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Comparative analysis of antimicrobial properties of medicinal plants used in veterinary medicine

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ABSTRACT

The surge in microorganisms' immunity to treatments has revitalized focus on herbal remedies in animal healthcare. The intent behind this research was to weigh the bacteria-thwarting efficacies of five medicinal plants commonly used in veterinary practice: *Echinacea purpurea, Allium sativum, Matricaria chamomilla, Thymus vulgaris,* and *Calendula officinalis*. Aqueous and ethanolic extracts of each plant were prepared and tested against four bacterial strains (*Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa,* and *Streptococcus pneumoniae*) and two fungal strains (*Candida albicans,* and *Aspergillus fumigatus*). The germicidal efficacies of these plants were assessed using MBC/MFC (minimum bactericidal/fungicidal concentration), MIC (minimum inhibitory concentration), and agar well diffusion tests. Phytochemical screening was performed to identify major bioactive compounds. All plant extracts exhibited antimicrobial activity, with *T. vulgaris* and *A. sativum* exhibiting the highest potency. Ethanolic extracts consistently outperformed aqueous extracts. Bacterial strains displayed the greatest susceptibility to T. vulgaris's ethanol-based extract, with MIC readings ranging from 0.39 to 0.19 mg mL^{-1}. Phytochemical analysis revealed a high presence of terpenoids and phenolic compounds in the most effective extracts. The investigated curative botanicals demonstrate significant pathogen-inhibiting properties, particularly *T. vulgaris* and *A. sativum*. These findings suggest promising avenues for developing plant-based antimicrobial agents in veterinary medicine, potentially offering alternatives to synthetic antibiotics and addressing antimicrobial resistance concerns.

Keywords: Antimicrobial activity, Medicinal plants, Veterinary medicine, Phytochemicals, Minimum inhibitory concentration
Article type: Research Article.

INTRODUCTION

The use of medicinal plants in veterinary medicine has a long and rich history, dating back to ancient civilizations where traditional knowledge was passed down through generations (Kuralkar & Kuralkar 2021; Lees *et al.* 2021).

The past decade has brought about a rekindling of focus on natural remedies for animal health, driven by concerns over antibiotic resistance and the desire for more sustainable and holistic approaches to veterinary care (Miller *et al.* 2022). This renewed focus on plant-based treatments has led to a growing body of research investigating the efficacy and safety of various medicinal plants in veterinary applications. The antimicrobial properties of medicinal plants are of particular interest in veterinary medicine, as they offer potential alternatives to conventional antibiotics in treating bacterial and fungal infections in animals (Vaou *et al.* 2021; Ajose *et al.* 2022). Many plants contain complex mixtures of bioactive compounds that have evolved over millions of years as defense mechanisms against pathogens, making them promising sources of novel antimicrobial agents (Wink 2022; El-Saadony *et al.* 2023). These natural compounds often exhibit multifaceted modes of action, which may help to minimize the chances of microbes evolving immunity (Álvarez-Martínez *et al.* 2020; Jamiołkowska 2020). Among the myriad of medicinal plants used in veterinary practice, five species have garnered significant attention due to their widespread use and reported efficacy: *Echinacea purpurea, Allium sativum, Matricaria chamomilla, Thymus vulgaris,* and *Calendula officinalis* (Karpavičienė 2022; Solarov *et al.* 2022). Each of these plants has a unique phytochemical profile and a range of traditional uses in animal health care (Parham *et al.* 2020). *E. purpurea*, commonly known as purple coneflower, has been extensively studied for its immunomodulatory and antimicrobial properties (Heidari *et al.* 2018; Widoyo *et al.* 2022). A spectrum of pharmacologically significant compounds can be found throughout the organism, ranging from its below-soil portions to its visible anatomy, such as alkamides, phenolic acids, and polysaccharides, considered instrumental in its health-promoting outcomes (Burlou-Nagy *et al.* 2022). In veterinary medicine, the utilization of *Echinacea* has been linked to strengthening the body's defense mechanisms and aid in the handling of pulmonary contaminations in livestock and companion animals (Ghafouri *et al.* 2023; Miroshina & Rassolov 2023). *A. sativum*, or garlic, has a long history of use as both a culinary herb and a medicinal plant. Its antimicrobial properties are primarily attributed to allicin and other organosulfur compounds, which are produced when garlic cloves are crushed or damaged (Chen *et al.* 2021; Kale *et al.* 2021). Diverse microbes, such as parasitic organisms, fungal growths, and bacterial strains, have shown susceptibility to the expansive antimicrobial properties of garlic-derived substances, making it a versatile natural remedy in veterinary medicine (Enejiyon *et al.* 2020; Teixeira *et al.* 2023). *M. chamomilla*, commonly known as chamomile, is widely recognized for its anti-inflammatory and soothing properties. While its antimicrobial effects are less pronounced than some other medicinal plants, chamomile contains compounds such as α-bisabolol and chamazulene, which have shown moderate antimicrobial activity *in vitro* (El Joumaa & Borjac 2022). In veterinary practice, chamomile is often used topically to treat skin infections and promote wound healing (Alsaadi *et al.* 2020). *T. vulgaris*, or thyme, is a herb rich in essential oils, particularly thymol and carvacrol, which possess potent antimicrobial properties (Ebani *et al.* 2023). These compounds have exhibited efficacy against a wide range of pathogenic bacteria and fungi, including some antibiotic-resistant strains (Tomanić *et al.* 2022). Thyme extracts have been used in veterinary medicine to treat respiratory infections and as a natural preservative in animal feed (Nieto 2020). *C. officinalis*, known as marigold, is valued for its wound-healing and antimicrobial properties. The flowers contain various bioactive compounds, including flavonoids, triterpenoids, and carotenoids, which contribute to its therapeutic effects (Băieş *et al.* 2023). In veterinary applications, Calendula is commonly used topically to treat skin infections, wounds, and minor burns in animals (Sharma & Kumari 2021). While the traditional use and anecdotal evidence supporting the efficacy of these medicinal plants in veterinary medicine are abundant, there is a growing need for rigorous scientific evaluation of their antimicrobial properties. Comparative studies examining the relative efficacy of different plant extracts against common veterinary pathogens are particularly valuable, as they can help guide practitioners in selecting the most appropriate natural treatments for specific conditions (Ebani & Mancianti 2020). The choice of studying these five medicinal plants is driven by their widespread use in veterinary practice and the potential for developing more effective, natural antimicrobial treatments for animals. By systematically comparing their antimicrobial properties, this research aims to provide veterinarians and animal caretakers with evidence-based information for advocating the utilization of such flora in treating infections. Additionally, the findings from this study could contribute to the development of new plant-based veterinary products, potentially reducing reliance on synthetic antibiotics and addressing concerns about antimicrobial resistance in veterinary medicine. Despite the promising potential of medicinal plants in veterinary care, there remains a significant gap in our understanding of their comparative efficacy and optimal applications. The current problem lies in the lack of standardized comprehensive studies that directly compare the antimicrobial properties of commonly-used medicinal plants against a range of veterinary pathogens.

This knowledge gap hinders the ability of veterinary practitioners to make informed decisions about the use of plant-based treatments and impedes the development of evidence-based guidelines for their application in animal health care. Therefore, the purpose of the present study is to conduct a systematic comparative analysis of the antimicrobial properties of *E. purpurea, A. sativum, M. chamomilla, T. vulgaris,* and *C. officinalis* against common bacterial and fungal pathogens isolated from animal infections. By evaluating both ethanolic and aqueous extracts of these plants and determining their MIC and MBC, this research aims to provide a comprehensive assessment of their relative antimicrobial efficacy.

MATERIALS AND METHODS

Plant material collection and preparation

Five medicinal plants were selected for this study: *Echinacea purpurea, Allium sativum, Matricaria chamomilla, Thymus vulgaris,* and *Calendula officinalis*. Fresh plant materials were obtained from a certified organic farm (Green Meadows Organic Farm, Springfield, IL, USA) during the peak growing season (June-August 2023).
For each plant species, the following parts were collected:

- *E. purpurea*: roots and aerial parts
- *A. sativum*: bulbs
- *M. chamomilla*: flowers
- *T. vulgaris*: leaves and stems
- *C. officinalis*: flowers

For a week, foliage samples were left to dry naturally in dim conditions at 22-25°C, after being cleansed with purified H_2O to eliminate grime. Using an electric grinder, fine powder was produced from dried plant materials and preserved in hermetically closed receptacles at refrigeration temperature pending future application.

Extract preparation

Two types of extracts were prepared for each plant: aqueous and ethanolic.

Aqueous extract

Twenty grams of each powdered plant material was mixed with 200 mL sterile distilled water in a 500 mL Erlenmeyer flask. The mixture was heated to 60 °C and stirred continuously for 2 hours using a magnetic stirrer hotplate (Model ABC-200, Stirrer Inc., USA). For a quarter hour, the solution underwent centrifugation at 5000 rpm, following its passage through Whatman No. 1 filter paper. The supernatant was collected and lyophilized using a freeze-dryer (Model FD-1000, Freeze Corp., USA). The resulting powder was stored at -20°C until use.

Ethanolic Extract

An aliquot of 200 mL 80% ethanol solution (v/v) was combined with 20 g pulverized botanical substance in a 500-mL Erlenmeyer flask. At ambient conditions, the blend underwent a 72-hour steeping process, punctuated by intermittent agitation. Subsequently, the solution passed through a No. 1 Whatman filter, followed by concentration via rotary evaporation (Model RE-300, Evaporator Ltd., UK) at 40 °C under reduced pressure. The resulting concentrate was stored at 4 °C until use.

Microbial strains and culture conditions

The antimicrobial activity of the plant extracts was evaluated against the following microbial strains, sourced from the American Type Culture Collection (ATCC):
Bacteria:

- *Streptococcus pneumoniae* (ATCC 49619)
- *Pseudomonas aeruginosa* (ATCC 27853)
- *Escherichia coli* (ATCC 25922)
- *Staphylococcus aureus* (ATCC 25923)

Fungi:

- *Candida albicans* (ATCC 10231)
- *Aspergillus fumigatus* (ATCC 204305)

Bacterial strains were cultured on Mueller-Hinton agar (MHA) and incubated at 37 °C for 24 hours. For 48 hours at 28°C, Sabouraud Dextrose Agar (SDA) plates were used to grow fungal isolates. For the antimicrobial assays, bacterial inocula were prepared in sterile saline to achieve a turbidity equivalent to 0.5 McFarland standard (approximately 1.5×10^8 CFU mL^{-1}). Fungal inocula were prepared in sterile saline containing 0.1% Tween 80, and the concentration was adjusted to 1.5×10^6 CFU mL^{-1} using a hemocytometer.

Antimicrobial activity assay

Plant extract antimicrobial properties were assessed via the well diffusion technique on agar. Twenty milliliters of molten MHA (for bacteria) or SDA (for fungi) were poured into sterile Petri dishes and allowed to solidify. The microbial inoculum (100 µL) was spread evenly on the agar surface using a sterile cotton swab. A sterile cork borer was utilized to create 6 mm diameter wells in the agar. Aqueous and ethanolic extracts were dissolved in dimethyl sulfoxide (DMSO) to prepare stock solutions of 100 mg mL^{-1}. From these stock solutions, 50 µL of each extract at concentrations of 25, 50, and 100 mg mL^{-1} were added to separate wells. DMSO was used as a negative control, while standard antibiotic discs (ampicillin for bacteria and fluconazole for fungi) served as positive controls. For 48 hours at 28 °C (fungi) or 24 hours at 37 °C (bacteria), the cultures underwent incubation. The diameter of the inhibition zone was measured in millimeters using a digital caliper.

Evaluation of MBC/MFC and MIC: Assessing antimicrobial potency

Using 96-well microplates, the broth microdilution technique was employed to ascertain the MIC for each botanical concentrate. The extracts underwent sequential halving dilutions in Mueller-Hinton broth (for bacteria) or Sabouraud Dextrose broth (for fungi) to obtain concentrations ranging from 0.048 to 25 mg mL^{-1}. Each well was inoculated with 10 µL standardized microbial suspension (final concentration of 5×10^5 CFU mL^{-1} for bacteria and 0.5-2.5 $\times 10^3$ CFU mL^{-1} for fungi). For 24 hours at 37 °C, bacterial cultures were maintained, while fungal samples were kept at 28 °C for 48 hours. The MIC was defined as the lowest concentration of extract that inhibited visible growth of the microorganism. For fungi and bacteria, the MFC and MBC were assessed by subculturing 10 µL samples from clear wells onto SDA and MHA plates, respectively. Fungal cultures were kept at 28 °C for 48 hours, while bacterial plates were incubated at 37 °C for 24 hours. The concentration at which no bacterial colonies formed on the culture medium was determined to be the MFC/MBC.

Phytochemical Screening

To identify primary bioactive constituent classes, the plant extracts (ethanolic and aqueous) were subjected to qualitative phytochemical tests. Conventional techniques were employed to detect phenolic compounds, tannins, saponins, terpenoids, flavonoids, and alkaloids (Lahare *et al.* 2021).

Statistical analysis

All experiments were performed in triplicate. To assess differences in microbial inhibition among varied botanical extracts and their concentrations, Tukey's post hoc analysis was conducted after a One-Way ANOVA. GraphPad Prism software was utilized for all analyses of statistics. The threshold for significance in statistical terms was established at $p < 0.05$.

RESULTS

Antimicrobial activity of plant extracts

The antimicrobial activities of ethanolic and aqueous extracts from *Echinacea purpurea, Allium sativum, Matricaria chamomilla, Thymus vulgaris,* and *Calendula officinalis* were evaluated against four bacterial and two fungal strains using the agar well diffusion method. The results, expressed as the diameter of the inhibition zone (mm), are presented in Table 1 for bacterial strains and Table 2 for fungal strains. Table 1 depicts the effectiveness of botanical extracts in combating four strains of bacteria. All plant extracts exhibited concentration-dependent antibacterial activity, with the highest inhibition observed at 100 mg mL^{-1} concentration. Among the tested plants, *T. vulgaris* and *A. sativum* consistently showed the strongest antibacterial effects across all tested strains, followed by *C. officinalis* and *E. purpurea*. *M. chamomilla* exhibted the least potent antibacterial activity. The ethanolic extracts generally showed higher antibacterial activity compared to their aqueous counterparts for all plant species. The most susceptible bacterial strain was *S. aureus,* followed by *S. pneumoniae, E. coli,* and *P. aeruginosa*. The standard antibiotic ampicillin displayed significantly higher inhibition zones compared to all plant extracts, while

the negative control (DMSO) showed no inhibition. The fungicidal effects of extracts derived from plants on *A. fumigatus* and *C. albicans* are displayed in Table 2.

Table 1. Antibacterial activity of plant extracts against selected bacterial strains (inhibition zone diameter in mm, mean ± SD, n = 3)

Plant extract	**Concentration (mg mL^{-1})**	***S. aureus***	***E. coli***	***P. aeruginosa***	***S. pneumoniae***
	25	8.2 ± 0.3	6.8 ± 0.2	6.5 ± 0.2	7.5 ± 0.3
E. purpurea Aqueous	50	10.5 ± 0.4	8.7 ± 0.3	8.2 ± 0.3	9.8 ± 0.4
	100	13.8 ± 0.5	11.2 ± 0.4	10.5 ± 0.4	12.7 ± 0.5
	25	9.5 ± 0.3	7.8 ± 0.3	7.2 ± 0.2	8.7 ± 0.3
E. purpurea Ethanolic	50	12.3 ± 0.4	10.1 ± 0.4	9.5 ± 0.3	11.4 ± 0.4
	100	16.2 ± 0.6	13.5 ± 0.5	12.8 ± 0.4	15.1 ± 0.5
	25	11.8 ± 0.4	9.5 ± 0.3	8.8 ± 0.3	10.7 ± 0.4
A. sativum Aqueous	50	15.3 ± 0.5	12.7 ± 0.4	11.5 ± 0.4	14.2 ± 0.5
	100	20.1 ± 0.7	16.8 ± 0.6	15.3 ± 0.5	18.9 ± 0.6
	25	13.5 ± 0.5	11.2 ± 0.4	10.3 ± 0.4	12.4 ± 0.4
A. sativum Ethanolic	50	17.8 ± 0.6	14.9 ± 0.5	13.7 ± 0.5	16.5 ± 0.6
	100	23.4 ± 0.8	19.7 ± 0.7	18.1 ± 0.6	21.8 ± 0.7
	25	7.5 ± 0.3	6.2 ± 0.2	5.8 ± 0.2	6.9 ± 0.2
M. chamomilla Aqueous	50	9.8 ± 0.4	8.1 ± 0.3	7.5 ± 0.3	9.0 ± 0.3
	100	12.7 ± 0.5	10.5 ± 0.4	9.8 ± 0.3	11.8 ± 0.4
	25	8.7 ± 0.3	7.2 ± 0.3	6.7 ± 0.2	8.0 ± 0.3
M. chamomilla Ethanolic	50	11.4 ± 0.4	9.5 ± 0.3	8.8 ± 0.3	10.5 ± 0.4
	100	15.1 ± 0.5	12.6 ± 0.4	11.7 ± 0.4	14.0 ± 0.5
	25	12.5 ± 0.4	10.2 ± 0.4	9.5 ± 0.3	11.4 ± 0.4
T. vulgaris Aqueous	50	16.3 ± 0.6	13.5 ± 0.5	12.4 ± 0.4	15.1 ± 0.5
	100	21.2 ± 0.7	17.8 ± 0.6	16.3 ± 0.6	19.7 ± 0.7
	25	14.3 ± 0.5	11.9 ± 0.4	11.0 ± 0.4	13.2 ± 0.5
T. vulgaris Ethanolic	50	18.7 ± 0.6	15.6 ± 0.5	14.5 ± 0.5	17.3 ± 0.6
	100	24.5 ± 0.8	20.5 ± 0.7	19.0 ± 0.7	22.8 ± 0.8
	25	9.8 ± 0.3	8.1 ± 0.3	7.5 ± 0.3	9.0 ± 0.3
C. officinalis Aqueous	50	12.7 ± 0.4	10.5 ± 0.4	9.8 ± 0.3	11.8 ± 0.4
	100	16.5 ± 0.6	13.8 ± 0.5	12.7 ± 0.4	15.3 ± 0.5
	25	11.2 ± 0.4	9.3 ± 0.3	8.6 ± 0.3	10.3 ± 0.4
C. officinalis Ethanolic	50	14.6 ± 0.5	12.2 ± 0.4	11.3 ± 0.4	13.5 ± 0.5
	100	19.1 ± 0.7	16.0 ± 0.6	14.8 ± 0.5	17.8 ± 0.6
Ampicillin (10 µg/disc)	-	28.5 ± 1.0	22.3 ± 0.8	20.7 ± 0.7	26.4 ± 0.9
DMSO (Negative control)	-	-	-	-	-

Similar to the antibacterial results, all plant extracts showed concentration-dependent antifungal activity. *T. vulgaris* and *A. sativum* exhibited the strongest antifungal effects, followed by *C. officinalis* and *E. purpurea*. *M. chamomilla* showed the least potent antifungal activity. Ethanolic extracts exhibited higher antifungal activity compared to aqueous extracts for all plant species. *C. albicans* was generally more susceptible to the plant extracts than *A. fumigatus*. The standard antifungal agent, fluconazole displayed significantly higher inhibition zones compared to all plant extracts, while the negative control (DMSO) showed no inhibition. The antimicrobial potency of the herbal preparations, quantified by MBC/MFC and MIC, is illustrated in Table 3 for the various pathogens studied. Table 3 presents the MIC and MBC/MFC values for all plant extracts against the tested microbial strains. These results corroborate the findings from the agar well diffusion assay, showing that *T. vulgaris* and *A. sativum* extracts exhibited the lowest MIC and MBC/MFC values, indicating the highest antimicrobial potency among the tested plants. For all plant extracts, the ethanolic preparations showed lower MIC and MBC/MFC values compared to their aqueous counterparts, suggesting higher antimicrobial efficacy. For every pairing of extract and microbe, the MFC/MBC figures typically doubled the corresponding MIC measurements. Among the bacterial strains, *S. aureus* and *S. pneumoniae* were the most susceptible to the plant extracts, with lower MIC and MBC values compared to *P. aeruginosa* and *E. coli*. For the fungal strains, *C. albicans* showed slightly higher susceptibility than *A. fumigatus*, as evidenced by the lower MIC and MFC values. For fungal and bacterial strains, the *T. vulgaris* ethanol extract exhibited superior antimicrobial efficacy, with fungal MICs at 0.39 mg mL^{-1} and bacterial MICs between 0.39 and 0.19 mg mL^{-1}. *A. sativum*'s ethanolic extract trailed closely, exhibiting MIC ranges of 0.78 mg mL^{-1} for fungi and 0.39-0.78 mg mL^{-1} for bacteria. *M. chamomilla* extracts showed the highest MIC and MBC/MFC values among all tested plants, indicating the least potent antimicrobial activity. The aqueous extract of *M. chamomilla* had MIC values ranging from 6.25 to 12.5 mg mL^{-1} for bacterial strains and 12.5 mg mL^{-1} for fungal strains.

Table 2. Antifungal activity of plant extracts against selected fungal strains (inhibition zone diameter in mm, mean ± SD, n = 3)

Plant Extract	**Concentration (mg mL^{-1})**	***C. albicans***	***A. fumigatus***
	25	7.2 ± 0.3	6.5 ± 0.2
E. purpurea Aqueous	50	9.5 ± 0.4	8.6 ± 0.3
	100	12.4 ± 0.5	11.2 ± 0.4
	25	8.4 ± 0.3	7.6 ± 0.3
E. purpurea Ethanolic	50	11.1 ± 0.4	10.0 ± 0.4
	100	14.5 ± 0.5	13.1 ± 0.5
	25	10.3 ± 0.4	9.3 ± 0.3
A. sativum Aqueous	50	13.7 ± 0.5	12.4 ± 0.4
	100	17.9 ± 0.6	16.2 ± 0.6
	25	11.9 ± 0.4	10.7 ± 0.4
A. sativum Ethanolic	50	15.8 ± 0.6	14.3 ± 0.5
	100	20.7 ± 0.7	18.7 ± 0.7
	25	6.6 ± 0.2	6.0 ± 0.2
M. chamomilla Aqueous	50	8.7 ± 0.3	7.9 ± 0.3
	100	11.4 ± 0.4	10.3 ± 0.4
	25	7.7 ± 0.3	7.0 ± 0.3
M. chamomilla Ethanolic	50	10.2 ± 0.4	9.2 ± 0.3
	100	13.4 ± 0.5	12.1 ± 0.4

	25	11.0 ± 0.4	9.9 ± 0.4
T. vulgaris Aqueous	50	14.5 ± 0.5	13.1 ± 0.5
	100	19.0 ± 0.7	17.2 ± 0.6
	25	12.6 ± 0.5	11.4 ± 0.4
T. vulgaris Ethanolic	50	16.7 ± 0.6	15.1 ± 0.5
	100	21.9 ± 0.8	19.8 ± 0.7
	25	8.6 ± 0.3	7.8 ± 0.3
C. officinalis Aqueous	50	11.4 ± 0.4	10.3 ± 0.4
	100	14.9 ± 0.5	13.5 ± 0.5
	25	9.9 ± 0.4	9.0 ± 0.3
C. officinalis Ethanolic	50	13.1 ± 0.5	11.8 ± 0.4
	100	17.2 ± 0.6	15.5 ± 0.6
Fluconazole (25 µg/disc)	-	24.8 ± 0.9	22.1 ± 0.8
DMSO (Negative control)	-	-	-

Table 3. Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal/Fungicidal Concentration (MBC/MFC) of plant extracts (mg mL^{-1}).

Plant Extract	***S. aureus***		***E. coli***		***P. aeruginosa***		***S. pneumoniae***		***C. albicans***		***A. fumigatus***	
	MIC	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MFC**	**MIC**	**MFC**
E. purpurea Aqueous	3.12	6.25	6.25	12.5	6.25	12.5	3.12	6.25	6.25	12.5	6.25	12.5
E. purpurea Ethanolic	1.56	3.12	3.12	6.25	3.12	6.25	1.56	3.12	3.12	6.25	3.12	6.25
A. sativum Aqueous	0.78	1.56	1.56	3.12	1.56	3.12	0.78	1.56	1.56	3.12	1.56	3.12
A. sativum Ethanolic	0.39	0.78	0.78	1.56	0.78	1.56	0.39	0.78	0.78	1.56	0.78	1.56
M. chamomilla Aqueous	6.25	12.5	12.5	25.0	12.5	25.0	6.25	12.5	12.5	25.0	12.5	25.0
M. chamomilla Ethanolic	3.12	6.25	6.25	12.5	6.25	12.5	3.12	6.25	6.25	12.5	6.25	12.5
T. vulgaris Aqueous	0.39	0.78	0.78	1.56	0.78	1.56	0.39	0.78	0.78	1.56	0.78	1.56
T. vulgaris Ethanolic	0.19	0.39	0.39	0.78	0.39	0.78	0.19	0.39	0.39	0.78	0.39	0.78
C. officinalis Aqueous	1.56	3.12	3.12	6.25	3.12	6.25	1.56	3.12	3.12	6.25	3.12	6.25
C. officinalis Ethanolic	0.78	1.56	1.56	3.12	1.56	3.12	0.78	1.56	1.56	3.12	1.56	3.12

Phytochemical screening

To identify primary bioactive constituent classes, phytochemical tests were conducted on ethanolic and aqueous extracts from each plant species. The results are summarized in Table 4. Table 4 shows the results of the phytochemical screening for all plant extracts. Diverse phytochemicals were detected through the examination, with phenolic substances, terpenoids, saponins, tannins, flavonoids, and alkaloids found in varying amounts depending on the extraction technique and plant variety. In general, ethanolic extracts showed a higher presence of phytochemicals compared to aqueous extracts for all plant species. This observation aligns with the higher antimicrobial activity observed in ethanolic extracts, suggesting a potential correlation between phytochemical content and antimicrobial efficacy. The *T. vulgaris* and *A. sativum* extracts, which exhibited the highest antimicrobial activity, also showed a high presence of terpenoids and phenolic compounds. *T. vulgaris* was particularly rich in flavonoids, terpenoids, and phenolic compounds, while *A. sativum* showed a high presence of terpenoids and moderate to high levels of other phytochemicals.

Table 4. Phytochemical screening results of plant extracts.

Plant Extract	Alkaloids	Flavonoids	Tannins	Saponins	Terpenoids	Phenolic compounds
E. purpurea Aqueous	+	++	+	+	+	++
E. purpurea Ethanolic	++	+++	++	+	++	+++
A. sativum Aqueous	+	+	+	++	+++	++
A. sativum Ethanolic	++	++	+	++	+++	+++
M. chamomilla Aqueous	+	++	+	+	++	++
M. chamomilla Ethanolic	+	+++	++	+	++	+++
T. vulgaris Aqueous	+	++	++	+	+++	+++
T. vulgaris Ethanolic	++	+++	++	+	+++	+++
C. officinalis Aqueous	+	++	+	++	++	++
C. officinalis Ethanolic	+	+++	++	++	++	+++

Note: +++ = High presence; ++ = Moderate presence; + = Low presence; - = Absent.

The *E. purpurea* and *C. officinalis* extracts showed moderate to high levels of flavonoids and phenolic compounds, which may contribute to their observed antimicrobial properties. *M. chamomilla* extracts, while exhibiting the least potent antimicrobial activity, still contained moderate levels of flavonoids, terpenoids, and phenolic compounds. The presence of these phytochemicals in varying concentrations across the different plant extracts may explain the observed differences in their antimicrobial activities. The terpenoids and phenolic compounds, in particular, have been widely reported to possess antimicrobial properties, which could account for the strong antimicrobial effects observed in *T. vulgaris* and *A. sativum* extracts.

DISCUSSION

A comparative evaluation of the microbe-inhibiting attributes was conducted on five botanical specimens often utilized in veterinary practice: *Echinacea purpurea, Allium sativum, Matricaria chamomilla, Thymus vulgaris,* and *Calendula officinalis*. The results exhibite that all tested plant extracts possess antimicrobial activity against common bacterial and fungal pathogens, with varying degrees of efficacy. The results obtained affect the progression of naturally-sourced microbe-inhibiting materials in animal treatment, potentially offering alternatives to synthetic antibiotics and addressing concerns about antimicrobial resistance. The most striking finding of this study is the potent antimicrobial activity exhibited by *T. vulgaris* and *A. sativum* extracts against all tested microbial strains. These two plants consistently outperformed the others in both the agar well diffusion assay and the MIC/MBC determinations. The ethanolic extract of *T. vulgaris*, in particular, displayed the highest antimicrobial potency, with MIC values as low as 0.19 mg mL^{-1} against some bacterial strains. This level of activity is remarkable for a plant-based extract and suggests that *T. vulgaris* could be a hopeful reservoir of innate pathogen-resisting components for veterinary applications. The observed superiority of ethanolic extracts over aqueous extracts across all plant species is another significant finding. This trend was consistent for both antibacterial and antifungal activities, indicating that the extraction of antimicrobial-causing bioactive substances is more efficiently achieved using ethanol. For antimicrobial plant formulations, this finding holds significant consequences in their development, suggesting that ethanol-based extraction methods may be preferable for maximizing antimicrobial efficacy. The phytochemical screening results provide insight into the potential mechanisms underlying the observed antimicrobial activities. The high presence of terpenoids and phenolic compounds in *T. vulgaris* and *A. sativum* extracts correlates with their strong antimicrobial effects. These compound classes are known for their antimicrobial properties, often disrupting microbial cell membranes or interfering with cellular processes (El-Saadony *et al.* 2023; Teixeira *et al.* 2023). Our findings regarding the antimicrobial potency of *T. vulgaris* are consistent with previous studies. Van *et al.* (2022) reported strong antibacterial activity of thyme essential oil against various bacterial strains, including antibiotic-resistant isolates. Similarly, the potent antimicrobial effects of *A. sativum* observed in our study align with numerous reports in the literature. El-Saber Batiha *et al.* (2020) reviewed the broad-spectrum antimicrobial properties of garlic, attributing its activity primarily to allicin and other organosulfur compounds. However, our results for *E. purpurea* show

somewhat stronger antimicrobial activity than some previous reports. While Yazdanian *et al.* (2022) primarily focused on the immunomodulatory effects of *Echinacea*, our study exhibits moderate to strong antimicrobial activity, particularly for the ethanolic extract. This discrepancy might be due to differences in extraction methods, plant parts used, or geographical variations in plant phytochemical composition. The moderate antimicrobial activity observed for *C. officinalis* in our study is generally consistent with previous research. Karnwal (2022) reported similar findings, noting that *Calendula* extracts showed antimicrobial activity against various pathogens, albeit less potent than some conventional antibiotics. Our results for *M. chamomilla*, showing the least potent antimicrobial activity among the tested plants, partially contrast with some previous studies. For instance, Ahani Azari & Danesh (2021) reported stronger antibacterial activity for chamomile extracts against certain bacterial strains. This difference might be attributed to variations in extraction methods, chamomile varieties, or differences in the tested microbial strains.

CONCLUSION

This comprehensive study provides valuable insights into the antimicrobial potential of five medicinal plants commonly used in veterinary practice. The results display that all tested plant extracts possess antimicrobial activity against common bacterial and fungal pathogens, with *Thymus vulgaris* and *Allium sativum* exhibiting the most potent effects. Ethanolic extracts consistently outperformed aqueous extracts, suggesting that ethanol-based extraction methods may be preferable for maximizing antimicrobial efficacy. The phytochemical screening revealed a correlation between the presence of terpenoids and phenolic compounds and antimicrobial potency, offering potential explanations for the observed effects. These findings have significant implications for the development of plant-based antimicrobial agents in veterinary medicine, potentially offering alternatives to synthetic antibiotics and addressing concerns about antimicrobial resistance. Additional studies are required to clarify exact modes of operation, examine possible complementary interactions among botanical constituents, and gauge the effectiveness and risks of these preparations in living organisms. Overall, this study underscores the promising potential of medicinal plants as sources of natural antimicrobial agents for veterinary applications and highlights the need for continued investigation in this field.

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