids.³⁵ The notable total phenolic content (10.03 mg GAE/g) and the presence of hydroxycinnamic derivatives in the extract likely compromise fungal membrane integrity, facilitating intracellular penetration of ecdysteroids or other bioactive constituents. This selective antifungal profile aligns with recent findings that non-albicans *Candida* species exhibit variable susceptibility to plant polyphenols as a result of variations in cell wall structure and drug-efflux capacity.³⁶

When comparing our results with European counterparts like *A. genevensis*, it becomes clear that while the latter may have higher absolute phenolic counts, the Central Asian *A. turkestanica* possesses a unique phytochemical “signature” that confers superior activity against specific yeast strains.²⁷ Furthermore, the lack of significant antibacterial activity against *E. coli* reinforces the idea that this extract is not a generic antiseptic, but rather a selective bio-modulator. Given its remarkable safety profile ($LD_{50} > 10,000$

mg/kg), *A. turkestanica* represents a promising, non-toxic candidate for developing targeted antifungal treatments.

The integrated biological response to *A. turkestanica* extract is characterized by a synergistic mechanism (Figure 3). While polyphenolic compounds primarily provide a defensive shield by neutralizing reactive oxygen species and inhibiting lipid peroxidation (reflected in reduced MDA levels), ecdysteroids like turkesterone promote metabolic adaptation. This dual action likely explains the observed 38.7% increase in survival under hypoxic conditions. Simultaneously, the destabilization of fungal membranes by phenolic acids complements the systemic antioxidant defense, confirming the extract’s multimodal efficacy.

DISCUSSION

The present study provides a comprehensive evaluation of the phytochemical composition and multimodal biological activities of *Ajuga turkestanica*, a Central Asian species that remains comparatively underexplored relative to its European congeners. The findings demonstrate that despite a moderate total phenolic content, the extract exhibits pronounced antioxidant, antihypoxic, cytoprotective, and selective antifungal activities, underscoring a mechanism of action that extends beyond simple phenolic abundance.

Phytochemical screening revealed a total phenolic content of 10.03 ± 1.68 mg GAE/g, which is lower than values reported for European species such as *A. genevensis* but remains biologically significant. Notably, the low flavonoid (0.46 mg QE/g) and phenolic acid (0.96 mg CAE/g) contents suggest that the antioxidant capacity of *A. turkestanica* is not driven by flavonoid density alone. Instead, the data indicate that a specific phenolic acid profile, acting in concert with phytoecdysteroids, is likely responsible for the observed biological effects. This supports emerging evidence that qualitative phytochemical composition may be more critical than total phenolic load in determining pharmacological efficacy.

The extract demonstrated remarkable antioxidant potency across multiple in vitro assays. Its exceptionally low IC₅₀ value against

hydroxyl radicals (0.04 mg/mL) highlights a strong capacity to neutralize highly reactive oxygen species that are central mediators of lipid peroxidation and membrane damage. This activity was corroborated by high total antioxidant capacity (800 mg TEAC/g) and effective inhibition of lipid peroxidation, reinforcing the extract's role as a chain-breaking antioxidant. Importantly, hydroxyl radical scavenging is considered a stringent indicator of antioxidant efficacy, as these radicals are among the most damaging ROS in biological systems.

The *in vivo* relevance of these antioxidant properties was validated through the significant suppression of malondialdehyde formation in multiple organ homogenates following alloxan-induced oxidative stress. The near-complete normalization of MDA levels in the brain and small intestine is particularly noteworthy. These organs are highly vulnerable to oxidative damage due to their lipid-rich composition and metabolic demands. The ability of the extract to restore MDA levels to near-intact values suggests not only direct radical scavenging but also effective stabilization of membrane lipids under severe pro-oxidant challenge.

The neuroprotective implications of these findings are substantial. The maintenance of brain MDA levels comparable to intact controls suggests systemic antioxidant activity; however, blood–brain barrier permeability and endogenous antioxidant modulation were not directly assessed in this study. This observation is consistent with previous reports suggesting the neuroprotective potential of phytoecdysteroids such as turkesterone and 20-hydroxyecdysone, which have been reported to modulate oxidative stress responses and support neuronal survival. Mechanistically, the observed systemic protection appears to arise from a synergistic “dual-layer” defense system, consistent with previous reports describing the biological activity of phytoecdysteroid-containing *Ajuga* species and their adaptogenic properties.^{32, 33} Polyphenolic compounds may contribute to ROS scavenging and inhibition of lipid peroxidation, as suggested by the *in vitro* antioxidant results. Simultaneously, ecdysteroids may promote metabolic adaptation by enhancing the activity of endogenous antioxidant enzymes, including superoxide dismutase and catalase.

Such a multi-target strategy is characteristic of phytoecdysteroid-containing plants and has been documented in experimental models of metabolic and oxidative stress.^{28, 34} This dual mechanism is further reflected in the pronounced antihypoxic activity observed, particularly in the hemic hypoxia model. The significant extension of survival time (up to 38.7%) suggests effective protection against chemically induced impairment of oxygen transport. Hemic hypoxia is strongly associated with oxidative damage to hemoglobin and erythrocyte membranes, conditions under which potent antioxidant activity is especially beneficial.^{2, 30} The extract's ability to preserve erythrocyte membrane integrity and limit oxidative hemoglobin conversion likely underlies this effect.

In contrast, the modest and statistically insignificant effects observed in the NaF-induced circulatory hypoxia model provide important mechanistic insight. Sodium fluoride disrupts cellular energy metabolism by inhibiting enolase and impairing glycolytic ATP production. The limited efficacy of *A. turkestanica* in this model suggests that its primary mode of action is cytoprotective and antioxidative rather than directly energetic or hemodynamic. This distinction reinforces the view that the extract enhances cellular resilience to oxidative stress rather than broadly stimulating metabolic flux.

The antimicrobial evaluation revealed a selective biological profile, with pronounced antifungal activity against non-*albicans* *Candida* species, particularly *C. glabrata* and *C. krusei*. These species are clinically relevant due to their reduced susceptibility to conventional antifungal agents.^{35, 36} The observed selectivity suggests that the extract does not function as a nonspecific antimicrobial but rather targets specific vulnerabilities in fungal membrane structure. Phenolic acids are known to disrupt lipid bilayers, increasing membrane permeability and facilitating intracellular penetration of other bioactive constituents. The heightened sensitivity of *C. glabrata* may be attributed to its comparatively limited efflux pump activity and membrane defense mechanisms.

Interestingly, the relatively weak antibacterial activity against *Escherichia coli* further supports the notion of selective bio-modulation rather than generalized antimicrobial

toxicity. Such selectivity is advantageous from a therapeutic perspective, as it reduces the likelihood of broad-spectrum microbial resistance and collateral disruption of beneficial microbiota.

When compared to European species such as *A. genevensis*, *A. turkestanica* appears to possess a distinct phytochemical signature that compensates for lower total phenolic levels through functional synergy. This qualitative advantage may explain its superior efficacy against specific fungal strains and its robust antihypoxic performance. Combined with its excellent safety profile (LD₅₀ > 10,000 mg/kg), these characteristics position *A. turkestanica* as a promising candidate for the development of non-toxic adaptogenic and antifungal formulations.

In summary, the present findings demonstrate that the biological activity of *A. turkestanica* is driven by an integrated, multimodal mechanism involving antioxidant defense, membrane stabilization, metabolic adaptation, and selective antimicrobial action. This synergy between polyphenols and ecdysteroids provides a strong scientific basis for the traditional use of this species and supports its further development as a functional adaptogen with targeted therapeutic potential.

CONCLUSION

In conclusion, this study confirms that the biological efficacy of the *Ajuga turkestanica* extract is the result of a synergistic interaction between its antioxidant polyphenols and its established adaptogenic components. The extract demonstrated a high safety profile (LD₅₀ > 10,000 mg/kg) and pronounced antihypoxic activity, extending survival time in the hemic hypoxia model by 38.7%. These findings expand the therapeutic scope of the plant, positioning it as a suitable platform for the formulation of multi-target therapeutic approaches designed to enhance physiological resilience against oxidative stress and hypoxic challenges. While the current study establishes a robust foundation for the multimodal bioactivity of *A. turkestanica*, future work should address its pharmacokinetic profile and long-term safety in higher mammals. Given its selective antifungal action against non-*albicans* *Candida* species and exceptional safety margin, this extract may serve

as a template for developing natural adjuvants in both hypoxia-related pathologies and antimicrobial therapy.

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Conflict of Interest

The author(s) do not have any conflict of interest.

Data Availability Statement

This statement does not apply to this article.

Ethics Statement

The protocols were specifically authorized by the Animal Ethics Committee of the Institute of Bioorganic Chemistry, AS RUz (Protocol Number: 133/1a/h, dated August 4, 2014).

Informed Consent Statement

This study did not involve human participants, and therefore, informed consent was not required.

Clinical Trial Registration

This research does not involve any clinical trials

Permission to reproduce material from other sources

Not applicable

Author Contributions

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