



RESEARCH ARTICLE

Antifungal activity of ethanol and methanol extracts from *Ageratum conyzoides* L. against *Fusarium oxysporum*

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Abstract

Billygoat weed (*Ageratum conyzoides* L.) is generally distributed in tropical and subtropical regions. This herb not only has activity against a range of crop pests and pathogens but also boasts a rich history of traditional medicinal use across various countries worldwide. Studies of *Ageratum conyzoides* have provided evidence of the presence of a wide variety of phytochemicals that are capable of demonstrating its pesticidal, antimicrobial properties. Therefore, this study aimed to investigate the impact of various extracts from the billygoat weed on the antifungal activity against *Fusarium oxysporum*, a fungus that causes diseases for many crops. The billygoat weed powder was extracted with 2 solvents, ethanol and methanol at 4 concentrations (30 %, 50 %, 70 % and 90 %) and the antifungal activity of the extracts was determined by measuring the inhibition of fungal growth diameter at different extract concentrations (0 %, 1 %, 2 %, 4 % and 8 %). The results demonstrate that the E90 and M90 extracts, at a concentration of 8 %, exhibit better antifungal activity compared to the other extracts at the same concentration, with inhibition rates of 87.21 % and 89.77 % respectively. Moreover, when conducting a 2-factor ANOVA analysis, there was an interaction between solvent strength and extract solution concentration on the growth of *Fusarium oxysporum*. Hence, *Ageratum conyzoides* has the potential to be applied in the control of some harmful fungi on plants, oriented as biological pesticides.

Keywords

Ageratum conyzoides; antifungal; billygoat weed; ethanol; *Fusarium oxysporum*; methanol extract

Introduction

Fungal diseases pose a significant global threat, impacting not only humans but also the growth and storage of vegetation. *Fusarium oxysporum* is a fungal species that inflicts diseases on numerous crop types, targeting all parts of plants, particularly their roots (1, 2). This result in symptoms such as vascular wilting, root rot, stem rot, fruit rot and seed damage. *F. oxysporum* is commonly present in soil, existing as chlamydospores or mycelia on plant residues and organic matter. Fusarium wilt, a prevalent disease caused by this fungus, affects a wide range of crops, including bananas, tomatoes, onions and cucumbers, leading to substantial losses in both yield and quality (3, 4). They render foodstuffs unsuitable for consumption by reducing their nutritional value and, at times, releasing harmful mycotoxins (5). Certain

mold metabolites present in food can have lasting health impacts on both humans and animals, leading to immune deficiencies and potentially inducing cancer. Effective and long-term management of this devastating disease presents a significant challenge (6, 7). Currently, the most widely employed control methods involve using chemical treatments to directly eradicate disease-causing pathogens in the soil. Although this approach has advantages in terms of speed, cost-effectiveness, simplicity and efficiency, its prolonged use can lead to the depletion of microbial diversity in the soil, adversely affecting the environment and food safety (8). Agricultural practices such as crop rotation and soil amendments require considerable time and effort, making them unsuitable for large-scale operations (9). In comparison to other preventive approaches, there is an urgent need for biologically derived products that efficiently hinder fungal growth while preserving environmental friendliness and safety for humans and warm-blooded animals (10, 11). This requirement is vital for the development of a sustainable and secure agriculture sector.

Researchers have long been drawn to new active ingredients, particularly those derived from herbs, for their noteworthy biological activities. These activities include insecticidal and antimicrobial properties, making them both safe and environmentally friendly (12, 13). Herbal fungicides, in particular, offer diverse mechanisms of action, broad-spectrum activities and limited resistance development and show promise as alternatives to synthetic fungicides (14, 15). *Ageratum conyzoides*, commonly known as goat weed, is an aromatic herb that belongs to the Asteraceae family and thrives in tropical and subtropical regions (16). It has been extensively studied for its diverse secondary metabolites, which encompass flavonoids, alkaloids, saponins, polyphenols, coumarins, precocene, eugenols, essential oils, alkaloids, tannins, sulfur, chromenes, benzofurans and terpenoids (monoterpenes and sesquiterpenes) (16). Beyond their medicinal properties, these secondary metabolites have drawn attention to their potential impact on agriculture, such as their antimicrobial activity against plant pathogens (17, 18) and their potential in pest control (19, 20). Despite its potential applications in agriculture, there remains a dearth of research on *A. conyzoides* in the field of plant protection. This study aimed to bridge this knowledge gap by examining the inhibitory activity of different *A. conyzoides* extracts against *F. oxysporum*, a fungal pathogen, through in vitro evaluation. This research seeks to expand our understanding of the potential benefits and applications of *A. conyzoides* in agriculture.

Materials and Methods

Collection and preparation of plant materials for extraction

Billygoat weed (*Ageratum conyzoides* L.) was collected in Da Huoai - Lam Dong province, Vietnam. The aerial parts were washed with tap water to remove the adhering dust particles, oven-dried at 50 °C to a constant weight and

crispy texture to aid grinding to powder using a blender and stored in a sterile air-tight labeled container until required.

Pathogen and culture

The *F. oxysporum* strain was provided by the Department of Transformational Biotechnology, Institute of Tropical Biology. Fungal strain isolated from diseased pepper in Dak Nong, Vietnam. Then, the strain was maintained on potato dextrose agar (PDA) in the dark at 25 °C.

Preparation for ethanolic extraction

Billygoat weed powder (30 g) was weighed and extracted with ethanol at 4 different concentrations (30 %, 50 %, 70 % and 90 %) using an ultrasonic bath at 50 °C for 3 h. The plant powder to ethanol was maintained at a ratio of 1:17. The mixture was filtered and all the liquid extracts were combined. The solvent was removed using a rotary evaporator under low pressure (100-160 mBar) at 60 °C and a speed of 100 rpm. This process produces four concentrated extracts, each labeled as E30, E50, E70 and E90. They are stored at 4 °C (5).

Preparation for methanolic extraction

Billygoat weed powder (30 g) was weighed and extracted with methanol at 4 different concentrations (30 %, 50 %, 70 % and 90 %) using ultrasonic waves for 3 h. The plant powder to methanol was maintained at a ratio of 1:17. The mixture was filtered and all the liquid extracts were combined. The solvent was removed using a rotary evaporator under low pressure (100-160 mBar) at 60 °C and a speed of 100 rpm. This process produces 4 concentrated extracts, each labeled as M30, M50, M70 and M90. They are stored at 4 °C (19).

Assessing inhibition of *F. oxysporum* fungal colony growth diameter

The study assessed the effects of the extracts on the growth of *F. oxysporum* based on morphology and colony diameter (21). The procedure included pouring 20 mL of PDA medium with various extracts (E30, E50, E70, E90, M30, M50, M70 and M90 at 1 %, 2 %, 4 % and 8 % concentrations) into 9 cm sterile petri dishes. Control plates consisted of PDA only. Each concentration is tested on 3 plates. Using a sterile hook, a fungal hypha from a 7 days old *F. oxysporum* cultured at room temperature and placed in the center of each petri dish. The petri dishes were incubated in the dark at room temperature in a completely randomized design. The fungal growth diameter (FGD) was measured after 3 days of cultivation.

Statistical analysis

The experiment was repeated three times. The data was analyzed using ANOVA in Minitab 21 software and the mean values were tested using the Tukey test at a significance level of 95 %.

Results and Discussion

Based on the growth rate of *F. oxysporum* mycelium diameter, the antifungal activity of the extracts from

A. conyzooides was determined. In this study, we found that both ethanol and methanol extracts in this study had a positive impact on reducing the growth and development of the fungus.

The effect of ethanol extract on the antifungal activity of *F. oxysporum*

The impact of ethanol extracts on fungal growth after 3 days of incubation was investigated. The results demonstrated that all extract solutions effectively inhibited the development of *F. oxysporum*. Remarkably, E90 displayed superior antifungal properties compared to other extracts, exhibiting the smallest mean colony diameter of 8 % (5.33 mm) when compared to both the control (41.67 mm).

In 2020, secondary compounds in *A. conyzooides*, such as phenols, alkaloids, flavonoids, sterols, tannins, glycosides, coumarins, saponins and anthraquinones were identified (22). Some of these compounds have demonstrated antifungal activity against certain fungi, for example, phenolic compounds against *Fusarium solani* (23) and flavonoids against *Fusarium moniliforme* (24). Based on the scientific evidence, the antifungal potential of *A. conyzooides* ethanol extracts against *F. oxysporum* may be attributed to the presence of these secondary compounds in this plant species.

Table 1. The effect of ethanol extracts at different concentrations on the growth of *F. oxysporum* colonies after 3 days

Extract concentration (%)	Fungal growth diameter (mm)				The average extract concentration
	E30	E50	E70	E90	
0 %	41,67 ^a	41,67 ^a	41,67 ^a	41,67 ^a	41,67 ^A
1 %	32,33 ^{bcd}	37,33 ^{ab}	27,67 ^{cdef}	26,33 ^{def}	31,00 ^B
2 %	30,00 ^{cde}	34,33 ^{bc}	25,33 ^{def}	21,33 ^{fg}	27,75 ^C
4 %	24,67 ^{efg}	25,33 ^{def}	14,00 ^{hi}	9,33 ^{ij}	18,33 ^D
8 %	18,00 ^{gh}	25,33 ^{def}	8,33 ^{ij}	5,33 ^j	14,25 ^E
The average solvent concentration	29,33 ^B	32,80 ^A	23,47 ^C	20,80 ^D	
P (solvent concentration)			< 0,05		
P (extract concentration)			< 0,05		
P (interaction)			< 0,05		

The average values with lowercase letters following each other have no statistical significance (95 %). The average values in the same column with uppercase letters following each other have no statistical significance (95 %). The average values in the same row with uppercase letters following each other have no statistical significance (95 %).

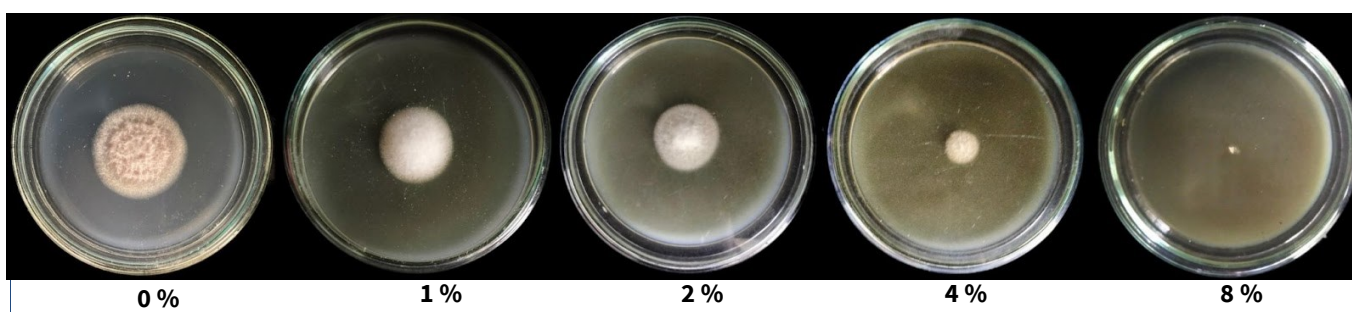


Fig. 1. *Fusarium oxysporum*'s morphology was cultivated on PDA medium supplemented with 90 % ethanol extract (E90 at different concentrations after 3 days. The medium becomes darker in color due to the addition of extracts from low to high concentrations.

The results of the survey on the impact of extract concentration show that there is an influence on the growth of fungi with a p-value < 0.05. The higher the extract concentration, the better the inhibitory effect on fungi. For instance, when using a 1 % E70 extract supplement, the observed FGD measured 27,67 mm, surpassing the measurement at an 8 % extract concentration (8,33 mm) (Table 1, Fig. 1). Similar outcomes were observed for the E30, E50 and E90 formulations.

This experiment was designed with 2 factors: one being the ethanol concentration for extracting the active substance and the other being the concentration of the extract added to the culture medium to assess the effects of these factors on the growth of *F. oxysporum*'s mycelium diameter. The experimental results, analyzed using the 2-factor ANOVA test, revealed a significant interaction effect (p-value < 0.05) between the ethanol concentrations and the extract concentrations. This interaction suggests that both factors have a combined effect on the growth of *F. oxysporum*.

The effect of methanol extract on the antifungal activity of *F. oxysporum*

Methanol extracts were also evaluated for their impact on fungal growth on agar plates after 3 days of incubation. All extract solutions demonstrated the ability to inhibit the development of *F. oxysporum*, with M90 extract showing better antifungal properties compared to the other extracts. It exhibited the lowest mean inhibition zone diameter (4.33 mm) at the same extract concentration in comparison to the control (42.33 mm). This difference was statistically significant with a p-value of < 0.05 (Table 2).

The active compounds in the methanol extract of *A. conyzoides* were analyzed using the GC-MS method, revealing the presence of 8 compounds, among which precocene II was the main compound, accounting for 59.5 % of the total (25). It inhibits the production of trichothecene by *Fusarium graminearum*, the pathogen responsible for leaf blight and trichothecene contamination in cereals (17, 27). Moreover, Caryophyllene, accounting for 7.6 % of the composition, demonstrated rapid and effective inhibition against fungi like *F. solani* (26).

Based on a significance level of $p < 0.05$, it can be concluded that there is a notable difference in the concentrations of the extract solution. Higher concentrations result in greater inhibition and impact the antifungal activity. Without the addition of the M70 extract, the antifungal

activity showed a robust development at 42.33 mm, which decreased to 7.67 mm as the concentration increased to 8 %. As the concentration of the concentrated extract solution increased, the diameter of *F. oxysporum* decreased, and the morphology of the fungal colonies underwent noticeable changes under the influence of this antifungal agent. Without the extract, the fungal mycelium exhibited vigorous growth characterized by fluffy and thick white and pinkish strands. However, in the presence of the extract, the fungal colonies became smoother and appeared brighter white. For instance, Fig. 2 demonstrates that the fungal colonies cultured on a medium containing the extract appeared brighter white and had finer mycelium compared to the control.

The *in vitro* antifungal activity of the methanol extracts from different parts of *A. conyzoides*, including flowers, leaves and stems, against *F. oxysporum* was tested at different concentrations (4 %, 6 %, 8 % and 10 %) over a 6 days cultivation period. The results showed that all plant extracts from different parts were able to inhibit the fungal growth of *F. oxysporum*, with the maximum inhibition observed in the 10 % flower extract with an inhibitory effect of 28.20 %. The leaf extract followed with a 22.4 % reduc-

Table 2. The effect of methanol extracts at different concentrations on the growth of *F. oxysporum* colonies after 3 days

Extract concentration (%)	Fungal growth diameter (mm)				The average of extract concentration
	Extract concentration (%)				
	M30	M50	M70	M90	
0 %	42,33 ^a	42,33 ^a	42,33 ^a	42,33 ^a	42,33 ^A
1 %	34,33 ^b	28,67 ^{cde}	29,67 ^{bcde}	30,33 ^{bcde}	30,75 ^B
2 %	31,00 ^{bcd}	16,00 ^g	21,00 ^f	27,67 ^{de}	23,92 ^C
4 %	33,33 ^{bc}	12,67 ^g	14,33 ^g	12,67 ^g	18,25 ^D
8 %	26,00 ^e	12,00 ^g	7,67 ^{hi}	4,33 ⁱ	12,50 ^E
The average solvent concentration	33,40 ^A	22,33 ^B	23,00 ^B	23,47 ^B	
P (solvent concentration)					$< 0,05$
P (extract concentration)					$< 0,05$
P (interaction)					$< 0,05$

The average values with lowercase letters following each other have no statistical significance (95 %). The average values in the same column with uppercase letters following each other have no statistical significance (95 %). The average values in the same row with uppercase letters following each other have no statistical significance (95 %).

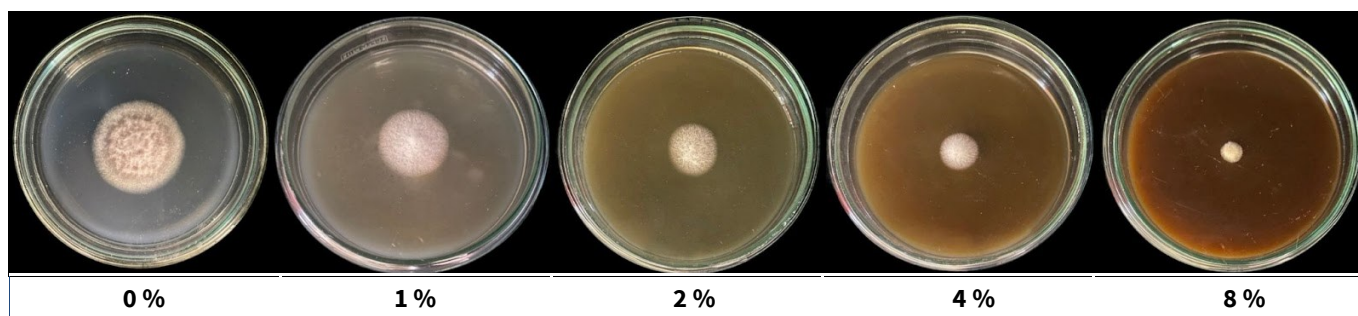


Fig. 2. *F. oxysporum* morphology was cultivated on PDA medium supplemented with 70 % methanol extract (E90) at different concentrations after 3 days. The medium becomes darker in color due to the addition of extracts from low to high concentrations.

tion at 8 % concentration, while the stem extract had the least effect of 13 % reduction at 10 % concentration (28).

When examining the interaction between methanol solvent concentration and extract concentration using a 2-factor ANOVA test, the p-value (interaction) was found to be <0.05. Therefore, both solvent concentration factors for extracting the active compound and additional extract concentration in the medium have an interaction effect on the growth of *F. oxysporum* in the agar medium.

In summary, the efficiency of extracting *F. oxysporum* antifungal compounds is positively influenced by the concentration of extractants and solvents. Solvent concentrations of 90 % ethanol and methanol are suitable for extracting antifungal compounds from *A. conyzoides* powder. The reason is that different solvents produce different natural compounds and therefore, their antibacterial abilities differ (29, 30). Additionally, when different natural compounds are present in the same high extractive type, they interact with each other in a synergistic manner (23, 31), resulting in higher antimicrobial activity.

Conclusion

The obtained results show that the extracts obtained from 90 % ethanol and 90 % methanol solvents have the most effective inhibition on *F. oxysporum* compared to other solvents at low concentrations. The higher the concentration of the extract, the higher the inhibitory effect on the fungus. This means that the extract concentration is directly proportional to the ability to suppress the fungus's growth. Hence, ethanol or methanol can be employed for bioactive compound extraction from the *A. conyzoides* plant, providing a potential biocide application against *F. oxysporum* in agricultural crops.

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Authors' contributions

TTTP participated in designing the study, supported all experiments and performed the statistical analysis, processing and writing of the paper. NBKT carried out experiments on antifungal activity and collected data. NDTN carried out methods of extracting active ingredients. NTNQ supported in collecting samples, guidance, editing

and reviewing papers.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None

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