



PHYTOCHEMICAL SCREENING AND ANTIMICROBIAL ACTIVITY OF AJUGA BRACTEOSA AND CIRSIUM HORRIDULUM FROM DISTRICT DIR LOWER KHYBER PAKHTUNKHWA PAKISTAN

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Abstract

The purpose of the current study was to carry out phytochemical characterization and evaluation of antimicrobial properties in ethanolic and methanolic extracts of *Cirsium horridulum* and *Ajuga bracteosa* against bacterial pathogens like, *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus pneumoniae* *Salmonella typhi* and fungal strains, like *Aspergillus flavus*, *Fusarium oxysporum*, *Fusarium graminearum*, *Aspergillus niger*, and *Candida albicans*. Qualitative and quantitative phytochemicals were assessed for bioactive constituents in both plants. The results of this study showed that some organism were resistant the plant extract such as *Bacillus subtilis* and *Pseudomonas aeruginosa*. The findings of the agar well-diffusion method demonstrated dose-dependent action of plant extracts against testes samples of bacteria and fungi. The highest antibacterial effect of *Ajuga bracteosa* (25.2 ± 1.23) and *Cirsium horridulum* (23.5 ± 1.51) was recorded against *Salmonella typhi*. The antifungal activity showed that the highest zone of inhibition (27.1 ± 1.26) and (28.4 ± 3.23) against *Aspergillus niger* were exhibited by the ethanolic and methanolic extracts of *Ajuga bracteosa* and *Cirsium Horridulum*, respectively. The phytochemical assessment of selected plants also revealed both plants contain highly important constituents like coumarins, flavonoids, proteins, phlobatanins, tannins, terpenoids, phenols and alkaloids.

Key Words: Antibacterial, antifungal, plant extract, *Ajuga bracteosa*, *Salmonella typhi*

Introduction

Plants are naturally God gifted for the isolation and characterization of medicinally active compounds which are used to cure human illnesses. Traditional medical procedures and modern scientific research support that, medicinal plants are important for therapeutic purposes to reduce illness and improve human vitality. Majority of the plants are assumed to be rich suppliers of materials for the synthesis and manufacturing of medicines (Oladeji et al., 2019). Components of medicinal plants, such as flavonoids, quinine, terpenoids etc have biological actions that enhance their therapeutic benefits, including anti-inflammatory, anti-mutagenic, anti-carcinogenic, antifungal, and antibacterial qualities (Batiha et al., 2020). As compare to synthetic drugs, natural products and their derivatives have rare adverse effects and greater efficacy (Batiha et al., 2020). Apart from the fact that natural products are highly valuable to treat common diseases and having less adverse effects, our ability to use these products for the treatment of common diseases is still very limited due to the development and spread of microorganisms which are resistant to synthetic antibacterial and antifungal drugs (Halat et al., 2022). It is very alarming that the speedy universal spread of infectious, multi-resistant bacteria and fungi which have adapted themselves with old antibacterial and antifungal drugs are not curable with available antibacterial and antifungal drugs (Chokkareddy et al., 2025). Only 6 out of the 32 antibiotics under clinical development that target the priority pathogens on the World Health Organization's (WHO) list were deemed novel in 2019. Additionally, one of the serious problems is the common people of under developed and developing countries are still not using high-quality antibiotics either due to unawareness or poverty. All levels of development are impacted by the lack of antibiotics and antifungals, particularly in the health care system. World Health Organization suggested in survey that, more than eighty percent of the world's population uses traditional medicines made from different plant fractions and their active ingredients, (Shahat, 2018).

Ajuga bracteosa is a perennial herb belonging to Lamiaceae family. It grows at elevations between 1200 and 2500 meters in grasslands, exposed slopes, and open fields in temperate and subtropical regions of the world. Because of the variety of active substances in it, it has considerable medical potential (Wasti et al., 2024). Previously done phytochemical analyses revealed that *A. bracteosa* has some important chemicals like clerodane diterpenes, iridoid glucosides, flavonoids, and various saturated and unsaturated long chain medicinally important compounds (Gao et al., 2025).

Cirsium, *horridulum* or Thistle belonging to Asteraceae family which is widely distributed worldwide. There are over 200 species of genus *Cirsium* throughout North and Central America, Asia, Europe, and North Africa (Mabberley & Mabberley, 2008). In agriculture, *Cirsium* species are viewed as invasive plants that must be controlled with strong measures. On the other hand, Some *Cirsium* species are regarded as edible plants in some nations, such as Turkey (Sener et al., 2021). Several nations traditional medical systems also employ several members of the genus *Cirsium* as therapeutic herbs. The leaves of certain *Cirsium* species are used to make tea, which has medicinal benefits in various European nations. This is the situation in Central Italy, where digestive problems and stomach aches are treated with a decoction of *Cirsium arvense* leaves. In China, more than 10 species root or entire plants extracts are used for the treatment treat of various of diseases, such as gastrointestinal issues, jaundice, and bleeding (Aggarwal et al., 2022). The present study investigates various bioactive compounds and antimicrobial activity of different extracts from medicinally important *Ajuga bracteosa* and *Cirsium*, *horridulum*.

2. Materials and Methods

Ajuga bracteosa and *Cirsium horridulum* plants were collected from different parts of district Dir lower (Goro, Bagh, Katan). Mr. Jelani recognized these plants at University of Peshawar, Pakistan. Collected plants were deposited in the department of Chemical & Life Sciences' herbarium with voucher numbers Salim Ullah 1-A and Salim Ullah 1-B.

Preparation of plant extract: Crude extracts both plants were prepared using techniques outlined by Madiha et al (2018) with little changes in the solvents. The antimicrobial activity of both methanolic and ethanolic extracts was assessed against some bacterial species. (*Bacillus subtilis*, *Staphylococcus pneumoniae*, *Salmonella typhi*, *Escherichia coli*, *Pseudomonas aeruginosa*) and some fungal species (*Aspergillus flavus*, *Fusarium oxysporum*, *Fusarium graminearum*, *Aspergillus niger* and *Candida albicans*).

Phytochemical screening and antimicrobial activity:

Phytochemical screening was performed by using protocols of Madiha et al. (2018) with some modifications. The unrefined ethanolic and methanolic extracts of *Ajuga bracteosa* and *Cirsium horridulum* were assessed for their antibacterial properties by using agar tube dilution method and the agar well diffusion approach (Ahmad et al., 2011). The antifungal activity *Ajuga bracteosa* and *Cirsium horridulum* was carried out by agar tube dilution method (Hussain et al., 2010).

Data analysis: The statistical data is represented as Significant variability at $p < 0.05$ was established by taking the mean of the original triple data and applying the Least Significant Difference (LSD) test (Steel et al., 1997).

3. Results

The results of the current phytochemical investigations of *Ajuga bracteosa* and *Cirsium horridulum* confirmed that both the plants were rich in phytochemicals like, proteins, tannins, coumarins, phlobatanins, flavonoids, phenols, terpenoids, and alkaloids (Table 1 & 2). The current study (phytochemical screening) of *Ajuga bracteosa* confirmed the presence of phytochemical constituents when its crude ethanolic extract evaluated. Ethanolic extract of the tested plant (*Ajuga bracteosa*) revealed the presence of chemical constituents like phytosterols, saponins, tri-terpenes, glycosides, flavonoids and protein. Similarly, crude methanolic extract of the same plant also confirmed the presence of active compound like anthraquinones, flavonoids, alkaloids, proteins and glycosides in appropriate amount. In ethanolic extract of *Cirsium horridulum* some valuable phytochemicals were found such as coumarins, proteins, terpenoids, tannins, flavonoids, phlobatanins and phenols. The same methanolic extract the selected plant (*Cirsium horridulum*) also revealed phytochemicals such as coumarins, tannin, flavonoids, phenols and alkaloids, proteins in high amount.

The findings of quantitative phytochemical screening in methanolic and ethanolic extracts of both the tested plants (*Ajuga bracteosa* and *Cirsium horridulum*) confirmed phytochemical constituents from highest degree to down to lowest degree of presence (Table 3 & 4). Quantitative phytochemical analysis in ethanolic extract of *Ajuga bracteosa* showed the high quantity (8.3mg/100g) of saponins in dry weight. While ethanolic extract of *Ajuga bracteosa* minimum amount of tannins (2.3mg/100mg) was reported. In the methanolic extract of *Ajuga bracteosa* concentrations of phytochemicals were determined. Tannins were reported in high amount (6.60mg/100g) while phenols were reported in low amount (1.20mg/100g). Similarly, *Cirsium horridulum* in the methanolic extract showed highest quantity of tannins (10.3mg/100g) and lowest quantity of phenols (5.0mg/100g). Highest quantity of saponins (8.6 mg/100g) were recorded in methanolic extract of *Cirsium horridulum* and lowest quantity of phenols (4.2 mg/100g) was observed in the same plant extract.

In the ethanolic extract, the antibacterial activity *Ajuga bracteosa* showed the highest zone of inhibition (25.2 ± 1.23) against *Salmonella typhi*, followed by *Pseudomonas aeruginosa* (23.5 ± 1.15). While ethanolic extract of *Ajuga bracteosa* showed the lowest zone of inhibition (10.36 ± 1.35) against *Bacillus subtilis* (Fig. 1; Table 5). Similarly, *Ajuga bracteosa* in methanolic extract showed the highest growth of inhibition against *Escherichia coli* (24.6 ± 1.45) and *Bacillus subtilis* (23.5 ± 1.45). In the same extract *Ajuga bracteosa* showed lowest growth of inhibition (13.5 ± 2.15) against *Salmonella typhi* (13.5 ± 2.15).

Antibacterial activity *Cirsium horridulum* was evaluated in of ethanolic extract which showed the highest zone of inhibition (23.5 ± 1.51) against *Salmonella typhi*, then *Staphylococcus aureus* (23.31 ± 3.45), and the lowest zone of inhibition (14.3 ± 2.31) was shown against *Pseudomonas aeruginosa*. Similarly, the methanolic extract of *Cirsium horridulum* showed maximum inhibition (25.4 ± 1.32) against *Staphylococcus aureus* followed by *Escherichia coli* (22.3 ± 2.25) and the lowest zone of inhibition (18.5 ± 2.45) was shown against *Pseudomonas aeruginosa*. (Fig. 1; Table 6)

In ethanolic extract, the antifungal activity *Ajuga bracteosa* showed significant effect (27.1 ± 1.26) to stop the growth *Aspergillus niger*, and *Fusarium oxysporum* (25.1 ± 2.30). *Ajuga bracteosa* in ethanolic extract revealed minimum antifungal activity against *Aspergillus flavus* (17.2 ± 2.21) (Fig. 2; Table 7). In the same way, the methanolic extract of the selected plant (*Ajuga bracteosa*) confirmed significant efficacy against *Aspergillus niger* and *Fusarium oxysporum* (29.4 ± 1.62) (25.2 ± 2.30) respectively. In methanolic extract *Ajuga bracteosa* showed lowest antifungal activity (18.3 ± 2.13) against fungal strains i.e *Aspergillus flavus* at the maximum concentration of plant extract (100 mg/ml).

Cirsium horridulum showed highest antifungal activity in the ethanolic extract (28.4 ± 3.23) against *Aspergillus niger* followed by *Fusarium graminearum* (24.4 ± 1.26). While *Cirsium horridulum* showed minimum antifungal activity (14.1 ± 2.28) in the ethanolic extract against *Fusarium oxysporum*. In the same way, *Cirsium horridulum* in methanolic extract showed profound antifungal activity (26.3 ± 2.10) against *Fusarium oxysporum* and *Fusarium graminearum* (24.4 ± 1.28). *Cirsium horridulum* in methanolic extract showed lowest antifungal activity (19.3 ± 2.10) against *Candida albicans* at highest quantity of plant extract (100 mg/ml) (Fig. 2; Table 8).

Table 1. *Ajuga bracteosa* Qualitative phytochemical screening

Phytochemicals	Ethanolic Extract	Methanolic Extract
Phytosterols.	+++	---
Reducing sugar.	+++	+++
Saponins.	+++	+++
Triterpenes.	+++	+++
Proteins.	+++	---
Flavonoids.	+++	+++
Anthraquinones.	---	+++
Glycosides	+++	+++

Table 2. *Cirsium horridulum* Qualitative phytochemical screening

Chemical constituents	Ethanolic Extract	Methanolic Extract
Coumarins.	+++	+++
Proteins.	+++	+++
Tannins.	+++	+++
Phlobatanins.	+++	---
Terpenoids.	+++	---
Proteins.	+++	+++
Flavonoids.	---	+++
Phenols.	+++	+++
Alkaloids.	+++	+++

Table 3. *Ajuga bracteosa* Quantitative phytochemical analysis

Chemical constituents	Ethanollic Extract	Methanollic Extract
Tannins.	2.3 (%)	1.2 (%)
Alkaloids.	4.0 (%)	2.6 (%)
Flavonoids.	6.2 (%)	5.2 (%)
Saponin.	8.3 (%)	6.6 (%)
Phenols.	3.0 (%)	2.2 (%)

Table 4. *Cirsium horridulum* Quantitative phytochemical analysis

Chemical constituents	Ethanollic Extract	Methanollic Extract
Tannins.	10.3 (%)	7.3 (%)
Alkaloids.	8.2 (%)	6.6 (%)
Flavonoids.	5.2 (%)	7.4 (%)
Saponins.	6.5 (%)	8.6 (%)
Phenols.	5.0 (%)	4.2 (%)

Table 5. *Ajuga bracteosa* Antibacterial Activity

Microbial Strains	Positive control	Negative control	Zones of Inhibition(mm)			
			Ethanollic Extract 75 mg/ml 100 mg/ml		Methanollic Extract 75mg/ml 100mg/ml	
Bacillus subtilis	33±2.26	0	6.3±1.15	10.36±1.3 5	16.43±1.1 5	23.5±1.4 5
Staphylococcus pneumonia	30±2.24	0	8.3±0.86	12.41±1.3 4	14.32±2.3 5	20.6±1.5 7
Salmonella typhi	28±2.43	0	16.5±1.15	25.2±1.23	10.6±1.43	13.5±2.1 5
Escherichia coli	28±1.54	0	17.5±1.42	22.3±1.34	15.36±1.2 7	24.6±1.4 5
Pseudomonas aeruginosa	30±2.14	0	13.43±2.6 5	23.5±1.15	9.63±1.15	15.6±2.3 5
Escherichia coli	28±1.54	0	17.5±1.42	22.3±1.34	15.36±1.2 7	24.6±1.4 5

Table 6. *Cirsium horridulum* Antibacterial Activity

Microbial Strains	Positive control	Negative control	Zones of Inhibition(mm)			
			Ethanollic Extract 75 mg/ml 100 mg/ml		Methanollic Extract 75mg/ml 100mg/ml	
<i>Pseudomonas aeruginosa</i>	27±1.43	0	10.5±2.45	14.3±2.31	12.43±1.15	18.5±2.45
<i>Staphylococcus aureus</i>	30±2.20	0	15.5±2.35	23.31±3.45	15.41±1.15	25.4±1.32
<i>Shigella flexneri</i>	35±2.22	0	18.6±2.65	22.3±1.63	16.5±1.14	20.3±1.15
<i>Escherichia coli</i>	30±1.24	0	13.2±2.41	20.4±1.42	14.3±1.27	22.3±2.25
<i>Salmonella typhi</i>	29±3.36	0	15.6±1.45	23.5±1.51	14.5±1.15	19.5±1.45

Table 7. *Ajuga bracteosa* Antifungal Activity

Fungal strains	Positive control	Negative control	Inhibition zone (mm)			
Extract concentration			Ethanolic Extract		Methanolic Extract	
			75mg/ml	100mg/ml	75mg/ml	100mg/ml
Aspergillus flavus	28±1.18	0	13.2±0.72	17.2±2.21	14.2±1.23	18.3±2.13
Fusarium oxysporum	31±3.28	0	16.4±2.13	25.1± 2.30	16.3±1.35	25.2±2.30
Fusarium graminearum	30±1.52	0	16.0±1.12	23.32±1.23	16.4±2.20	23.3±1.25
Aspergillus nigar	33±2.52	0	18.0±1.21	27.1±1.26	20.1±3.25	29.4±1.62
Candida albicans	28±1.35	0	17.3±1.16	24.2±2.12	15.4±1.52	20.5±2.30

Table 8. *Cirsium horridulum* Antifungal Activity

Fungal strains	Positive Control	Negative control	Inhibition zone (mm)			
Extract Concentrations			Ethanolic Extract		Methanolic Extract	
			75mg/ml	100mg/ml	75mg/ml	100mg/ml
Aspergillus flavus	25±1.30	0	16.2±1.62	23.2±1.10	15.4±2.11	20.1±1.18
Candida albicans	28±1.52	0	18.5±2.62	21.0±1.20	13.2±2.18	19.3±2.10
Fusarium graminearum	26±2.18	0	17.4±1.42	24.4±1.26	19.0±1.50	24.4±1.28
Aspergillus nigar	32±3.56	0	21.31±1.32	28.4±3.23	14.31±1.42	22.4±1.42
Fusarium oxysporum	32±2.24	0	9.0±0.51	14.1±2.28	17.31±2.17	26.3±2.10

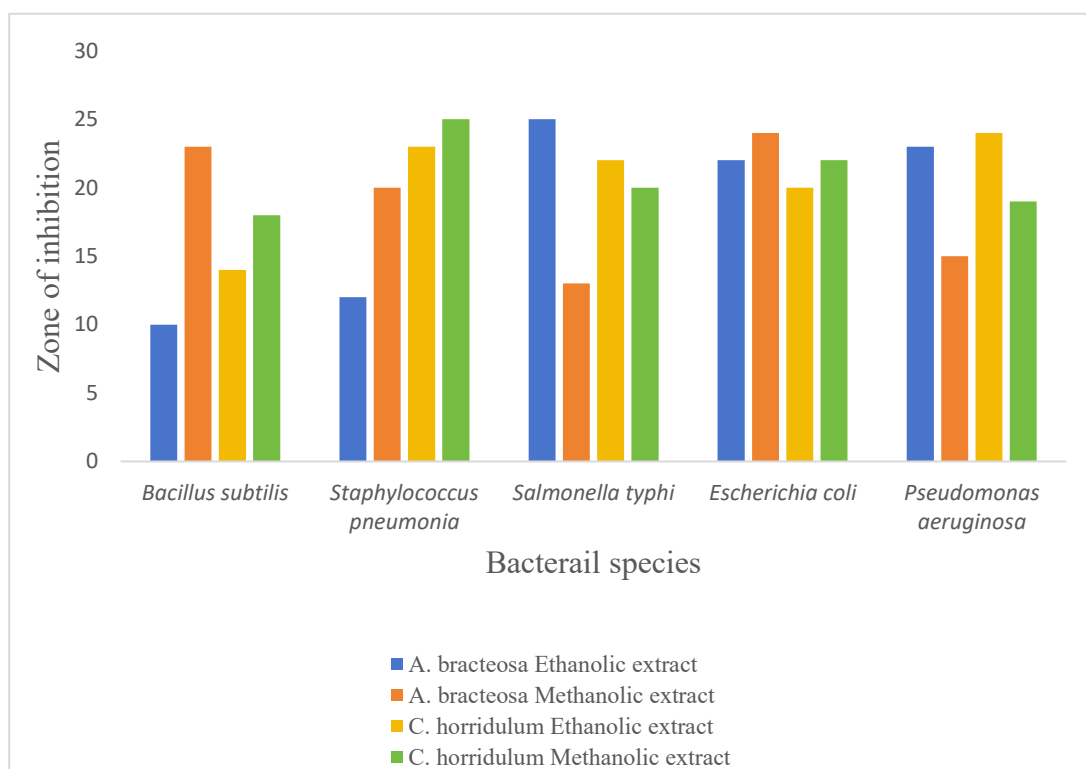


Figure 1. Antibacterial activity of *Ajuga bracteosa* and *Cirsium horridulum* in crude ethanolic extract and methanol extract against different bacterial strains. (Bar shows LSD at p<0.05)

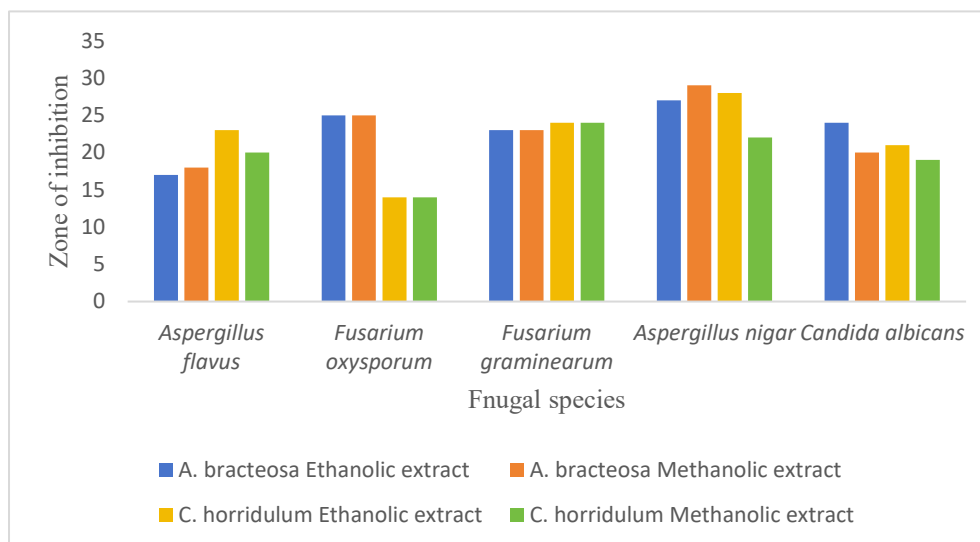


Figure 2. Antifungal activity of *Ajuga bracteosa* and *Cirsium horridulum* in crude ethanolic extract and methanoli extracts of against a varity Fungal strainsc (Bar shows LSD at $p < 0.05$)

Discussion

The phytochemical screening indicated that both plants exhibited Coumarins, protiens, tannins, phlobatanins, terpenoids, flavonoids, phenols and alkaloids (Table 1). The antibacterial activity showed that both plant extracts reduced bacterial growth in a dose-dependent way. *Salmonella typhi* was the bacteria that responded more to the methanolic extract of *Cirsium horridulum* in comparison with ethanolic extract. Similar results are also reported by ((Aguilar *et al.*, 2025) who revealed that these bacterial strains were more sensitive to the methanolic extract of *Chaerophyllum villosum* as compare to ethanolic extract. These results showed that *Escherichia coli* was most vulnerable bacteria to the ethanolic extract of *Ajuga bracteosa*. Whereas, *Pseudomonas aeruginosa* was more susceptible bacteria to ethanolic extract of *Ajuga brecteosa*. These findings concur with the research by Al-Tawalbeh *et al.* (2025) stating “*P. aeruginosa* is the most susceptible to the methanolic extract *Cannabis sativa*.”

These findings demonstrated that, in comparison to Gram negative bacteria, extracts from each of the investigated plants had strong antibacterial action against Gram positive bacteria. Amin *et al.* (2023), also conducted a similar kind of study and found that gram positive bacteria are more vulnerable to *Artemisia vulgaris* extracts than gram negative bacteria. Nourbakhsh *et al.* (2025) stated that the resistivity of gram-negative bacteria to plant extract may be due to the fact that these bacteria have an outer membrane which chemically consists phospholipids and lipopolysaccharides, which function is to prevent the entrance of any antimicrobial agent, which may be the cause of the bacterial resistance to plant extracts.

The ethanolic extract of *Cirsium horridulum* showed highest antifungal activity against *Aspergillus niger*. While, in methanolic extract *cirsium horridullum* showed maximum antifungal activity against *Fusarium oxysporum*. These findings in line with the results of (Soumia *et al.*, 2024) who also reported highest antifungal properties of a methanolic extract of *Brucea antidysenterica* and *Justicia schimperiana* against *A. niger*. Both the tested plant extract contains secondary metabolites, including flavonoids, phenols, and alkaloids, which have been shown to have antibacterial action. Alkaloids are chemical constituents that come in a variety of forms and are known to possess antibacterial qualities by inhibiting the activity of certain enzymes. Similarly, (Rongai *et al.*, 2015) reported that extract of *Salvia guaranitica* significantly inhibit mycelial growth and spore germination of *F. oxysporum*, and this antifungal activity is depending on the permeability of the cellular walls of fungi.

Ajuga bracteosa in ethanolic extract showed highest antifungal activity against *Aspergillus niger* and in methanolic extract *Ajuga bracteosa* also revealed maximum antifungal activity against *Aspergillus niger*. These results agree with (Koka *et al.*, 2018) who screened eight commonly used spices for their antifungal properties against *A. niger* growth through the use of poisoned food. There was a notable variation in each extract's capacity to inhibit *A. niger* growth.

The results of the present study indicated that both the tested plants were rich in certain medicinally important phytochemical constituents like coumarins, proteins, tannins, phlobatanins, terpenoids, flavonoids, phenols and alkaloids. The presence of these phytochemicals may be the cause of their use as antimicrobial plants. These findings concur with those of Pikhtirova *et al.* (2023), who found that glycosides, tannins, saponins, and alkaloids all had therapeutic effectiveness against a variety of microbial infections. Saponins damage all structured tissues by changing the permeability of cell walls. Saponins causes morphological changes in cell membrane that result in splitting of cells, hence Saponins has some antibacterial effects (Ibrahim *et al.*, 2025).

Conclusion

In order to kill, or stop the growth of different pathogenic bacterial and fungal strains, we used ethanolic and methanolic extracts of two plants (*Ajuga bracteosa* and *Cirsium horridulum*) to evaluate their antibacterial and antifungal activities. The experimental evidence confirmed the presence of distinct phytochemical compounds in ethanolic and methanolic extracts of *Cirsium horridulum* and *Ajuga bracteosa* that may have therapeutic implications. Ethanolic extract of *Ajuga bracteosa* showed strongest antibacterial properties as compare to the methanolic extract of *Ajuga bracteosa* against *Salmonella typhi*. On the other hand, *Staphylococcus aureus* is more inhibited by the methanolic extract of *Cirsium horridulum* than by the ethanolic extract. The results of the current study encourage the use of methanolic and ethanolic preparations of *Ajuga bracteosa* and *Cirsium horridulum* to treat various bacterial and fungal infections.

Declarations:

Competing Interest;

The authors have no relevant financial or non-financial interests to disclose.

Author Contributions:

Material preparation and data collection were performed by [Salim Ullah], and [Muhammad Adil]. Whole research was supervised by [Muhammad Adil]. The first draft of the manuscript was written by [Salim Ullah] and analysis was done by [Muhammad Naseer], Faten Zubair did the graphs and tables, funding and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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All data is available in the main manuscript.

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