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Phytochemical Screening and In vitro antibacterial activity of Petroleum Ether extract of *Ageratum conyzoides*

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Abstract: This study investigates the phytochemical composition and antibacterial activity of *Ageratum conyzoides* petroleum ether extract. Phytochemical screening revealed the presence of bioactive compounds, including alkaloids, flavonoids, Terpenoids, and Saponins, which are known for their therapeutic properties. The antibacterial activity was evaluated against Gram-positive (*Staphylococcus aureus, Streptococcus pneumoniae*) and Gram-negative (*Escherichia coli, Klebsiella pneumoniae*) bacteria using the agar well diffusion method. The various concentrations of petroleum ether leaf extract of *Ageratum conyzoides* demonstrated significant antibacterial effects, 400 µg/ml showed ZOI 13.5 mm against *E. coli* and 600 µg/ml showed the highest ZOI 12.5 and 12 mm against *S. aureus* and *S. pneumonia* respectively, while 1000 µg/ml concentrations, showed the highest ZOI of 12mm against the *K. pneumonia.* These findings highlight the potential of *Ageratum conyzoides* petroleum ether extract as a natural source of antibacterial agents and support its traditional use in herbal medicine. Further studies are recommended to isolate and characterize the active compounds responsible for the observed antibacterial properties.

Keywords: *Ageratum conyzoides* • phytochemical analysis • Antibacterial activity • petroleum ether

Introduction

Various plant and various plant species are present on the earth and these plants are playing various important roles for the society. *Ageratum conyzoides*, is an invasive plant commonly known as "Billy goat weed," is a tropical herbaceous plant belonging to the Asteraceae family (Patil et al 2019, Yadav et al 2019). This plant is recognized for its distinctive blue-purple flowers and utilized in traditional medicine for its therapeutic properties (Gang et al 2023). Traditional healers across various cultures have employed *Ageratum conyzoides* to treat a range of ailments, including wounds, inflammation, fever, gastrointestinal disorders, and skin diseases (Permawati et al 2019). The plant's widespread use in folk medicine highlights its potential as a source of bioactive compounds with medicinal significance (Gang et al 2023, Kumar et al 2024).

In African traditional medicine, *Ageratum conyzoides* is often used to treat malaria, rheumatism, and wounds due to its purported anti-inflammatory and antimicrobial effects (Pintong et al 2020). In India, it has been employed to manage skin infections and digestive issues. Similarly, in Brazil and other parts of South America, the plant is utilized to alleviate pain, stop bleeding, and combat bacterial infections (Gang et al 2023). The diverse applications of *Ageratum conyzoides* across different cultures suggest the presence of a rich phytochemical profile responsible for its therapeutic properties (Ramasamy et al 2021).

Phytochemical investigations of *Ageratum conyzoides* have explained the presence of various phytochemicals, including alkaloid, flavonoids, terpenoids, saponins, and coumarins. These bioactive compounds are known to possess antimicrobial, anti-inflammatory, antioxidant, and analgesic properties (Chahal et al 2021). Various phytochemical also help for the synthesis of nanoparticles in the green synthesis methods of nanoparticles (Agrawal et al 2024, Kumar et al 2025). Among the different extraction

methods, the use of petroleum ether can isolate non-polar compounds, which may exhibit significant antibacterial effects (Huo et al 2024). Understanding the phytochemical composition and antimicrobial potential of *Ageratum conyzoides* is crucial for exploring its use as a natural alternative to synthetic antibiotics. Antibiotic resistance undermines the efficacy of conventional antibiotics, resulting in prolonged illnesses, increased mortality rates, and escalating healthcare costs. The World Health Organization (WHO) has highlighted the urgent need to discover and develop novel antimicrobial agents to combat resistant bacterial strains. In this context, medicinal plants present a promising avenue for identifying new bioactive compounds with antibacterial activity (Vaou, et al, 2021, Agrawal et al 2023).

Ageratum conyzoides, with its rich ethno pharmacological background, offers a potential source of novel antibacterial agents. Although several studies have reported the antimicrobial properties of this plant, further investigation is required to understand the efficacy of different extracts and their activity against specific bacterial strains. PE extraction targets non-polar compounds, which may include potent antibacterial agents that have not been fully explored (Singh et al 2024).

Investigating the phytochemical and antibacterial properties of *Ageratum conyzoides* is essential for several reasons. Firstly, it may lead to the discovery of new natural compounds capable of combating antibiotic-resistant bacteria. Secondly, it can provide scientific validation for the plant's traditional uses in treating infections. Finally, understanding the plant's bioactive components may pave the way for the development of plant-based pharmaceuticals or complementary therapies (Rana et al 2022). This study seeks to address these gaps by evaluating the phytochemical profile and antibacterial activity of the PE extract of *Ageratum conyzoides*.

To perform phytochemical screening of *A. conyzoides* PE extract: This objective involves identifying and characterizing the bioactive compounds present in the petroleum ether extract through qualitative analysis (Sonam, et al 2017, Puro et al 2018,).

To evaluate the antibacterial activity against selected bacterial strains: This objective entails testing the petroleum ether extract against pathogenic bacteria. By addressing these objectives, the study aims to contribute to the growing body of knowledge on plant-based antimicrobials and their potential role in overcoming antibiotic resistance.

Material and methods

Sample collection and preparation

Leave samples of *Ageratum conyzoides* were collected from the Pauri district of Uttarakhand (Fig. 1). After the collection, the sample was washed 2-3 times with tap water and then distilled. Leaves dried at room temperature and grind them with the help of mortar and pestle.

Fig. 1 Picture of *Ageratum conyzoides* at sampling site

Extraction Process

The plant extract was prepared using petroleum ether solvent. To prepare plant extract, 2 grams of plant leaf powder was added to 20 ml of petroleum ether and then kept in the orbital shaker at 150 rpm for 48 hrs. After that, the plant extract was filtered with the help of Whatman's filter paper No. 41, after that evaporate the solvent by using water bath and the final concentration of 1mg/ ml was prepared, and the phytochemical screening and antibacterial activity were performed.

Phytochemical Screening

The preliminary qualitative phytochemical screening of *Ageratum conyzoides* leaf, extracts was performed to identify the presence of various bioactive compounds. The screening was performed to detect the presence of alkaloids, carbohydrates, reducing sugar, glycosides, cardiac glycosides, amino acids, flavonoids, phenolic compounds, Tannins, phlobatannins, phytosterols, Terpenoids, triterpenoids, and Saponins. The screening process followed the methods described by Banu and Catherine (2015) with minor modifications (Agarwal et al 2023).

Qualitative analysis for the presence of various phytochemical

Alkaloids: For the identification of alkaloids, Wagner's test was performed. A few milliliters of the plant extract and a few drops of Wagner's reagent were added to it. Brown and reddish precipitate indicates the presence of alkaloids.

Carbohydrate: for the detect the presence of carbohydrates, 5 ml of 5% potassium hydroxide (KOH) solution was added in 1ml of the plant extract. If canary-yellow color appears, indicating the presence of carbohydrates.

Detection of reducing sugar: First, 1 ml of the plant extract was mixed with 1 ml each of Fehling's solution A and Fehling's solution B, and the mixture was then boiled in a water bath. The presence of red precipitate indicates reducing sugars in the plant sample.

Glycosides: For the detection of glycosides, Borntrager's test was performed. First, 2 ml of the plant extract was taken and mixed with 3 ml of chloroform, followed by thorough shaking. The chloroform layer was then separated, and a 10% ammonium solution was added to it. If rose-pink to blood-red color appeared, it indicates the presence of glycosides.

Cardiac Glycosides: The Keller-Killani test was performed to detect the presence of cardiac glycosides. For this test, 1.5 ml of glacial acetic acid was added to 1 ml of the plant extract. This was followed by the addition of one drop of 5% ferric chloride solution and a few drops of concentrated sulfuric acid (H_2SO_4). If greenish plant extract changed into a blue-colored solution, it indicates the presence of cardiac glycosides in the petroleum ether plant extract.

Amino Acid: To detect the presence of amino acids, the xanthoproteic test was performed by adding a few drops of concentrated nitric acid to the plant extract. The appearance of a yellow-colored solution confirmed the presence of amino acids.

Flavonoids: To detect flavonoids, the ferric chloride test was performed by adding a few drops of 10% ferric chloride solution to the plant extract. The formation of a green precipitate indicated the presence of flavonoids.

Phenolic Compounds: For the detection of Phenolic compounds, 5% ferric chloride solution was added to the plant extract. Dark green colour indicates the presence of Phenolic compound.

Tannins: A 10% sodium hydroxide (NaOH) test was performed to detect tannins. In this experiment, 4 ml of 10% NaOH was added to 0.4 ml of the plant extract and the mixture was shaken well. If emulsion form, it suggests that Tannin is present.

Phlobatannins: For the detection of Phlobatannins, 2 ml of the plant extract mixed with 2 ml of 1% hydrochloric acid (HCl),

followed by boiling. Red precipitate indicates that Phlobatannins absent in the plant extract.

Phytosterols: Hesse's reaction was carried out to detect Phytosterols. For this, 5 ml of the plant extract was mixed with 2 ml of chloroform, followed by the addition of 2 ml of concentrated sulfuric acid (H_2SO_4).The solution color changes to blue, indicating that Phytosterols is present in the plant extract.

Terpenoids: To detect Terpenoids, 2 ml of chloroform was added to 5 ml of the plant extract and the mixture was evaporated using a water bath. After evaporation, 3 ml of concentrated sulfuric acid (H_2SO_4) was added, and the mixture was boiled in the water bath for a few minutes. The appearance of a gray-colored solution indicated the presence of Terpenoids.

Triterpenoides: Salkowski's test was performed for the detection of Triterpenoides by adding a few drops of concentrated sulfuric acid (H_2SO_4) to the plant extract. The mixture was then shaken properly and allowed to stand. a golden yellow layer found at the bottom of the solution, which indicates the presence of Triterpenoides.

Saponins: A foam test was conducted to detect Saponins. In this experiment, 0.5 mg of the plant extract was taken, 2 ml of distilled water was added, and the mixture was vigorously shaken.

Antibacterial Activity

Gram-positive and gram-negative bacterial strains were used to check the antibacterial activity of the *Ageratum conyzoides* plant extract. Two strains were gram-positive (*Staphylococcus aureus,* Streptococcus pneumoniae), and two were Gram-negative (*Escherichia coli, Klebsiella pneumoniae*). The antibacterial activity was assessed using the well diffusion method. Initially, nutrient agar and nutrient broth were prepared and sterilized using an autoclave. After sterilization, the nutrient agar was poured into Petri plates and allowed to solidify. Inoculums of bacteria were then streaked over the surface of the nutrient agar plates to ensure uniform coverage following incubation.

Wells were made in the agar using a 100 mm diameter cork borer. Each well was filled with 100 µl of various concentrations of the test substances. Streptomycin (25 µg) and Ampicillin (10 µg) served as positive controls, while petroleum ether was used as the negative control. The plates were left at room temperature for two hours to allow pre-diffusion of the substances, after which they were incubated at 37°C for 24 hours.

Statistical analysis

Each test was conducted in triplicate, and the data are presented as mean values with standard deviations (mean ± SD).

Results

Phytochemical analysis of *Ageratum conyzoides*

Ageratum conyzoides is an annual herb and it containing various phytochemical which are essential for the various bioactivities. Petroleum ether extract of *Ageratum conyzoides* shows presence of various phytochemical (Table:1) such as Alkaloids, Carbohydrate, Reducing sugar, Glycosides, Cardiac glycosides, Amino acid, Flavonoids, Phenolic compound, Phytosterols, Terpenoids, Triterpenoides and Saponins. Tannins and Phlobatannins were found to be absent in the PE extract of *Ageratum conyzoides.*

Table 1: Phytochemical screening of PE leaf extract of *Ageratum conyzoides*

Serial No.	Phytochemical	Crude (PE) *A. conyzoides*
1.	**Alkaloids**	Present
2.	**Carbohydrate**	Present
3.	**reducing sugar**	Present
4.	**Glycosides**	Present
5.	**Cardiac Glycosides**	Present
6.	**Amino Acid**	Present
7.	**Flavonoids**	Present
8.	**Phenolic compound**	Present
9.	**Tannins**	Absent
10.	**Phlobatannins**	Absent
11.	**Phytosterols**	Present
12.	**Terpenoids**	Present
13.	**Triterpenoides**	Present
14.	**Saponins**	Present

Antibacterial potential of *Ageratum conyzoides*

Ageratum conyzoides petroleum ether leaves extract shows various antibacterial effects against the bacterial strains. Table: 2 represent the antibacterial activity of petroleum ether leaf extract of *Ageratum conyzoides* and positive and negative control, ZOI measured in mm.

Table: 2 Antibacterial activity of PE leaf extract of *Ageratum conyzoides*

Plant	Concentration µg/ml	Bacterial strain zone of inhibition (mm)			
		K. pneumonia	*E. coli*	*S. pneumonia*	*S. aureus*
PE extract of *Ageratum conyzoides*	200	00.0 ± 0.0	00.0 ± 0.0	00.0 ± 0.0	00.0 ± 0.0
	400	00.0 ± 0.0	13.5 ± 0.5	10.5 ± 0.5	11.0 ± 0.5
	600	10.5 ± 0.5	13.0 ± 0.0	12.0 ± 0.7	12.5 ± 0.5
	800	11.5 ± 0.5	11.5 ± 0.5	11.5 ± 1.3	11.0 ± 0.0
	1000	12.0 ± 1.0	10.0 ± 0.0	11.5 ± 0.5	11.0 ± 1.0
Control	Petroleum ether	00.0 ± 0.0	00.0 ± 0.0	00.0 ± 0.0	00.0 ± 0.0
	Ampicillin	10.0 ± 0.0	00.0 ± 0.0	00.0 ± 0.0	10.0 ± 0.0
	Streptomycin	30.0 ± 0.0	23.0 ± 0.0	25.0 ± 0.0	25.0 ± 0.0

The antibacterial activity test reveals that the tested compound exhibits moderate inhibition against *E. coli, S. pneumoniae,* and *S. aureus* at 400 µg/ml, while *K. pneumoniae* remains resistant until 600 µg/ml (fig.2). As the concentration increases, the zone of inhibition stabilizes between 10 mm and 12.5 mm for all bacteria, indicating a dose-dependent response. However, the compound is significantly less potent than streptomycin, which exhibits inhibition zones between 23 mm and 30 mm. Ampicillin shows limited effectiveness, only inhibiting *K. pneumoniae* and *S. aureus* (10 mm each), whereas petroleum ether demonstrates no antibacterial activity, confirming that the solvent does not contribute to the observed effects. Overall, the compound has potential as an antibacterial agent but requires higher concentrations for stronger inhibition, particularly against *K. pneumoniae*.

Discussion

Phytochemical screening confirmed the presence of various bioactive compounds in the *Ageratum conyzoides* plant leaves. These phytochemicals are responsible for many biological activities. The phytochemical screening and antibacterial activity tests indicate that the sample contains several bioactive compounds with potential medicinal applications. The presence of alkaloids, flavonoids, phenolic compounds, glycosides, and saponins suggests strong antimicrobial potential, which correlate with the observed antibacterial effects (Latiffah et al 2024). The antibacterial test results showed that the sample exhibits moderate inhibition against

Escherichia coli, Streptococcus pneumoniae, and *Staphylococcus aureus* at 400 µg/ml, while *Klebsiella pneumoniae* shows resistance until 600 µg/ml. As the concentration of plant extract increases, inhibition zones range between 10 mm and 12.5 mm, indicating a dose-dependent antibacterial effect.

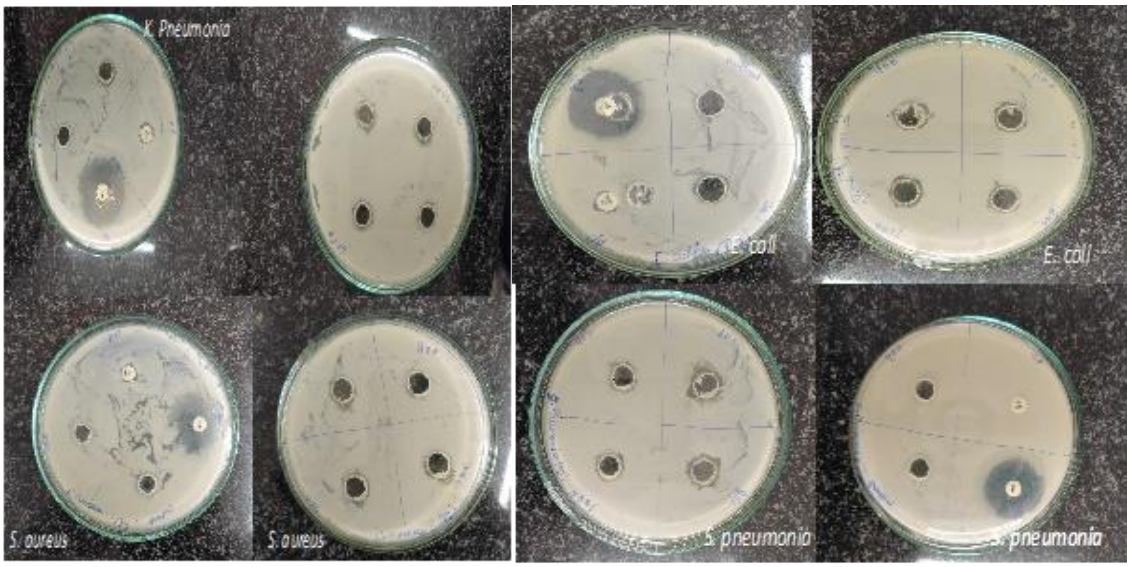

Fig.2 Antibacterial effect of *Ageratum conyzoides* leaf extract

Flavonoids and phenolic compounds are known to disrupt bacterial cell membranes and inhibit bacterial enzymes, contributing to the antimicrobial activity observed (Latiffah et al 2024). Alkaloids, Phytosterols and Saponins may further enhance antibacterial effects by interfering with bacterial metabolism and cell wall integrity (Huang et al 2022, Bakrim et al 2022). The presence of terpenoids and triterpenoids also suggests possible antimicrobial benefits, which might help in combating infections (Wiart et al 2023).

Despite its moderate antibacterial activity, the test sample is significantly less potent than streptomycin, which exhibited inhibition zones of 23–30 mm across all bacterial strains. The sample performed better than ampicillin against *E. coli* and *S. pneumoniae*, indicating selective antibacterial potential.

Conclusion

PE extract of *Ageratum conyzoides* showed the presence of various phytochemicals and antibacterial activity against the gram-positive and gram-negative bacteria. Though the PE extract showed moderate antibacterial effects compared to standard antibiotics, diverse bioactive compounds indicate possible therapeutic applications, particularly in treating bacterial infections and oxidative stress-related diseases. Further studies can be done to assess its potential for pharmaceutical development.

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