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Antioxidant potential and phytochemical analysis of medicinal plants used for livestock treatment in Kazakhstan

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ABSTRACT

Traditional veterinary practices in Kazakhstan rely heavily on medicinal plants for treating livestock diseases. However, scientific validation of these plants' phytochemical composition and antioxidant properties remains limited. This research's objective was to assess the antioxidant properties and examine the phytochemical composition of commonly used medicinal plants in Kazakhstan for livestock treatment: *Glycyrrhiza uralensis*, *Ephedra equisetina*, *Rhodiola rosea*, *Hypericum perforatum*, and *Artemisia absinthium*. Fresh samples of the selected plants were collected from local markets in Kazakhstan. Phytochemical screening was conducted using standard methods to detect tannins, flavonoids, alkaloids, saponins, and terpenoids. Total phenolic and flavonoid contents were determined spectrophotometrically. Antioxidant activity was assessed using DPPH radical scavenging and ferric reducing antioxidant power (FRAP) assays. To pinpoint and measure important phenolic components in the extracts exhibiting highest activity, High-Performance Liquid Chromatography (HPLC) examination was conducted. All five plants exhibited diverse phytochemical profiles, with flavonoids and terpenoids being ubiquitous. *G. uralensis* and *R. rosea* demonstrated the highest total phenolic (82.7 ± 3.5 and 75.9 ± 2.8 mg GAE/g, respectively) and flavonoid (45.3 ± 1.9 and 40.2 ± 1.7 mg QE/g, respectively) contents. These two species also showed the most potent antioxidant activities, with *G. uralensis* exhibiting the lowest IC_{50} value (42.8 ± 1.7 µg mL^{-1}) in the DPPH assay and the highest FRAP value (1285.6 ± 52.3 µmol Fe(II)/g). The analysis revealed robust positive associations linking total phenolic content ($r > 0.95$, $p < 0.01$) and antioxidant activities. HPLC analysis revealed distinct phenolic profiles for *G. uralensis* and *R. rosea*, with high concentrations of rutin and chlorogenic acid, respectively. This research presents experimental corroboration, lending credence to the traditional use of these medicinal plants in livestock treatment, highlighting their rich phytochemical composition and potent antioxidant properties. *G. uralensis* and *R. rosea* emerged as particularly promising sources of natural antioxidants.

Keywords: Medicinal plants, Livestock, Phytochemicals, Antioxidants, Kazakhstan.
Article type: Research Article.

INTRODUCTION

Traditional veterinary medicine, rooted in centuries of empirical knowledge and cultural practices, has played a pivotal role in maintaining livestock health across diverse geographical regions (Baharvand Ahmadi *et al.* 2023; Uzakovna *et al.* 2024). In Kazakhstan, a country with a rich nomadic heritage and a significant livestock sector, the use of medicinal plants for treating animal ailments remains a cornerstone of traditional veterinary practices. This reliance on plant-based remedies is not unique to Kazakhstan but reflects a global trend, particularly in regions where modern veterinary services may be less accessible or prohibitively expensive for small-scale farmers and herders (Aldayarov *et al.* 2022). The use of medicinal plants in animal healthcare is deeply ingrained

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in the cultural fabric of many societies, with knowledge passed down through generations. This traditional ecological knowledge represents a valuable resource, not only for its cultural significance but also for its potential contributions to modern veterinary pharmacology (Geck *et al.* 2020; Shafi *et al.* 2021; Bibon 2022). Recently, there is an escalating international interest in exploring and validating traditional plant-based remedies, driven by the need for sustainable, cost-effective, and locally available treatment options for livestock diseases. Kazakhstan, with its vast steppes and diverse ecosystems ranging from mountains to deserts, boasts a rich flora that has been utilized for centuries in both human and veterinary medicine. The country's geographical position at the crossroads of Europe and Asia has contributed to a unique blend of medicinal plant knowledge, incorporating influences from Russian, Chinese, and Central Asian traditions (Nendissa *et al.* 2023). This rich botanical heritage, combined with the country's significant livestock industry, presents a compelling case for scientific investigation into the efficacy and safety of traditionally used medicinal plants. The livestock sector plays a crucial role in Kazakhstan's economy and rural livelihoods. As the country seeks to modernize and expand its agricultural output, ensuring the health and productivity of livestock becomes paramount. Traditional plant-based remedies offer a potential complement to modern veterinary practices, particularly in remote areas where access to conventional medicines may be limited. Moreover, the growing global concern over antimicrobial resistance in livestock has sparked renewed interest in alternative treatment modalities, including phytotherapy (Abdikerimova & Moldabekov 2021). Among the myriad plant species used in Kazakhstani traditional veterinary medicine, several stand out for their widespread use and purported efficacy. *Glycyrrhiza uralensis* (licorice), native to Central Asia, has a long history of application in addressing respiratory ailments in both humans and animals. *Ephedra equisetina*, found in the mountainous regions of Kazakhstan, is traditionally employed as a stimulant and to treat asthma-like conditions in livestock. *Rhodiola rosea*, known for its adaptogenic properties, is used to enhance animal resilience to stress and improve overall health. *Hypericum perforatum* (St. John's wort) and *Artemisia absinthium* (wormwood) are valued for their anti-inflammatory and antiparasitic properties, respectively (Fabricant & Farnsworth 2001). Despite the long-standing use of these plants in traditional veterinary practices, scientific validation of their efficacy and safety remains limited. This gap between traditional knowledge and scientific evidence presents both a challenge and an opportunity for researchers. The phytochemical composition of medicinal plants can vary significantly based on elements including regional position, soil characteristics, and cultivation techniques. Therefore, comprehensive studies of locally-sourced plants are essential to understand their potential therapeutic properties and optimize their use in veterinary applications (Sarwar *et al.* 2023; Arshad *et al.* 2024). One of the key areas of interest in plant-based medicine is the antioxidant potential of various species. Oxidative stress is crucial in numerous animal diseases, and natural antioxidants found in plants could offer protective effects. Phenolic compounds and flavonoids, in particular, have been associated with a wide range of biological activities, including anti-inflammatory, antimicrobial, and immunomodulatory effects (May *et al.* 2023). Understanding the antioxidant profiles of traditionally-used medicinal plants could provide insights into their mechanisms of action and guide the development of standardized herbal preparations for veterinary use. The scientific investigation of traditional remedies also aligns with global efforts to promote sustainable agriculture and reduce reliance on synthetic drugs in livestock management. As consumers increasingly demand organic and naturally raised animal products, the validation and standardization of plant-based veterinary treatments could contribute to more sustainable and environmentally friendly farming practices (Krzywonos & Piwowar-Sulej 2022; McGaw 2023). Furthermore, the exploration of medicinal plants incorporated in veterinary medicine has implications beyond animal health. A significant number of these plant species have been utilized in folk remedies throughout human history, and insights gained from veterinary applications could inform research in human pharmacology. This One Health approach, recognizing the interconnectedness of human, animal, and environmental health, underscores the broader significance of ethnopharmacological research in veterinary contexts (Asaaga *et al.* 2024). However, the scientific validation of traditional plant-based remedies faces several challenges. The complex phytochemical composition of plants makes it difficult to attribute therapeutic effects to specific compounds. Moreover, the synergistic interactions between various plant constituents may contribute to their overall efficacy, necessitating holistic approaches to research and development. Standardization of herbal preparations and ensuring consistent quality are additional hurdles that need to be addressed for the wider acceptance and integration of plant-based remedies in modern veterinary practice (Alam *et al.* 2022; Abdallah *et al.* 2023). Despite these challenges, the potential benefits of validating and optimizing traditional plant-based treatments for livestock are substantial. In addition to providing affordable and accessible healthcare options for

animals, this research could lead to the discovery of novel bioactive compounds with applications in both veterinary and human medicine. It also offers an opportunity to document and preserve traditional ecological knowledge, contributing to the conservation of cultural heritage and biodiversity. This research's objective is to address the gap between traditional knowledge and scientific evidence by conducting a comprehensive phytochemical analysis and evaluation of the antioxidant potential of five medicinal plants commonly used in Kazakhstan for livestock treatment. By focusing on locally-sourced plants and employing standardized scientific methodologies, this research seeks to provide a foundation for the rational use of these plants in veterinary practice. This research's findings could inform the development of evidence-based herbal formulations, contribute to the sustainable use and conservation of medicinal plants, and potentially open new avenues for natural product research in the context of animal health.

MATERIALS AND METHODS

Plant material collection and preparation

Five medicinal plants commonly used in traditional veterinary practices in Kazakhstan were selected for this study: *Glycyrrhiza uralensis* (licorice), *Ephedra equisetina*, *Rhodiola rosea*, *Hypericum perforatum* (St. John's wort), and *Artemisia absinthium* (wormwood). Fresh samples of each plant were collected from local markets in Almaty, Nur-Sultan, and Shymkent between May and July 2023. The plant materials were identified and authenticated by a botanist at the Institute of Botany and Phytointroduction, Almaty. Voucher specimens (GU-2023-01, EE-2023-02, RR-2023-03, HP-2023-04, and AA-2023-05) were deposited in the herbarium of the institute. The collected plant materials were thoroughly washed with distilled water to remove any surface contaminants. Roots of *G. uralensis*, aerial parts of *E. equisetina* and *H. perforatum*, rhizomes of *R. rosea*, and leaves of *A. absinthium* experienced natural dehydration in a dim environment at 22 ± 2 °C for 14 days. Using a 40-mesh sieve, the samples, after being finely powdered with an electric grinder, were filtered in their dried state. The powdered materials were kept in sealed receptacles at 4 °C for future utilization.

Extraction procedure

The powdered plant materials (100 g each) were subjected to sequential extraction using solvents of increasing polarity: hexane, chloroform, ethyl acetate, and methanol. Each extraction was performed using a Soxhlet apparatus for 8 hours. The balance between the botanical substance and the dissolving agent was preserved at 1:10 (w/v). Using Whatman No. 1 filter paper, the extracts underwent filtration before being condensed at 40 °C via a rotary evaporator under lowered pressure. The concentrated extracts were then lyophilized using a freeze-dryer and stored at -20 °C until further analysis.

Phytochemical screening

The presence of principal secondary metabolites in all extracts was investigated through qualitative phytochemical tests, adhering to recognized procedures delineated by Harborne (1998) with slight modifications.

Alkaloids: Mayer's and Wagner's tests were performed. Alkaloid detection involved adding 2 mL of either Mayer's or Wagner's reagent to an equal volume of extract. Alkaloid presence was indicated by a cream-colored precipitate (Mayer's test) or a reddish-brown precipitate (Wagner's test). *Flavonoids*: The extract (2 mL) received a few drops of 20% NaOH. A vivid yellow color emerged, which upon introduction of diluted HCl, became colorless - indicating flavonoids. *Tannins*: To 2 mL of extract, a few drops of 1% ferric chloride solution were added. The formation of a blue-black or brownish-green color indicated the presence of tannins. *Saponins*: Saponins were detected by the presence of persistent froth after vigorously agitating equal volumes (5 mL each) of extract and distilled H_2O in a test tube for half a minute. *Terpenoids*: Terpenoids were detected when a reddish-brown hue appeared at the interface after carefully layering 3 mL of concentrated H_2SO_4 over a mixture of 2 mL extract and 2 mL $CHCl_3$.

Quantitative phytochemical analysis

Total phenolic content (TPC)

The Folin-Ciocalteu method, with slight modifications, was used to determine the TPC, as described by Lawag *et al.* (2023). The process involved combining diluted extract (0.5 mL) with 10% Folin-Ciocalteu reagent (2.5 mL), allowing a 5-minute reaction. Subsequently, 7.5% Na_2CO_3 solution (2 mL) was introduced. This mixture

underwent 2-hour dark incubation at ambient temperature. A UV-Vis spectrophotometer measured absorbance at 765 nm. Using gallic acid as a reference, results were presented as mg GAE/g dry extract.

Total flavonoid content (TFC)

The aluminium chloride colorimetric method, as detailed by Chang *et al.* (2002), was used to determine the TFC. The assay required the preparation of a solution containing 0.5 mL diluted extract, 1.5 mL methanol, 0.1 mL each of 10% $AlCl_3$ and 1 M potassium acetate, as well as distilled water (2.8 mL). At 415 nm after 30 minutes of incubation at room temperature, the absorbance was measured. The results, compared to quercetin, were presented as mg QE/g of dry extract.

Antioxidant activity assays

DPPH radical scavenging assay

The DPPH radical scavenging activity was evaluated according to the method of Brand-Williams *et al.* (1995) with slight modifications. Various concentrations of extracts (25-200 µg mL^{-1}) were prepared in methanol. A methanol solution of 0.1 mM DPPH was prepared fresh daily. The process involved blending equal volumes (2 mL each) of extract solution and DPPH solution. Following vortexing, the mixture underwent 30-minute dark incubation at ambient temperature. Absorbance readings were taken at 517 nm. DPPH radical scavenging percentage was determined using equation:

% DPPH scavenging = [(A control – A sample) / A control] × 100

In this equation, A control represents the absorbance reading of the DPPH solution lacking extract, while A sample denotes the absorbance measurement of DPPH solution containing extract. The IC_{50} value, representing the concentration of extract required to scavenge 50% of DPPH radicals, was calculated from the plotted graph of scavenging activity against extract concentration. Ascorbic acid was used as a positive control.

Ferric reducing antioxidant power (FRAP) assay

With certain adjustments, the FRAP assay followed the protocol outlined by Benzie & Strain (1996). The FRAP reagent composition included acetate buffer (300 mM, pH 3.6), TPTZ (10 mM) in HCl (40 mM), and $FeCl_3 \cdot 6H_2O$ solution (20 mM), combined at a volumetric ratio of 10:1:1. Daily preparation and pre-use heating to 37 °C were essential steps.

The process involved combining 1.5 mL FRAP reagent with 50 µL extract solution. After 30 minutes of dark incubation at 37 °C, absorbance readings were taken at 593 nm. $FeSO_4 \cdot 7\ H_2O$ solution was utilized to create a standard curve. Data were conveyed as µmol Fe(II) equivalents relative to one gram of dehydrated extract (µmol Fe(II)/g).

HPLC analysis

To detect and measure the most important phenolic compounds in the most potent extracts, HPLC analysis was conducted. An Agilent 1260 Infinity II HPLC system with a DAD was employed. The separation process utilized a Zorbax Eclipse XDB-C18 column (250 × 4.6 mm, 5 µm) kept at 30 °C. A dual-solvent mobile phase was utilized, consisting of (A) 0.1% formic acid in aqueous solution and (B) acetonitrile. The gradient elution protocol was set as: 0-5 min at 10% B; 5-15 min, linear increase to 30% B; 15-25 min, ramping to 50% B; 25-30 min, escalation to 80% B; 30-35 min, descent to 10% B; 35-40 min, and maintained at 10% B. The injection volume was 10 µL, with the flow rate set at 1 mL min^{-1}. The detection wavelengths were set at 280, 320, and 360 nm. The identification of phenolic constituents was achieved by matching their UV spectral profiles and retention times against those of verified reference standards. Quantification was performed using external calibration curves of the standards.

Statistical analysis

Statistical analysis was performed using SPSS version 26.0. All experiments were performed in triplicate. To identify significant differences among means, One-Way ANOVA was performed, followed by Tukey's post hoc analysis. The correlation between antioxidant activities, TPC, and TFC was evaluated using Pearson's correlation coefficient. Results were deemed statistically significant when the associated p-value was less than 0.05.

RESULTS

Phytochemical Screening

The qualitative phytochemical screening of the five medicinal plants revealed the presence of various secondary metabolites across different solvent extracts (Table 1). All plant extracts showed the presence of flavonoids and terpenoids, while alkaloids were detected in all plants except *Hypericum perforatum*. Tannins were present in all plants except *Ephedra equisetina*, while saponins were found in *Glycyrrhiza uralensis*, *Rhodiola rosea*, and *Artemisia absinthium*.

Table 1. Qualitative phytochemical screening of medicinal plant extracts

Plant Species	Alkaloids	Flavonoids	Tannins	Saponins	Terpenoids
G. uralensis	+	+	+	+	+
E. equisetina	+	+	-	-	+
R. rosea	+	+	+	+	+
H. perforatum	-	+	+	-	+
A. absinthium	+	+	+	+	+

Note: +: Present, -: Absent.

The phytochemical screening results indicated that all five medicinal plants contain a diverse array of secondary metabolites. The ubiquitous presence of flavonoids and terpenoids across all plant species suggests their potential contribution to the plants' therapeutic properties. The absence of alkaloids in *H. perforatum* and the lack of tannins in *E. equisetina* highlight the unique phytochemical profiles of these species.

TPC and TFC

Methanolic extractions from five medicinal botanical sources were analysed for their TPC and TFC, with results compiled in Table 2. Among the studied plants, *G. uralensis* exhibited the highest TPC (82.7 ± 3.5 mg GAE/g), followed closely by *R. rosea* (75.9 ± 2.8 mg GAE/g). *A. absinthium* showed the lowest TPC (45.6 ± 2.1 mg GAE/g). For TFC, *G. uralensis* again demonstrated the highest content (45.3 ± 1.9 mg QE/g), while *E. equisetina* the lowest (28.7 ± 1.4 mg QE/g).

Table 2. Total phenolic and flavonoid content of methanol extracts.

Plant Species	TPC (mg GAE/g)	TFC (mg QE/g)
G. uralensis	82.7 ± 3.5	45.3 ± 1.9
E. equisetina	58.4 ± 2.3	28.7 ± 1.4
R. rosea	75.9 ± 2.8	40.2 ± 1.7
H. perforatum	69.3 ± 2.6	36.8 ± 1.5
A. absinthium	45.6 ± 2.1	31.5 ± 1.3

Note: Values are expressed as mean ± SD (n = 3). GAE: Gallic Acid Equivalents, QE: Quercetin Equivalents.

The results demonstrated considerable variation in phenolic and flavonoid content among the studied plants. *G. uralensis* and *R. rosea* consistently showed higher levels of both phenolics and flavonoids, suggesting their potential as rich sources of these bioactive compounds. The relatively lower TPC and TFC observed in *A. absinthium* and *E. equisetina* indicate species-specific differences in secondary metabolite accumulation.

Antioxidant activity

DPPH radical scavenging activity

Fig. 1 displays the IC_{50} values representing the DPPH radical scavenging capacity of the methanol extracts, which was assessed in this study. Lower IC_{50} values indicate higher antioxidant activity. *G. uralensis* exhibited the strongest DPPH radical scavenging activity with an IC_{50} of 42.8 ± 1.7 μg mL^{-1}, followed closely by *R. rosea* (50.3 ± 2.1 μg mL^{-1}). *E. equisetina* displayed the weakest activity with an IC^{50} of 89.5 ± 3.6 μg mL^{-1}. The positive control, ascorbic acid, demonstrated an IC_{50} of 5.2 ± 0.2 μg mL^{-1}. The DPPH assay results reveal significant differences in the free radical scavenging potential of the studied plant extracts. *G. uralensis* and *R. rosea* demonstrated superior antioxidant activity, which correlates with their higher phenolic and flavonoid contents. While all plant extracts showed antioxidant activity, none matched the potency of ascorbic acid, highlighting the strong antioxidant properties of this vitamin.

FRAP

Fig. 2 illustrates the outcomes of evaluating the ferric reducing antioxidant capacity of the methanolic extracts. *G. uralensis* showed the highest FRAP value (1285.6 ± 52.3 μmol Fe(II)/g), followed by *R. rosea* (1147.2 ± 47.9 μmol Fe(II)/g), while *A. absinthium* showed the lowest FRAP value (689.5 ± 28.7 μmol Fe(II)/g).

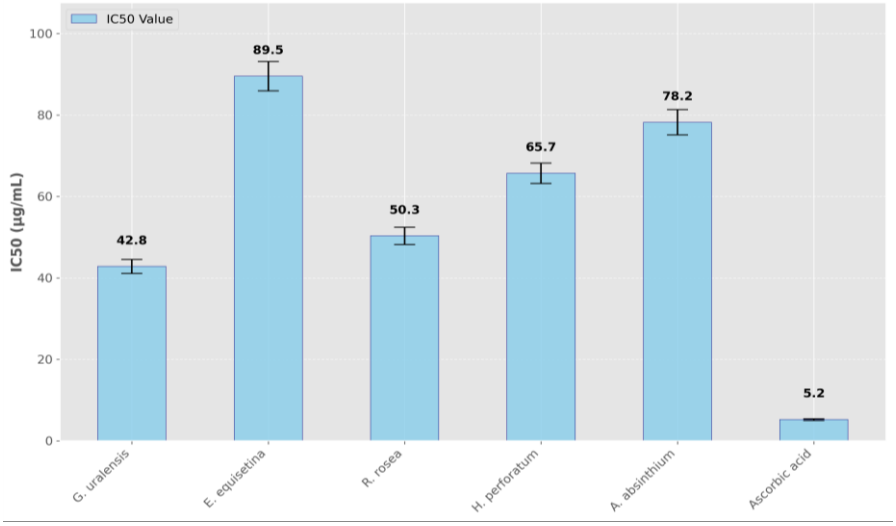

Fig. 1. DPPH radical scavenging activity (IC_{50}) of methanol extracts.

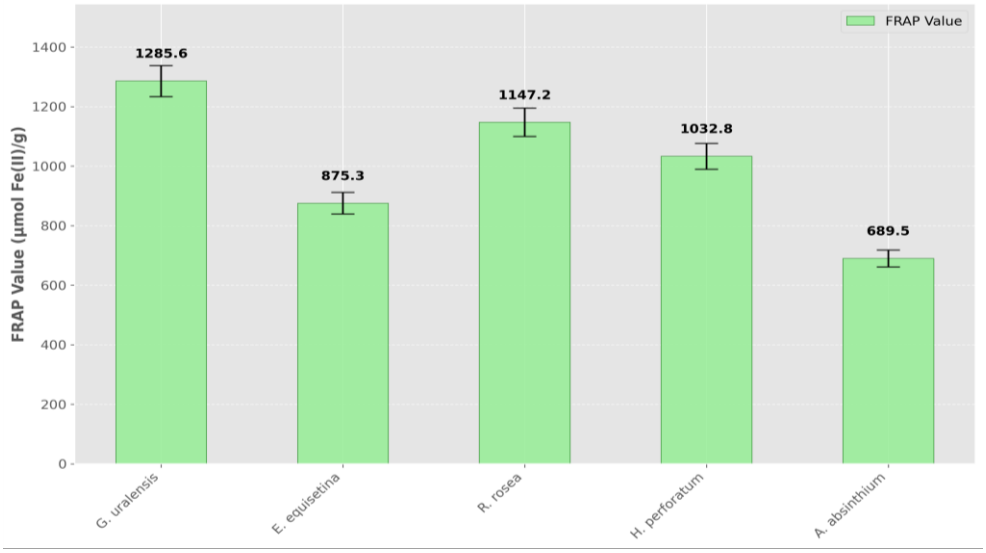

Fig. 2. Ferric reducing antioxidant power (FRAP) of methanol extracts.

The FRAP assay results corroborate the findings from the DPPH assay, with *G. uralensis* and *R. rosea* demonstrating the highest antioxidant capacity. The consistent performance of these two species across different antioxidant assays suggests their potential as rich sources of natural antioxidants. The lower FRAP values observed for *A. absinthium* align with its lower phenolic and flavonoid contents.

Correlation analysis

To understand the relationship between the phytochemical content and antioxidant activities, Pearson's correlation analysis was performed (Table 3). Strong positive correlations were observed between TPC and DPPH radical scavenging activity ($r = 0.953$, $p < 0.01$), and between TPC and FRAP ($r = 0.978$, $p < 0.01$). TFC also showed significant positive correlations with both antioxidant assays, but to a slightly lesser extent than TPC.

Table 3. Pearson's correlation coefficients between phytochemical content and antioxidant activities.

Correlation	TPC	TFC	DPPH	FRAP
TPC	1.000			
TFC	0.968*	1.000		
DPPH	0.953*	0.921*	1.000	
FRAP	0.978*	0.945*	0.967*	1.000

Note: Correlation is significant at the 0.01 level (2-tailed).

The strong correlations between phenolic content and antioxidant activities indicate that the noted antioxidant effects of the plant extracts are primarily attributable to phenolic constituents. The slightly weaker correlations

for flavonoids indicate that while they contribute significantly to antioxidant activity, other phenolic compounds may also play important roles.

HPLC Analysis

HPLC analysis was performed on the methanol extracts of *G. uralensis* and *R. rosea*, which showed the highest antioxidant activities. The major phenolic compounds identified and quantified are presented in Table 4.

Table 4. Major phenolic compounds identified in *G. uralensis* and *R. rosea* methanol extracts.

Compound	*G. uralensis* (mg g^{-1})	*R. rosea* (mg g^{-1})
Gallic acid	3.85 ± 0.17	2.73 ± 0.12
Chlorogenic acid	7.62 ± 0.31	12.45 ± 0.52
Caffeic acid	2.14 ± 0.09	1.87 ± 0.08
Rutin	15.73 ± 0.65	8.96 ± 0.37
Quercetin	5.28 ± 0.22	3.61 ± 0.15
Kaempferol	2.97 ± 0.12	1.54 ± 0.06

Note: Values are expressed as mean ± SD (n = 3).

The HPLC analysis revealed distinct phenolic profiles for *G. uralensis* and *R. rosea*. The former showed higher concentrations of rutin, quercetin, and kaempferol, that are famous for their strong antioxidant activity. The latter, on the other hand, contained a notably high amount of chlorogenic acid. These differences in phenolic composition may contribute to the specific antioxidant characteristics and potential therapeutic properties of each plant.

DISCUSSION

The present study provides a comprehensive analysis of the phytochemical composition and antioxidant properties of five medicinal plants traditionally used in Kazakhstan for livestock treatment: *Glycyrrhiza uralensis*, *Ephedra equisetina*, *Rhodiola rosea*, *Hypericum perforatum* and *Artemisia absinthium*. Our findings reveal significant variations in the bioactive compound content and antioxidant potential among these plants, with *G. uralensis* and *R. rosea* consistently demonstrating superior antioxidant activities. These results not only provide scientific support for the traditional use of these plants in veterinary practices, but also highlight their potential as sources of natural antioxidants for broader applications. The phytochemical screening revealed the presence of various secondary metabolites across all plant extracts, with flavonoids and terpenoids being ubiquitous. This diversity in phytochemical profiles is consistent with the complex nature of plant-based remedies and suggests that the therapeutic effects of these plants may result from the synergistic action of multiple compounds rather than a single active ingredient. The presence of alkaloids in all plants except *H. perforatum* is particularly noteworthy, as alkaloids are known for their diverse pharmacological activities, including anti-inflammatory and antimicrobial properties (Vincent *et al.* 2021). The absence of alkaloids in *H. perforatum* aligns with previous studies on this species and may contribute to its unique medicinal properties (Rizzo *et al.* 2020). Quantitative analysis of TPC and TFC content revealed *G. uralensis* and *R. rosea* as the richest sources of these compounds among the studied plants. The high TPC values observed for *G. uralensis* (82.7 ± 3.5 mg GAE/g) and *R. rosea* (75.9 ± 2.8 mg GAE/g) are comparable to or higher than those reported for other medicinal plants known for their antioxidant properties. For instance, Le Phuong Ha *et al.* (2022) demonstrated TPC values spanning from 1.3 to 92.6 mg GAE/g for various Indian medicinal plants, placing our findings at the upper end of this range. The relatively lower TPC and TFC values observed in *A. absinthium* and *E. equisetina* suggest that while these plants may possess valuable medicinal properties, their therapeutic effects may rely on mechanisms other than direct antioxidant activity. The antioxidant assays (DPPH and FRAP) corroborated the trends observed in phenolic content, with *G. uralensis* and *R. rosea* demonstrating the most potent antioxidant activities. The robust positive associations among TPC, TFC and antioxidant efficacy ($r > 0.92$, $p < 0.01$) suggest that phenolic compounds, including flavonoids, are major contributors to the observed antioxidant properties. This relationship has been widely reported in the literature (Suleria *et al.* 2020; Muflihah *et al.* 2021; Speisky *et al.* 2022) and underscores the importance of these compounds in the medicinal properties of plants. The DPPH radical scavenging activity of *G. uralensis* (IC_{50} = 42.8 ± 1.7 μg mL^{-1}) and *R. rosea* (IC_{50} = 50.3 ± 2.1 μg mL^{-1}) is particularly impressive when compared to other medicinal plants. For instance, Soheili *et al.* (2023) reported IC_{50} values spanning from 23.6 to 352.0 μg mL^{-1} for various Iranian medicinal plants, placing our findings among the more potent antioxidants. Although, noteworthy, these plant extracts showed strong antioxidant activity, they did not match the potency of ascorbic acid (IC_{50} = 5.2 ± 0.2 μg mL^{-1}), a known powerful antioxidant. This comparison highlights the potential of these plant extracts as natural antioxidant sources, while also setting realistic expectations for their potency. The HPLC analysis of *G. uralensis*

and *R. rosea* provided valuable insights into the specific phenolic compounds contributing to their antioxidant properties. The high concentrations of rutin, quercetin, and kaempferol in *G. uralensis* align with previous studies on this species (Liu *et al.* 2022) and may explain its superior antioxidant activity. The notable presence of chlorogenic acid in *R. rosea* is consistent with other reports (Polumackanycz *et al.* 2022) and may contribute to its adaptogenic properties. These findings not only help explain the observed antioxidant activities, but also provide a basis for standardization of herbal preparations from these plants. The strong antioxidant properties demonstrated by *G. uralensis* and *R. rosea* have significant implications for their use in veterinary medicine. Oxidative stress plays a crucial role in various livestock diseases, including mastitis, respiratory disorders, and heat stress (Lykkesfeldt & Svendsen 2007). The potent antioxidant activities of these plant extracts suggest that they could potentially mitigate oxidative damage in animals, supporting overall health and disease resistance. Moreover, the high phenolic content of these plants may contribute to other positive effects, like anti-inflammatory and immunomodulatory activities, which are relevant to animal health (May *et al.* 2023). While our study provides valuable insights into the antioxidant and phytochemical properties of these traditional medicinal plants, it is important to acknowledge its limitations. Firstly, our analysis focused on methanol extracts, which may not fully represent the compounds extracted through traditional preparation methods. Future studies should compare different extraction techniques to better mimic traditional uses. Secondly, while *in vitro* antioxidant assays provide useful preliminary data, they may not accurately reflect the *in vivo* efficacy of these extracts. Animal researches are required to confirm the biological relevance of our results and to assess the safety and efficacy of these plant extracts in livestock. Additionally, our study was limited to five plant species, which, while important in Kazakhstani traditional veterinary medicine, represent only a fraction of the plants used. A more comprehensive survey of medicinal plants used in the region could provide a broader understanding of the phytochemical resources available. Furthermore, seasonal and geographical variations in plant composition were not addressed in this study. Future research should consider these factors to ensure consistent quality and efficacy of plant-based preparations. Another limitation is the focus on antioxidant properties, which, while important, may not encompass all the potential therapeutic effects of these plants. Future studies should explore other biological activities, such as antimicrobial, anti-inflammatory, and immunomodulatory effects, to provide a more comprehensive understanding of their medicinal value.

CONCLUSION

This comprehensive study on the phytochemical composition and antioxidant properties of five medicinal plants traditionally used in Kazakhstan for livestock treatment has yielded valuable insights into their potential therapeutic value. Our findings provide strong scientific support for the traditional use of these plants, particularly *Glycyrrhiza uralensis* and *Rhodiola rosea*, in veterinary practices. The phytochemical screening revealed a diverse array of secondary metabolites across all plant extracts, with flavonoids and terpenoids being ubiquitous. This diversity underscores the complex nature of plant-based remedies and suggests that their therapeutic effects may result from the synergistic action of multiple compounds. The quantitative analysis of TPC and TFC identified *G. uralensis* and *R. rosea* as exceptionally rich sources of these bioactive compounds, with values comparable to or exceeding those of many other known medicinal plants. The antioxidant assays (DPPH and FRAP) corroborated these findings, with *G. uralensis* and *R. rosea* demonstrating potent antioxidant activities. The strong positive correlations between phenolic content and antioxidant activities ($r > 0.95$, $p < 0.01$) highlight the crucial role of these compounds in the plants' medicinal properties. The HPLC analysis of *G. uralensis* and *R. rosea* provided further insights into their specific phenolic profiles, revealing high concentrations of compounds like rutin, quercetin, and chlorogenic acid, that are famous for their strong antioxidant and other beneficial features. These findings have significant implications for veterinary medicine and animal health. The potent antioxidant properties of *G. uralensis* and *R. rosea* suggest their potential in mitigating oxidative stress-related conditions in livestock, such as mastitis, respiratory disorders, and heat stress. Moreover, the high phenolic content of these plants may contribute to other beneficial effects, including anti-inflammatory and immunomodulatory activities, which are crucial for maintaining animal health and productivity. Our study also contributes to the broader field of ethnopharmacology by providing scientific validation for traditional knowledge. It demonstrates the value of investigating traditional remedies as potential sources of new therapeutics or as complementary treatments to conventional veterinary medicine. The identification of specific bioactive compounds in these plants paves the

way for the development of standardized herbal preparations, ensuring consistent quality and efficacy in veterinary applications.

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