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PHYTOCHEMICAL EVALUATION AND ANTIOXIDANT CAPACITY OF PLANT EXTRACTS AND COMPLEX POLYHERBAL FORMULATIONS FOR VETERINARY APPLICATIONS

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The development of effective feed additives with various types of action based on plant materials is a promising field, as plant extracts contain metabolites that act in a complex way.

Aim. To screen for metabolites, quantify the contents of polyphenolic compounds, flavonoids, and amino acids in plant extracts and complex polyherbal formulations, and evaluate their antioxidant activity.

Materials and Methods. Qualitative chemical reactions were used to identify metabolites. The contents of polyphenols, flavonoids, and amino acids were determined spectrophotometrically using standard methods. Antioxidant activity was evaluated using the phosphomolybdenum method, while the ferric-reducing capacity of the studied samples was determined. Statistical analysis and calculation of the Pearson correlation coefficient (r) were performed using Microsoft Excel.

Results. Qualitative analysis confirmed the presence of polyphenolic compounds, flavonoids, tannins, and amino acids in the studied samples. The highest polyphenol content was found in the *Salvia officinalis* extracts (2.1466 ± 0.2594 and 1.3936 ± 0.1468 mg/mL in the ethanolic and aqueous extracts, respectively), while high flavonoid contents were detected in the ethanolic extracts of *Salvia officinalis* (2.9626 ± 0.1676 µg/mL) and *Calendula officinalis* (3.4179 ± 0.0430 µg/mL). A high amino acid concentration was recorded in the ethanolic extract of *Valeriana officinalis* (0.4318 ± 0.0305 mg Gly Eq./mL). The ethanolic extract of *Salvia officinalis* demonstrated the highest total antioxidant capacity (1.0906 ± 0.0069 mg AA Eq./mL), whereas its aqueous extract exhibited the highest ferric-reducing power (2.2231 ± 0.0596 mg AA Eq./mL). Correlation analysis revealed a strong positive relationship between the concentration of polyphenolic compounds and the antioxidant activity of the samples.

Conclusions. The studied extracts demonstrate considerable therapeutic potential and may be used as functional components in the development of feed additives for animals. The manufacturing conditions of complex.

Keywords: feed additive, extracts, complex herbal preparation, polyphenols, antioxidant activity.

Today, there is a growing demand for feed additives that improve the metabolism and health of farm and companion animals [1]. According to Ukrainian legislation, feed additives are intended to exert beneficial effects on animal welfare, particularly by influencing the gastrointestinal microbiota and feed digestibility [2]. Schmid et al. reported that approximately 30% of dogs have experienced a gastrointestinal disorder at least once [3], while the prevalence of gastrointestinal disorders in cats increases with age, reaching up to 4.2% [4]. Excessive production of reactive oxygen species leads to oxidative stress, which is a key factor in the development of inflammatory processes in the gastrointestinal tract [5].

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To prevent gastrointestinal disorders in animals and improve feed digestibility, the use of natural additives is warranted. Natural feed additives based on plant-derived materials have been recognized as a promising alternative to conventional drugs in veterinary medicine because of their potential for multiple biological effects [6]. Plant extracts contain metabolites with antifungal, antioxidant, antimicrobial, anti-inflammatory, antiviral, and immunomodulatory activities and can improve nutrient digestibility (Table 1) [1, 7–18].

Natural feed additives with antibacterial activity are associated with a lower risk of resistance development, which represents a significant advantage [6]. Triterpenoids from *Calendula officinalis*, used as feed additives for poultry, have been shown to enhance nutrient absorption and improve gut health [9]. *Silybum marianum* extracts contain silymarin, a complex of polyphenolic compounds that includes flavonolignans such as silychristin, silydianin, silybin, and isosilybin [15]. Silymarin is used to manage acute and chronic hepatic disorders in dogs and cats at a dose of 20–50 mg/kg per day [16]. *Valeriana officinalis* root extracts are used in feed additives to improve palatability and reduce stress in animals [17].

Plant-derived raw materials (Fig. 1) are widely used in functional animal feeds to address digestive disorders (e.g., *C. officinalis* in wet and dry foods marketed by Carnilove, LaVital, Lider Petfood, and VetLife) and support urinary tract health (*Salvia officinalis* and *S. marianum* in Happy Cat feeds). Commercial feed additives often contain single plant extracts or binary combinations intended to support liver function (*Artemisia absinthium* and *S. marianum* in products by Doctor By, Novoform Pharma GmbH, and Animal Essentials), improve digestion and immune function (*C. officinalis* extract by Hawaii Pharm; *Althaea officinalis* extracts by Animal Essentials and Karnlea), exert anti-inflammatory effects and support skin and coat health (*Bidens tripartita* extract in AnimAll

Table 1. **Metabolite contents and biological activities of plant extracts [1, 7–18]**

Plant Raw Material	Metabolites	Biological Activity	Refs.
Bidens tripartita L.	Flavonoids, phenolic acids, hydroxycoumarins, sterols, essential oils, tridecane-derived polyacetylenes, tannins, and triterpenes	Anti-inflammatory, antioxidant, antimicrobial, immunomodulatory, blood-purifying, antiulcer, antispasmodic, and hemostatic activities.	7, 8
Calendula officinalis L.	Saponins, flavonoids, phenolic acids, coumarins, quinones, carotenoids, peptides, sesquiterpenoids, monoterpenes, triterpenic alcohols, and vitamin C	Anti-inflammatory, hepatoprotective, antioxidant, immunostimulatory, nephroprotective, gastroprotective, antiparasitic, antibacterial, antifungal, and antiviral activities	9, 10
Althaea officinalis L.	Peptides, starch, flavonoids, tannins, coumarins, mucilage, scopoletin, tannins	Antimicrobial, anti-inflammatory, antioxidant, gastroprotective, immunomodulatory, antibacterial, antifungal, anti-mycobacterial	11
Salvia officinalis L.	Phenolic acids, flavonoids, terpenoids, alkaloids, sulphur compounds	Antioxidant, anti-inflammatory, antibacterial, antifungal, anticancer, antimutagenic, antidiabetic, metabolic effects	12
Artemisia absinthium L.	Sesquiterpene lactones, flavonoids, coumarins, phenolic acids, terpenoids	Antiparasitic, antioxidant, hepatoprotective, gastroprotective, immunostimulating, neuroprotective, antidepressant, digestion-stimulating	13
Silybum marianum L.	Flavonoids, essential oils, vitamins.	Hepatoprotective, antioxidant, antimicrobial, anti-inflammatory, immunomodulating, anti-tumour, cardiovascular-protective, neuroprotective, anti-diabetes.	14–16
Valeriana officinalis L.	Flavonoids, alkaloids, terpenes, organic acids, iridoids.	Hypnotic, sedative.	17, 18

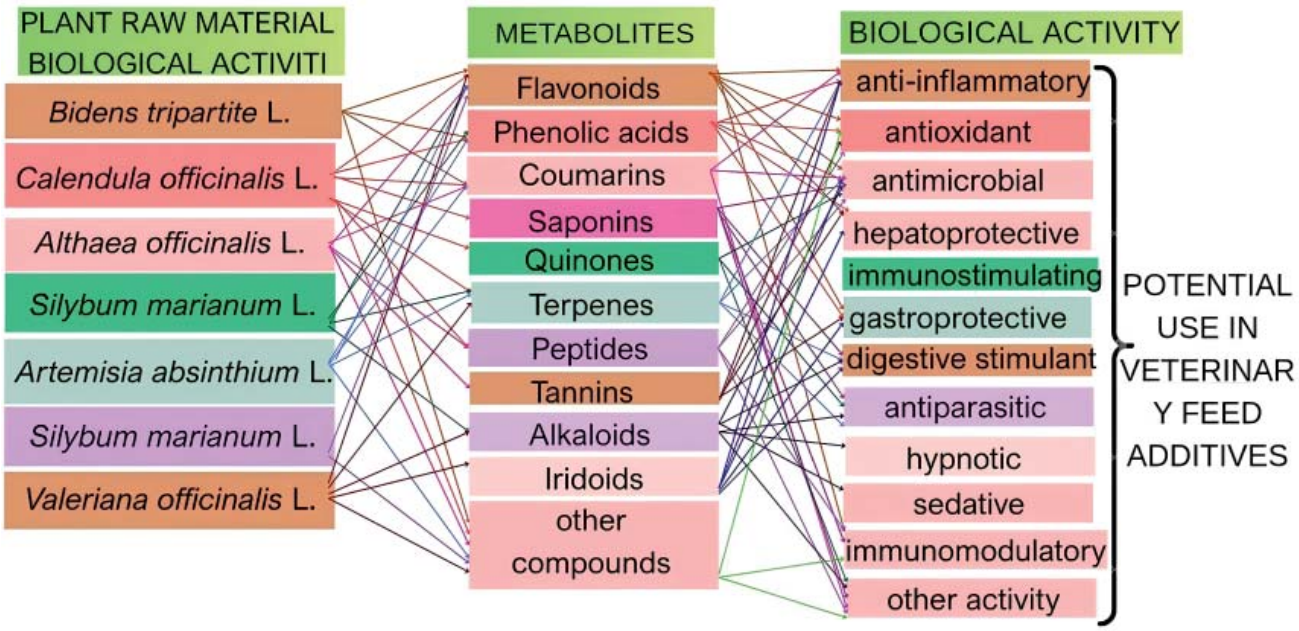

Fig. 1. **Metabolite content and biological activity of plant extracts [1, 7–18]**

Anti-itch and Superium products), or produce sedative effects (*V. officinalis* in Dorwest and Helpet products). The widespread use of these plants in veterinary products provides a basis for their consideration in the development of multifunctional feed additives.

Some plant extracts have limited applications in veterinary medicine because it is difficult to achieve effective doses without causing adverse effects. In such cases, feed supplements containing combinations of extracts with complementary effects can be used. *C. officinalis*, *A. officinalis*, *S. marianum*, *Crataegus monogyna* Jacq., *V. officinalis*, *Echinacea purpurea* L., *Mentha piperita* L., and *Melissa officinalis* L. are considered safe, whereas the use of *B. tripartita*, *A. absinthium*, and *S. officinalis* is subject to certain restrictions [19]. To minimize the risks associated with the potential toxicity of metabolites present in plant extracts, these extracts can be incorporated into complex feed additives at safe doses. Combining extracts from several plants may promote synergistic effects and, consequently, allow the concentration of restricted plant extracts to be reduced without compromising the overall biological activity of the formulation.

Despite the available data on the content of specific compounds in plant raw materials, a comprehensive assessment of key metabolite groups and their total antioxidant capacity is still needed to substantiate the composition of new phytoadditives.

The aim of this study was to qualitatively and quantitatively characterize key metabolites in plant extracts and complex polyherbal formulations and to evaluate their antioxidant activity as a basis for the development of functional veterinary feed additives.

Materials and Methods

Plant Materials and Formulations

The study utilized dried, commercially available plant raw materials, including *Bidens tripartita* herb, *Calendula officinalis* flowers, *Artemisia absinthium* herb (manufacturer: Ecocode, Ukraine), *Althaea officinalis* roots (manufacturer: Liktravy, Ukraine), *Salvia officinalis* leaves (manufacturer: Ternofarm, Ukraine), *Silybum marianum* meal, and *Valeriana officinalis* roots (manufacturer: Dary Karpat, Ukraine).

Ready-to-use complex polyherbal formulations in liquid and paste forms were also studied. The liquid polyherbal formulation was prepared by evaporating ethanol from extracts of *B. tripartita*, *C. officinalis*, *A. absinthium*, *A. officinalis*, *S. officinalis*, *Echinacea purpurea*, and *Zingiber officinale* L. in a water bath at 40 ± 1 °C until complete removal of the ethanol. The concentrated extracts were then mixed in predetermined ratios, and apple cider vinegar was added.

The polyherbal paste formulation was prepared by mixing *S. marianum* seed powder (Poland), dry *V. officinalis* extract (Germany),

Rosa canina L. fruit powder (Germany), *Z. officinale* root powder, and *Cucurbita pepo* L. fruit powder. The pumpkin and ginger raw materials were previously ground and dried in a thermostat at 40 °C for 10 days. The resulting mixture was supplemented with unfiltered apple cider vinegar, *S. marianum* oil, and a concentrated ethanol extract of *E. purpurea*.

Extract Preparation

To prepare the ethanol extracts, 40% ethanol was added to the plant materials without additional grinding at a raw material-to-solvent ratio of 1:10 (w/v). The samples were macerated for 30 days in the dark with periodic shaking. After extraction, the mixtures were filtered through paper filters.

Fresh aqueous extracts were prepared by adding distilled water to the plant materials at a raw material-to-water ratio of 1:10 (w/v). The mixtures were brought to a boil, allowed to cool completely to room temperature, and then filtered through paper filters.

An aqueous extract of the paste formulation was also prepared. Briefly, 2 g of the paste formulation was suspended in 20 mL of distilled water and shaken at 37 ± 1 °C for 40 min at 150 rpm.

The liquid complex polyherbal formulation was analyzed in its original form.

Qualitative Phytochemical Screening

To identify the key classes of metabolites in the extracts, qualitative phytochemical screening was performed according to standard methods.

Polyphenolic compounds:

• **Ferric chloride reaction (PC1):** A freshly prepared 3% ferric chloride solution (1–2 drops) was added to 5 drops of the extract. The appearance of a green or blue-green color indicated the presence of phenolic compounds [20].

• **Potassium ferricyanide test (PC2):** A freshly prepared mixture of equal volumes of 1% ferric chloride and 1% potassium ferricyanide solutions (3–5 drops) was added to 1 mL of the extract. The solution turned deep blue, indicating the presence of phenolic compounds [21].

Flavonoids:

• **Lead acetate reaction (F1):** A 10% lead acetate solution (3–5 drops) was added to 1 mL of the extract. The formation of a bright yellow or brown precipitate indicated the presence of flavonoids [20].

• **Ammonia test (F2):** 1 mL of the extract was mixed with 1 mL of 1% dilute ammonia solution, followed by the addition of concentrated sulfuric acid. The development of a yellow color indicated the presence of flavonoids [20].

• **Alkaline reagent test (F3):** A 5% sodium hydroxide solution (2 drops) was added to 1 mL of the extract. A yellow color developed and disappeared after the addition of a few drops of dilute acid, confirming the presence of flavonoids [20].

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Quantitative Metabolite Determination

• *Quantitative Determination of Polyphenolic Compounds*

• The total polyphenolic content was determined spectrophotometrically using an APEL PD-307 spectrophotometer and the Folin–Ciocalteu reagent. The results were expressed as gallic acid equivalents (GAE). Briefly, 1.58 mL of distilled water and 100 μL

of the Folin–Ciocalteu reagent were added to 20 μL of the extract, and the mixture was incubated in the dark for 5 min. Subsequently, 300 μL of a 7% aqueous Na_2CO_3 solution was added. The mixture was incubated in the dark for 2 h at room temperature. Absorbance was measured at 760 nm. A calibration curve was constructed under the same conditions using solutions of gallic acid (pharmaceutical grade, HLR, Ukraine) at known concentrations to determine the total phenolic content of the extracts [22].

Quantitative Determination of Flavonoids

• The total flavonoid content was determined spectrophotometrically using an APEL PD-307 spectrophotometer and an aluminum chloride solution. The results were expressed as rutin equivalents. Briefly, 0.2 mL of a 3% aluminum chloride solution, 0.01 mL of diluted acetic acid, and 1.09 mL of 96% ethanol were added to 0.2 mL of the extract. The mixture was incubated in the dark for 40 min. Absorbance was measured at 420 nm. In parallel, the absorbance of a rutin solution (pharmaceutical grade, HLR, Ukraine) at a known concentration was determined under identical conditions.

• The flavonoid content (C, mg/mL) in the analyzed extracts was calculated using the following formula:

$$X = \frac{A_1 \times m_0 \times 0.2 \times 2.5}{A_0 \times 50 \times 2.5 \times 2} \quad \text{mg/mL}, \quad (1)$$

where is the optical density of the extract; is the optical density of the rutin solution; is the mass of rutin (g) [22].

Quantitative Determination of Amino Acids

The total amino acid content was determined spectrophotometrically using an APEL PD-307 spectrophotometer and the ninhydrin method. The results were expressed as glycine equivalents (Gly Eq.). Briefly, 0.11 mL of freshly prepared 0.2% ninhydrin solution was added to 0.1 mL of the extract, and the mixture was heated for 20 min in a boiling water bath. After cooling to room temperature, the volume was adjusted to 10 mL with distilled water, and the solution was mixed thoroughly. After 1 h, the absorbance of the test and reference solutions was measured against a blank at 567 ± 2 nm. The amino acid content (X, mg Gly Eq./mL) in the extracts was calculated using the following formula:

$$X = \frac{A_x \times m_0}{A_0 \times m} \times 1000 \text{ mg Gly Eq./mL} \quad (2),$$

where is the optical density of the test solution; is the optical density of the standard glycine sample; is the mass of the standard glycine sample (g); is the mass of the extract taken for analysis (g) [23].

Antioxidant Activity Determination

Total Antioxidant Capacity (TAC)

Total antioxidant capacity was determined spectrophotometrically using an APEL PD-307 spectrophotometer and the phosphomolybdenum method. Briefly, 1 mL of a reagent solution containing equal volumes of 0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate was added to 0.1 mL of the extract. The mixtures were incubated at 95 °C for 90 min. After cooling, the absorbance of the solutions was measured at 695 nm. In parallel, the absorbance of an ascorbic acid solution at a known concentration was determined under identical conditions.

Total antioxidant capacity (mg AA/mL) was calculated using the following formula:

$$\text{TAC} = \frac{A \times C_{AA}}{A_{AA}}, \quad \text{mg AA/mL} \quad (3),$$

where A is the optical density of the test solution; is the optical density of the reference ascorbic acid solution; is the concentration of the ascorbic acid solution (mg/mL) [24].

Reducing power was determined spectrophotometrically using an APEL PD-307 spectrophotometer. Briefly, 2.5 mL of phosphate buffer (0.2 M, pH 6.6) and 2.5 mL of a 1% potassium ferricyanide solution were added to 0.5 mL of the extract. The mixture was incubated at 50 °C for 30 min. Subsequently, 2.5 mL of 10% trichloroacetic acid was added, and the resulting mixture was centrifuged at 3000 rpm for 10 min. Then, 2.5 mL of distilled water and 0.5 mL of freshly prepared 0.1% ferric chloride solution were added to 2.5 mL of the supernatant. After thorough mixing, the absorbance was measured at 700 nm. In parallel, the absorbance of an ascorbic acid solution at a known concentration was determined under identical conditions.

The reducing power was calculated using the following formula:

$$\text{RPA} = \frac{A \times C_{AA}}{A_{AA}}, \quad \text{mg AA/mL} \quad (4),$$

where A is the optical density of the test solution; is the optical density of the reference ascorbic acid solution; is the concentration of the ascorbic acid solution (mg/mL) [25].

Statistical Analysis

All experiments were performed in triplicate. The data were statistically analyzed using Microsoft Excel. The results are presented as the mean ± standard error (SE). Pearson's correlation analysis was used to assess the relationship between metabolite content (polyphenolic compounds and flavonoids) and the antioxidant activity of the extracts.

Results and Discussion

Phytochemical screening was performed using qualitative reactions to detect polyphenolic compounds, including flavonoids and tannins, as well as amino acids in ethanolic and aqueous extracts and in complex polyherbal formulations in liquid and paste forms (Table 2). Polyphenolic compounds were detected in all samples. Flavonoids were not detected in the paste formulation, while weakly positive reactions were observed for both ethanolic and aqueous extracts of *Valeriana officinalis*. Tannins were detected only in extracts of *Artemisia absinthium*, *Althaea officinalis*, *Salvia officinalis*, and *Silybum marianum*, as well as in the paste formulation. Although tannins are not the predominant group of active metabolites in these extracts, they may contribute to the overall biological activity of the feed additives. Amino acids were more effectively extracted from the plant materials using water, as indicated by positive ninhydrin reactions in all aqueous extracts. Amino acids were also detected in the ethanolic extracts of *S. marianum* and *V. officinalis*. Amino acids were not detected in the liquid polyherbal formulation, which may be related to its manufacturing process involving ethanol extraction followed by solvent evaporation.

The absence of flavonoids in the paste complex polyherbal formulation may be related to the limited solubility or incomplete release of these metabolites under the aqueous extraction conditions used. Currently, data on the gastrointestinal transit time of feed and drugs in cats remain limited, making it difficult to assume that the oral absorption kinetics of metabolites are comparable between cats and humans. According to Telles et al., the median total transit time of feed additives and drugs through the feline gastrointestinal tract when administered with food is 2,795 min, including 1,068 min in the stomach and 1,534 min in the intestine [26].

Therefore, further studies of the paste complex polyherbal formulation should focus on the release and availability of metabolites throughout gastrointestinal transit in cats, taking into account the specific electrolyte composition and pH conditions at each stage.

The highest concentration of polyphenolic compounds was found in *S. officinalis* extracts, reaching 2.1466 ± 0.2594 mg/mL and 1.3936 ± 0.1468 mg/mL in the ethanolic and aqueous extracts, respectively (Fig. 2, ***a***). For most of the studied plants, 40% ethanol was a more effective extractant than water, which may be related to differences in solvent polarity and extraction duration. Hbika et al. reported that solvents of intermediate polarity are the most effective for extracting polyphenols and flavonoids, whereas highly polar solvents such as water are more suitable for tannin extraction [27].

The only exception in the present study was *A. absinthium*, for which a higher polyphenolic content was recorded in the aqueous extract. According to the phytochemical screening results (Table 2), tannins were detected exclusively in the aqueous extract of *A. absinthium*, suggesting that they may constitute a substantial proportion of the total polyphenolic content in this sample.

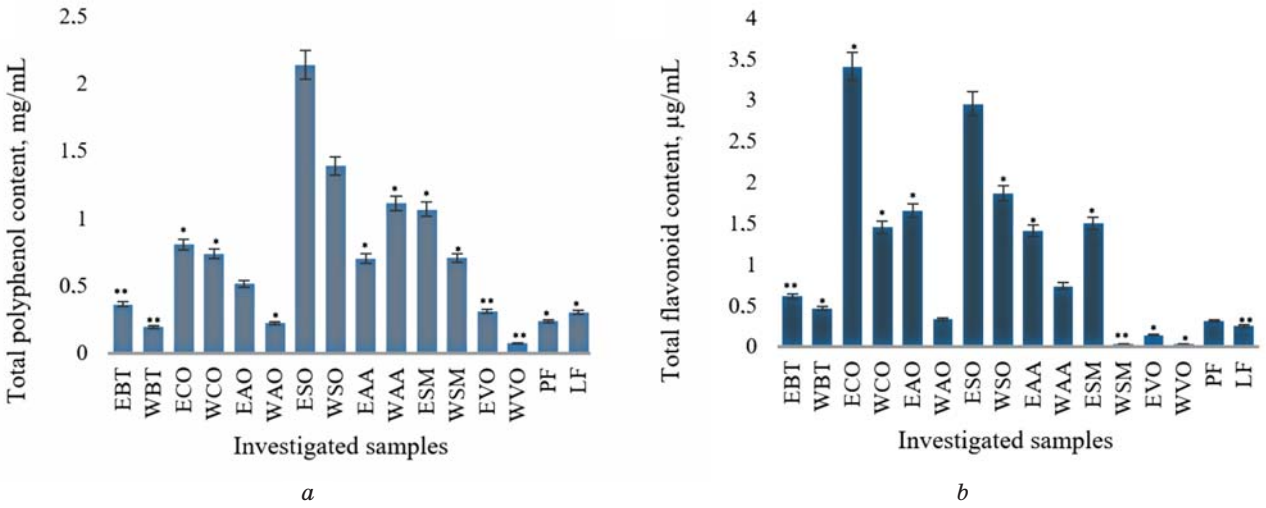
The lowest polyphenolic content was observed in the aqueous extracts of *B. tripartita*, *A. officinalis*, and *V. officinalis*, as well as in the extract of the paste complex polyherbal formulation. As noted above, the low metabolite content in the aqueous extract of the paste formulation may be associated with the relatively short extraction time and the high fiber content of the formulation, which may have limited the release of polyphenolic compounds into the aqueous phase. The polyphenolic content of the liquid complex polyherbal formulation was also low, which may be related to partial degradation or loss of polyphenolic compounds during solvent evaporation.

The flavonoid content was determined spectrophotometrically and expressed as rutin equivalents (Fig. 2, ***b***). The highest flavonoid content was found in the ethanolic extracts of *C. officinalis* and *S. officinalis* (3.4179 ± 0.0430 and 2.9626 ± 0.1676 µg/mL, respectively). The flavonoid content was low in the extracts of *V. officinalis* and in the aqueous extracts of *B. tripartita* and *A. officinalis*, as well as in the aqueous extract of the paste complex polyherbal formulation, and was consistent with the total polyphenolic content. In the aqueous extract of *S. marianum*, a low flavonoid content was observed despite a moderate total polyphenolic content. This may indicate that the polyphenolic compounds

Table 2. **Phytochemical screening of ethanolic and aqueous plant extracts and complex herbal preparations**

Reaction	*B. tripartita*		*C. officinalis*		*S. officinalis*		*A. absinthium*		*A. officinalis*		*S. marianum*		*V.officinalis*		PF	LF
	E	W	E	W	E	W	E	W	E	W	E	W	E	W		
PC1	+	+	+	+	+	+	+	+	+	+	+	+	+	+–	+–	+
PC2	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
F1	+	+	+	+	+	+	+	+	+	+	+	+	–	–	–	+
F2	+	+	+	+	+	+	+	+	+	+	+	+–	+	+–	–	+
F3	+	+	+	+	+	+–	+	+	+	+–	+	+–	+	+–	–	+
T1	–	–	–	–	+	+	–	+	+	+	–	–	–	–	+	–
T2	–	–	–	–	+	+	+–	+	+	+	+	+	–	–	–	–
AmA	–	+	–	+	–	+	–	+	–	+	+	+	+	+	+	–

Note: E — ethanolic extract; W — aqueous extract; PF — paste complex polyherbal formulation; LF — liquid complex polyherbal formulation. PC1 and PC2 — qualitative reactions for polyphenolic compounds; F1–F3 — qualitative reactions for flavonoids; T1 and T2 — qualitative reactions for tannins; AmA — qualitative reaction for amino acids. "+" — positive reaction; "–" — negative reaction; "±" — weakly positive reaction.



a *b*

Fig. 2. **Total phenolic (2a) and flavonoid (2b) contents in the studied samples:**
EBT and WBT — ethanolic and aqueous extracts of *B. tripartita*, respectively; ECO and WCO — ethanolic and aqueous extracts of *C. officinalis*, respectively; EAO and WAO — ethanolic and aqueous extracts of *A. officinalis*, respectively; ESO and WSO — ethanolic and aqueous extracts of *S. officinalis*, respectively; EAA and WAA — ethanolic and aqueous extracts of *A. absinthium*, respectively; ESM and WSM — ethanolic and aqueous extracts of *S. marianum*, respectively; EVO and WVO — ethanolic and aqueous extracts of *V. officinalis*, respectively; PF — aqueous extract of the paste complex polyherbal formulation; LF — liquid complex polyherbal formulation (* — $P \leq 0.05$; ** — $P \leq 0.01$; *** — $P \leq 0.001$; $M \pm m$; $n = 3$)

detected by the assay include flavonolignans such as silychristin, silydianin, silybin, and isosilybin, which are major constituents of the silymarin complex [15]. Chromatographic methods, particularly high-performance liquid chromatography (HPLC), are required for the reliable identification and quantitative determination of individual silymarin components.

Quantitative determination of amino acids was performed spectrophotometrically only for samples that showed a positive qualitative reaction with ninhydrin. A high amino acid content was found in the ethanolic extract of *V. officinalis* (0.4318 ± 0.0305 mg Gly Eq./mL) and in the aqueous extracts of *A. officinalis* and *S. marianum* (0.3414 ± 0.0075 and 0.3539 ± 0.0112 mg Gly Eq./mL, respectively) (Fig. 3). The roots of *V. officinalis* contain substantial amounts of γ-aminobutyric acid (GABA), which may contribute to the sedative effects of its extracts; tyrosine and glutamine have also been reported in the roots [28]. Although water is commonly used for the effective extraction of GABA from *V. officinalis*, the relatively high amino acid content observed in our ethanolic extract may also be associated with the prolonged extraction time in 40% ethanol. The low amino acid content in the paste complex polyherbal formulation, similar to the low polyphenolic content, may be related to the relatively short extraction time at the selected temperature, as well as to the presence of acetic acid and fiber in the formulation.

Plant-based feed additives may exhibit antioxidant activity due to their content of vitamin C and polyphenolic compounds, potentially contributing to the protection of the cardiovascular and digestive systems against oxidative stress. Oxidative stress can cause oxidative damage to proteins, DNA, and lipids and may contribute to the development of chronic diseases [27].

The antioxidant properties of the samples were evaluated spectrophotometrically and expressed as ascorbic acid equivalents (AA Eq.) (Fig. 4). The ethanolic extracts of most plants demonstrated high total antioxidant capacity (TAC). In particular, the *S. officinalis* extracts exhibited the highest TAC, which was consistent with their high polyphenolic content. In the ferric-reducing power assay (RPA), the aqueous plant extracts generally exhibited greater reducing power, with the aqueous extract of *S. officinalis* showing the highest value (2.2231 ± 0.0596 mg AA Eq./mL). The paste and liquid complex polyherbal formulations demonstrated moderate TAC and low reducing power. The observed antioxidant properties were generally consistent with the polyphenolic and flavonoid contents of the samples. The possible reasons for the low metabolite content and, consequently, the lower antioxidant capacity of the complex formulations are discussed above.

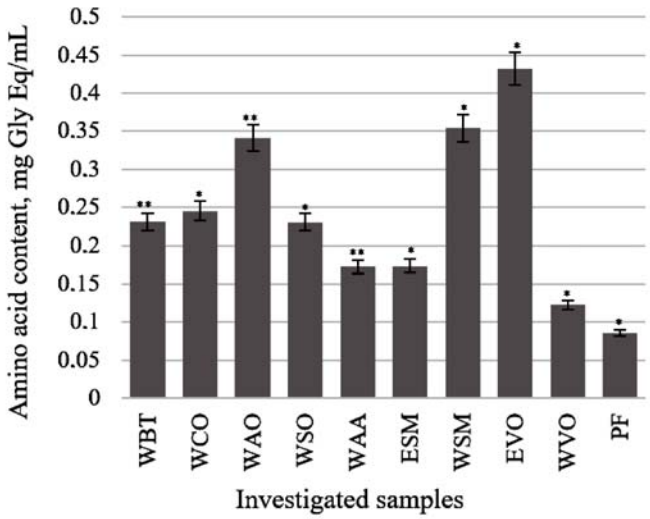


Fig. 3. **Total amino acid content in the studied samples, expressed as glycine equivalents:**
WBT — aqueous extract of *B. tripartita*; WCO — aqueous extract of *C. officinalis*; WAO — aqueous extract of *A. officinalis*; WSO — aqueous extract of *S. officinalis*; WAA — aqueous extract of *A. absinthium*; ESM and WSM — ethanolic and aqueous extracts of *S. marianum*, respectively; EVO and WVO — ethanolic and aqueous extracts of *V. officinalis*, respectively; PF — aqueous extract of the paste complex polyherbal formulation (* — $P \le 0.05$; ** — $P \le 0.01$; *** — $P \le 0.001$; $M \pm m$; $n = 3$).

The antioxidant activity of the aqueous and ethanolic extracts, as evaluated by the TAC and RPA methods, was influenced by the nature and polarity of the extractant. With increasing solvent polarity, greater ferric-reducing power was observed, which may be associated with more efficient extraction of polar compounds with antioxidant properties. In the phosphomolybdenum assay, the polarity of the solvent can affect the extraction of metabolites with similar polarity, thereby influencing the overall antioxidant capacity of the extracts [29]. Evaluation of antioxidant activity under *in vitro* conditions provides only an indication of the basic antioxidant potential of the samples, whereas their actual efficacy depends on the bioavailability and stability of the active compounds in the gastrointestinal tract of animals. Since polyphenols and flavonoids may undergo degradation or transformation due to enzymatic activity and variations in pH, further studies using *in vitro* gastrointestinal digestion models, followed by *in vivo* studies, are needed to assess their stability, bioavailability, and overall efficacy.

The results were statistically analyzed using Microsoft Excel, and Pearson's correlation coefficients (r) were calculated to assess the relationships between metabolite content and antioxidant activity. A strong positive correlation was observed between the contents of polyphenolic compounds and flavonoids (r = 0.7077), between polyphenolic content and antioxidant activity determined by the TAC (r = 0.7121) and RPA (r = 0.6198) methods, and between flavonoid content and antioxidant activity determined by the TAC method (r = 0.6741). A strong positive correlation was also observed between the antioxidant activity values obtained using the different methods (r = 0.6471). A moderate positive correlation was found between flavonoid content and antioxidant activity determined by the RPA method (r = 0.5534). These findings indicate a close association between the content of polyphenolic compounds and flavonoids and the antioxidant properties of the extracts.

Conclusions

The study demonstrated the presence of polyphenolic compounds, flavonoids, tannins, and amino acids in aqueous and ethanolic extracts of *Bidens tripartita*, *Calendula officinalis*, *Artemisia absinthium*, *Althaea officinalis*, *Salvia officinalis*, *Silybum marianum*, and *Valeriana officinalis*, as well as in the paste and liquid complex polyherbal formulations. The highest polyphenolic content was found in the extracts of *S. officinalis* and *A. absinthium*, whereas the highest flavonoid content was detected in the extracts of *S. officinalis* and *C. officinalis*. Amino acids were detected in all aqueous plant extracts, as well as in the ethanolic extracts of

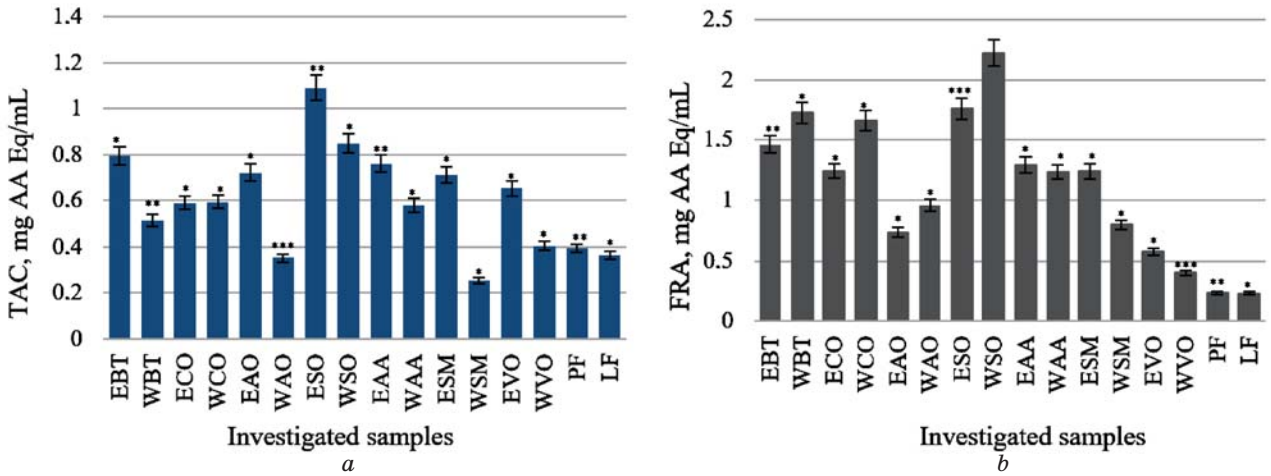


Fig. 4. **Total antioxidant activity (TAC) (*a*) and reducing power assay (FRA) (*b*) of the studied samples**

AA — ascorbic acid (control standard), EBT and WBT — ethanolic and aqueous extracts of *B. tripartita*, ECO and WCO — ethanolic and aqueous extracts of *C. officinalis*, EAO and WAO — ethanolic and aqueous extracts of *A. officinalis*, ESO and WSO — ethanolic and aqueous extracts of *S. officinalis*, EAA and WAA — ethanolic and aqueous extracts of *A. absinthium*, ESM and WSM — ethanolic and aqueous extracts of *S. marianum*, EVO and WVO — ethanolic and aqueous extracts of *V. officinalis*, PF — aqueous extract of the paste complex herbal preparation, LF — liquid complex herbal preparation (* — $P \le 0.05$; ** — $P \le 0.01$; *** — $P \le 0.001$; $M \pm m$; $n = 3$).

S. marianum and *V. officinalis*. The contents of polyphenolic compounds and flavonoids were positively associated with the antioxidant properties of the studied samples.

The aqueous extract of the paste complex polyherbal formulation contained relatively low levels of the studied metabolites. Further research should therefore investigate the release, stability, and bioavailability of these metabolites under simulated gastrointestinal conditions and in relevant *in vivo* models. Additional studies should focus on the identification and quantitative determination of individual metabolites using chromatographic methods, particularly HPLC, as well as on the safety assessment of the formulations using appropriate *in silico*, *in vitro*, and *in vivo* approaches. Future research should also aim to optimize the technological processes used for formulation preparation to maximize the retention of bioactive metabolites and, consequently, the biological activity of the resulting feed additives.

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Conflict of interest

The authors declare that they have no conflict of interest.

Authors' contribution

Hutsko, K. I. — conceptualization, methodology, formal analysis, investigation, writing — original draft, writing — review editing, visualization; Tsudna, S. I. — formal analysis, investigation, writing — original draft.

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ФІТОХІМІЧНА ОЦІНКА ТА АНТИОКСИДАНТНИЙ ПОТЕНЦІАЛ РОСЛИННИХ ЕКСТРАКТІВ І КОМПЛЕКСНИХ ПОЛІФІТОПРЕПАРАТІВ ДЛЯ ВЕТЕРИНАРНОГО ЗАСТОСУВАННЯ

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Створення ефективних кормових добавок різного типу дії на основі рослинної сировини є перспективним напрямом як екстрактів рослин, які мають метаболіти, що діють комплексно.

Мета. Провести скринінг метаболітів, визначити кількісний вміст поліфенольних сполук, флаваноїдів та амінокислот у екстрактах рослин та комплексних рослинних препаратів, оцінити їхню антиоксидантну активність.

Матеріали й методи. Для ідентифікації метаболітів використано відомі якісні хімічні реакції. Кількісний вміст поліфенолів, флаваноїдів та амінокислот визначено спектрофотометрично із використанням стандартних методик. Антиоксидантну активність визначено фосформолібденовим методом та визначено відновлювальну здатність заліза досліджуваних зразків. Статистичну обробку та розрахунок коефіцієнта кореляції Пірсона (r) проведено за допомогою програми *Microsoft Excel*.

Результати. Якісний аналіз підтвердив наявність поліфенольних сполук, флаваноїдів, танінів, амінокислот у досліджуваних зразках. Найвищий вміст поліфенолів виявлено у екстрактах *Salvia officinalis* (2.1466 ± 0.2594 та 1.3936 ± 0.1468 мг/мл у спиртовому та водному екстракті, відповідно), а високий вміст флавоноїдів у спиртових екстрактах *Salvia officinalis* (2.9626 ± 0.1676 мкг/мл) та *Calendula officinalis* (3.4179 ± 0.0430 мкг/мл). Високий вміст амінокислот виявлено в етанольному екстракті *Valeriana officinalis* (0.4318 ± 0.0305 мг екв. гліцину/мл). Спиртовий екстракт Salvia officinalis продемонстрував найвищу загальну антиоксидантну активність (0,0069 ± 1.0906 мг екв. АК/мл), а водний екстракт — найвищу активність до відновлення заліза (2,2231 ± 0,0596 мг екв. АК/мл). Розрахунок кореляції виявив міцний позитивний зв'язок між концентрацією поліфенольних сполук і антиоксидантною активністю зразків.

Висновки. Досліджувані екстракти мають високий терапевтичний потенціал і можуть бути використані як функціональні компоненти при розроленні кормових добавок для тварин. Умови виготовлення комплексних рослинних препаратів необхідно оптимізувати, для збільшення їхньої ефективності.

Ключові слова: кормова добавка, екстракти, комплексний рослинний препарат, поліфеноли, антиоксидантна активність.

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