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ORIGINAL ARTICLE



In-vitro Piscicidal Activity and Antifungal Activity of *Ageratum conyzoides*

Prakash Pant¹

Abstract

Ageratum conyzoides L., a tropical invasive herb of the Asteraceae family, is traditionally used for its antimicrobial and insect-repellent properties across South America, Africa, and Asia. This study investigates the piscicidal and antifungal activities of its ethanolic leaf extracts to explore its potential as a natural biocontrol agent. Leaves were collected from Udham Singh Nagar, India, authenticated, and extracted with 70% ethanol using a Soxhlet apparatus. Phytochemical screening identified flavonoids, saponins, terpenoids, tannins, and alkaloids as key bioactive constituents. Piscicidal activity was assessed against Rose Danio fish at concentrations of 100, and 200 mg/L over 48 hours, monitoring mortality, time to death. Antifungal activity was evaluated against *Marasmiellus cocophilus* via disc diffusion at 10 and 20 mg/mL, measuring inhibition zones. The extract exhibited dose-dependent piscicidal effects, achieving 100% mortality at 200 mg/L within 48 hours, and notable antifungal activity, with inhibition zones of 7–8 mm at 20 mg/mL or 10 mg/L, approaching fluconazole's efficacy. Terpenoids and flavonoids likely contribute to gill disruption in fish and fungal cell membrane damage. These results suggest *A. conyzoides* as a viable eco-friendly agent for aquaculture and antifungal applications, though further in vivo and toxicity studies are needed.

Key words: *Ageratum conyzoides* L, Piscicidal Activity, Antifungal activity, Rose Danio fish, *Marasmiellus cocophilus*, Phytochemical screening

1 | INTRODUCTION

The word "ageratum" comes from the Greek word "a geras," which means "non-aging," and refers to the longevity of the entire plant. The Greek word "konyz," on the other hand, is the source of the term *Conyzoides*, describing the plant's similarity to *Inula helenium*. In the Asteraceae family, *Ageratum conyzoides* is a member of the Eupatoriae tribe. This family is unique due to its well-defined traits. There are very few trees or shrubs in this family; most plants are herbaceous. The genus *Ageratum* contains around 30 species, of which only a tiny portion have been studied phytochemically. Across Asia, West Africa, and South America, the tropical plant *A. conyzoides* grows. The annual

branching herb can reach a height of approximately one meter (1).

There are tiny white hairs on the stems and leaves of the ovate leaves, which can reach a maximum length of 7.5 cm. (2, 3) The purple to white blooms is grouped in close terminal inflorescences and have a diameter of less than 6 mm. The achene fruits spread effectively; however, the photoblastic seeds are frequently lost in the third month of the year. The plant is widely distributed in waste areas and on abandoned sites, grows well in any garden soil, and is frequently found close to populated areas. Because of its unusual scent, which is compared to that of a male goat, it is known as "goat weed" or "billy goat weed" in Australia (4, 5).

¹Department of Pharmacy, Six Sigma Institute of Technology and Science, Jafarpur -Dineshpur Road, Khanpur Purab, Uttarakhand, 263153, Rudrapur.

Supplementary information The online version of this article contains supplementary material, which is available to authorized users.

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Fig. 1: Flower of *Ageratum Conyzoides* (2)

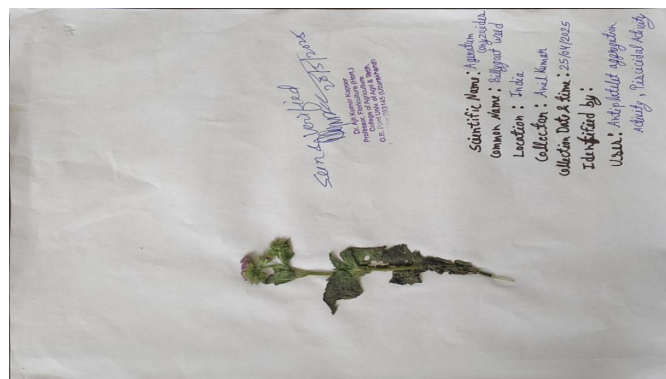


Fig. 2: Verified plant of *Ageratum conyzoides*

2 | MATERIAL AND METHOD

Plant Material Collection and Authentication

Plant Collection

The best phytochemical content was ensured by gathering fresh *Ageratum conyzoides* leaves from a botanical during the growing season (March–April 2025). The plants' brilliant green, variegated leaves and well-developed tuberous roots were indicators of their health, which led to their selection. Several plants were used to harvest about 2 kg of leaves to account for biological variability (6).

The collecting site was picked because of its ideal soil and climate, which encourage strong *A. conyzoides* growth. To stop moisture loss, samples were kept at 4°C until processing and carried in airtight plastic bags.

Authentication

The botanical specimen was verified by a botanist at College of Agriculture, G.B. Pant University of agri. and tech. Representative samples were pressed and dried to create voucher specimens, which were then placed in the College of Agriculture's herbarium at G.B. Pant University of Agribusiness and Technology.

Processing

Collected plant material was washed thoroughly with distilled water to remove soil and debris, then air-dried in a shaded, well-ventilated area at 25–30°C for 7–8 days to prevent degradation of thermolabile compounds. Dried samples were pulverized into a fine powder using a mechanical grinder and stored in airtight containers at room temperature until extraction (7).



Fig. 3: Powder of *Ageratum conyzoides*

Preparation of *Ageratum conyzoides* Extracts

Soxhlet Extraction : 20 g of powdered material was extracted with 100ml ethanol using a Soxhlet apparatus for 8 hours. The extract was concentrated and dried as described above, yielding a crude ethanolic extract stored at 4°C.

Procedure

Weigh the Sample: Measure ~20g of dried, powdered *Ageratum conyzoides*.

Load the Thimble: Place the powder in a thimble and insert it into the Soxhlet chamber.

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Add Solvent: The round bottom flask was filled with 100mL of Ethanol.

- The flask was attached to the Soxhlet apparatus.
- Assemble the Setup: Connect the flask, extractor, and condenser. Heat the flask gently using a heating mantle.
- Start the Extraction.
- The solvent boils, evaporates, and condenses in the condenser.
- Condensed solvent drips into the thimble and extracts soluble compounds.
- Once the chamber is full, it siphons back into the boiling flask.
- This cycle repeats for 6–8 hours.

Post-Extraction

- After completion, the apparatus was allowed to cool.
- The ethanol extract was carefully removed from the round bottom flask.
- Concentrate the Extract: After completion, the solvent is evaporated using a rotary evaporator to get the concentrated crude extract.

Filtration: The extract was filtered through Whatman filter paper to remove any particulate matter.

Dry and Store: The extract is dried, weighed, and stored in a desiccator or at 4°C for further use.



Fig. 4: Group of Rose Danio fish

PHYTOCHEMICAL ANALYSIS

Qualitative Screening

Preliminary phytochemical screening was conducted to identify major classes of bioactive compounds in the aqueous, methanolic, and ethanolic extracts of *A. conyzoides*, following standard protocols (Harborne, 1998) (8) :

Saponins: Froth test – 1 mL of extract was mixed with 5 mL of distilled water and shaken vigorously. Persistent foam for 10 minutes indicated saponins.

Flavonoids: Shinoda test – 1 mL of extract was treated with magnesium turnings and concentrated HCl, producing a pink or red color for flavonoids.

Phenolic Compounds: Ferric chloride test – 1 mL of extract was mixed with 2% FeCl₃, forming a blue-green color for phenolics.

Steroids and Triterpenoids: Salkowski test – 1 mL of extract in chloroform was treated with concentrated H₂SO₄, producing a red color in the chloroform layer for steroids.

Alkaloids: Dragendorff's test – 1 mL of extract was treated with Dragendorff's reagent, forming an orange precipitate for alkaloids.

PISCICIDAL ACTIVITY

Test Organism

The freshwater fish Rose Danio (6.50±0.6 cm in total length and 469 mg wet weight) was collected from the Acquired from a certified hatchery or local aquatic research facility.

Size and Weight: Average length 6–8 cm, weight 5–10 g.

Acclimatization: Fish were acclimatized in laboratory tanks for 7 days before experimentation under standard conditions (25–28°C, 12 h light/dark cycle).

Piscicidal Bioassay Procedure

Experimental Design

- Fish were divided into 2 groups 5 for each concentration.
- Crude extract of *Ageratum conyzoides* was dissolved in Water or a suitable solvent.



Fig. 5: Rose Danio fish and Crude Extract Solution of *Ageratum conyzoides*

Test concentrations: Two concentrations 100, 200 mg/L.

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Fig. 6: Two test Concentrations like:100 and 200mg

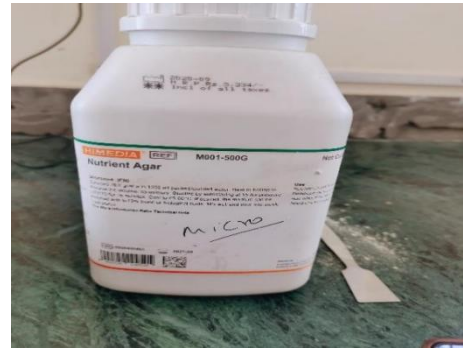


Fig. 9: Preparation of Fungal Culture Medium



Fig. 10: Autoclaving

Exposure Duration: 48 hours



Fig. 7: Exposure Duration of Fish



Fig. 11: Autoclaving



Fig. 8: Preparation of Fungal Culture Medium

ANTIFUNGAL ACTIVITY

In vitro antifungal activity of extracts

Preparation of Fungal Culture Medium

- 15g dextrose and 5g peptone, 11.3g of agar were added in distilled water.
- The final volume was adjusted to 500ml.
- The medium was autoclaved at 121°C and 15 psi for 15 minutes.
- After cooling to about 45°C, it was poured into sterile Petri dishes and allowed to solidify.

Inoculation of Fungal Strains

- Fungal cultures of *Marasmiellus cocophilus* were prepared.
- Each PDA plate was inoculated with the fungal suspension using a sterile cotton swab to ensure uniform distribution.



Fig. 12: Inoculation of Fungal Strains



Fig. 13: Inoculation of Fungal Strains

Application of Extract Using Filter Paper Discs

Sterile Whatman No. 1 filter paper discs (6 mm diameter) were soaked with 10 mg/mL extract solution, 20 mg/mL extract solution.



Fig. 14: Extract using Filter Paper Discs

Discs were air-dried slightly under sterile conditions to avoid excess solvent. The discs were placed onto the surface of inoculated agar plates using sterile forceps. Positive control discs with standard antifungal (e.g., fluconazole) and negative control discs with

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ethyl acetate extract alone were also applied.



Fig. 15: Discs were air-dried slightly under sterile conditions

Incubation and Observation

The plates were incubated at 28°C for 48–72 hours. After incubation, the zones of inhibition around each disc were measured in millimetres using a ruler or calliper. The antifungal activity was compared across the two extract concentrations and controls.



Fig. 16: Incubation and Observation

3 | RESULTS

Piscicidal Activity

Mortality Rates: The ethanolic leaf extract of *A. conyzoides* demonstrated dose-dependent piscicidal activity against Rose Danio. At 100 mg/L, no mortality occurred within 48 hours. At 200 mg/L, 100% mortality was achieved within 48 hours.

Time to Death: The mean time to death decreased with higher concentrations in 200mg/L in 48 hours and 100mg/L no death of any Rose Danio fish.



Fig. 17: Time to death of Fish

Table 1. Time to death of Fish

Sample	Start Time	Time to Death
Ageratum Conyzoides Ethanol Extract (100mg/L)	1:50 PM	No death
Ageratum Conyzoides Ethanol Extract (200mg/L)	1:50 PM	48 hours

4 | ANTIFUNGAL ACTIVITY

Zones of Inhibition: The in vitro antifungal activity of *Ageratum conyzoides* ethanol extract was evaluated against *Marasmiellus cocophilus*. The *Ageratum conyzoides* extract exhibited significant antifungal activity, with higher zones of inhibition. The higher concentration (20 mg/mL) showed more potent antifungal activity compared to the lower concentration (10 mg/mL). The positive control (Fluconazole) showed the highest zone of inhibition.



Fig. 18: Observation of Zone of Inhibition

Table 2. Zone of inhibition of Test and Standard

Sample	Zone of Inhibition (mm)
<i>Ageratum Conyzoides</i> Ethanol Extract (10 mg/mL)	7 mm
<i>Ageratum Conyzoides</i> Ethanol Extract (20 mg/mL)	8 mm
Fluconazole (10 mg/mL)	18 mm
Fluconazole (20 mg/mL)	22 mm

5 | DISCUSSION

The piscicidal activity of *A. conyzoides* ethanolic extract is likely driven by its phytochemical constituents. The dose-dependent mortality, with 100% mortality at 20 mg/L within 48 hours, suggests potential for controlling unwanted fish populations in aquaculture. However, the high concentration required raises concerns about environmental persistence and non-target effects, necessitating ecological impact assessments.

The antifungal activity against *Marasmiellus cocophilus*. The larger inhibition zones in *Marasmiellus cocophilus*. *Marasmiellus cocophilus* showed that the *Ageratum conyzoides* extract exhibited significant antifungal activity, with higher zones of inhibition. The higher concentration (20 mg/mL) showed more potent antifungal activity compared to the lower concentration (10 mg/mL). The positive control (Fluconazole) showed the highest zone of inhibition.

6 | CONCLUSION

The ethanolic leaf extract of *Ageratum conyzoides* exhibits significant biological activity, demonstrating both piscicidal and antifungal properties. The

extract showed a clear dose-dependent piscicidal effect on Rose Danio fish, with complete mortality at 200 mg/L within 48 hours, while no mortality occurred at 100 mg/L, indicating its potential as a natural piscicide at higher concentrations. In terms of antifungal activity, the extract effectively inhibited the growth of *Marasmiellus cocophilus* in vitro. The zones of inhibition increased with extract concentration, confirming a dose-dependent response. Although Fluconazole, the positive control, exhibited the highest antifungal effect, *A. conyzoides* still displayed considerable antifungal potential, particularly at 20 mg/mL.

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