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Weeds in oil palm plantations and their antifungal activity against *Ganoderma boninense*

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Abstract. *Sinong GF, Sahrir MAS, Yusoff N, Adiwena M, Ali NS, Ibrahim MH, Awang A, Rakib MRM. 2026. Weeds in oil palm plantations and their antifungal activity against* Ganoderma boninense*. Asian J Agric 10: g100102. https://doi.org/10.13057/asianjagric/g100102.* Basal Stem Rot (BSR) caused by *Ganoderma boninense* remains the most destructive disease of oil palm, threatening global palm oil production. Current management strategies have proven largely ineffective in providing long-term disease control, highlighting the urgent need for sustainable approaches based on natural bioactive compounds. Plant-derived metabolites represent a promising alternative due to their natural antifungal properties, environmental safety, and potential compatibility with integrated disease management systems. Weeds, in particular, are often resilient to pathogens and may serve as unexplored reservoirs of bioactive compounds with antifungal potential. The present study aimed to identify weed species associated with healthy oil palm trees and evaluate their extracts' in vitro antifungal properties against *G. boninense*. Three weed species, namely *Hoya carnosa* (W16), *Ischaemum muticum* (W18), and *Polygala paniculata* (W19), were found exclusively in association with healthy oil palm trees. Their crude extracts were evaluated in vitro against *G. boninense* using solvents of varying polarity. Among them, *P. paniculata* exhibited the strongest antifungal activity, with both aerial and below-ground parts showing pronounced inhibition. Below-ground part extracts consistently outperformed aerial parts, particularly when extracted with methanol, which yielded the highest inhibition and lowest EC_{50} values, suggesting a higher concentration of bioactive metabolites in root tissues. The observed antifungal efficacy correlated with solvent polarity, emphasizing the importance of targeted extraction in isolating effective phytochemicals. These findings indicate that *P. paniculata*, especially its methanolic root extract, represents a promising source of natural antifungal compounds. The study highlights the potential of weed plants as unconventional reservoirs of bioactive metabolites and provides a foundation for developing eco-friendly, broad-spectrum fungicides to combat *G. boninense*, thereby advancing sustainable disease management in oil palm plantations.

Keywords: Effective concentration, *Elaeis guineensis*, in vitro inhibitory, methanol extract, *Polygala paniculata*

Abbreviations: ANOVA: Analysis of Variance, BSR: Basal Stem Rot, CRD: Completely Randomised Design; DMSO: Dimethyl sulfoxide, EC: Effective Concentration, FFB: Fresh Fruit Bunch, ITS: Internal Transcribed Spacer, MIC: Minimum Inhibition Concentration, PDA: Potato Dextrose Agar, SAS: Statistical Analysis System

INTRODUCTION

Weed plants exert allelopathic effects, influencing the growth and development of neighbouring organisms through the release of secondary metabolites. Allelopathy is a naturally occurring phenomenon whereby plants and associated organisms such as algae, fungi, and bacteria secrete bioactive compounds that either suppress or stimulate other species (Kong et al. 2019). These allelochemicals are structurally diverse, encompassing terpenes, alcohols, phenolic acids, flavonoids, lactones, coumarins, polyphenols, glycosides, alkaloids, and aldehydes (Tran et al. 2016). Importantly, several of these chemical classes have demonstrated antifungal activity against phytopathogens (Meela et al. 2019), highlighting their potential for exploitation in crop protection. For example, extracts from *Melianthus comosus* have shown significant antifungal activity against various phytopathogens in both in vitro assays and field trials (Eloff et al. 2018), while *Withania somnifera* extracts effectively suppressed *Ascochyta rabiei* infections (Javaid et al. 2020). Such findings indicate that weeds, long regarded as agricultural nuisances, may instead serve as promising sources of antifungal metabolites for developing environmentally sustainable, plant-derived fungicides.

This potential is especially relevant for oil palm (*Elaeis guineensis*) production, which faces a major threat from Basal Stem Rot (BSR) caused by *Ganoderma boninense*

Pat. The disease is regarded as the most destructive disease of oil palm, severely compromising plantation productivity and profitability. The pathogen colonises root and stem tissues, causing progressive canopy wilting, yield decline, and eventual palm death (Rakib et al. 2015). Economic losses are both direct, through palm mortality, and indirect, through reduced Fresh Fruit Bunch (FFB) weight. Yield reductions of 0.04-4.34 tons ha^{-1} have been reported in palms aged 10-20 years (Midot et al. 2019; Olaniyi and Szulczyk 2020). The economic impact is equally severe, with losses reaching up to 68.73%, translating into an average of USD 4,112.78 per hectare annually (Kamu et al. 2021). Despite extensive research, conventional control strategies such as sanitation (removal of infected palms), preventive barriers, and chemical control have shown limited success in providing long-term management of the disease. Chemical approaches, such as the application of systemic fungicides such as hexaconazole has been evaluated in oil palm plantations, but generally provide inconsistent and short-term suppression rather than effective disease eradication (Maznah et al. 2015; Khoo and Chong 2023). Moreover, prolonged and repeated fungicide applications increase production costs and raise concerns regarding environmental contamination, non-target effects on beneficial soil microorganisms, and the potential development of pathogen resistance (Siddiqui et al. 2021). These limitations highlight the urgent need for alternative, eco-friendly disease management strategies that can be integrated into sustainable oil palm plantation practices.

In the exploration of bioactive compounds, the choice of solvent plays a crucial role in determining the yield, composition, and biological activity of plant extracts (Saini et al. 2025). Solvents differ in polarity, which directly affects their ability to dissolve and extract specific groups of phytochemicals such as alkaloids, flavonoids, terpenoids, or phenolic compounds (Jibhkate et al. 2023; Ghaffar and Perveen 2025). Consequently, solvent selection not only influences extraction efficiency but also determines the spectrum and potency of antifungal activity observed in vitro (Ghaffar and Perveen 2025).

One promising avenue lies in harnessing the antifungal potential of weed-derived secondary metabolites. Plants naturally produce a wide range of bioactive compounds that enhance their survival by inhibiting pathogenic colonisation (Franzoni et al. 2019; Riseh et al. 2022). Weeds frequently found in association with healthy oil palm stands may possess unique phytochemicals that contribute to disease suppression in the rhizosphere, either by directly inhibiting *G. boninense* or by altering microbial interactions in ways that reduce pathogen establishment. Their demonstrated resistance to pathogens suggests that these species may act as a reservoir of antifungal compounds, with possible applications in biological control strategies. However, research investigating the antifungal properties of weeds against *G. boninense* remains limited, representing a significant knowledge gap.

The present study addresses this gap by identifying weed species consistently associated with healthy oil palm trees and evaluating their in vitro antifungal activity against *G. boninense*. Ultimately, the study contributes to the broader goal of developing environmentally acceptable fungicides based on natural products. Leveraging the phytochemical diversity of weeds offers a novel, sustainable strategy for managing *G. boninense*, with the potential to transform a major agricultural challenge into an opportunity for eco-innovation in oil palm disease management.

MATERIALS AND METHODS

Weed selection and quantitative analysis

The selection of weeds to be examined for their antifungal potential was conducted using quantitative analysis in an oil palm plantation at the Faculty of Sustainable Agriculture, Universiti Malaysia Sabah, Malaysia (5.9297° N, 118.0107° E), with matured oil palm trees (14-year-old), to discriminate the weed species to be selected for in vitro assays. The weeds found adjacent to the healthy (symptomless) and *Ganoderma*-infected oil palm trees were recorded, where each category of oil palm trees used 10 circle quadrants with a radius of one meter around an oil palm tree (Zanella et al. 2017; Ashraf et al. 2018; Kanwal 2024). The quantitative analysis of weeds includes the frequency, the relative frequency, the density, and the relative density quantified according to Akhter et al. (2019), using the equations below. The weeds growing adjacent to the healthy and *Ganoderma*-infected oil palm trees were compared to find any distribution pattern or association. Weeds found exclusively or relatively abundance adjacent to the healthy oil palm trees were collected for further analysis.

$$\text{Frequency (\%)} = \frac{\text{Number of quadrat in which the species occurred}}{\text{Total number of quadrats studied}} \times 100$$

$$\text{Relative frequency (\%)} = \frac{\text{Frequency of the species}}{\text{Total frequency of all the species}} \times 100$$

$$\text{Density (individual per m}^2\text{)} = \frac{\text{Total no.of individuals of the species in all the quadrats}}{\text{Total number of quadrats studied} \times \text{area of the quadrat}} \times 100$$

$$\text{Relative density (\%)} = \frac{\text{Density of the species}}{\text{Total density of all the species}} \times 100$$

Collection and processing of weed samples

Weeds were collected based on the exclusiveness and relative abundance of the weeds adjacent to healthy oil palm trees. The whole plants were harvested and washed with running tap water to remove soil and dirt. Subsequently, the plants were dried for 48 hours at 70°C (Bayat et al. 2019). The dried weed samples were then separated into two partitions, i.e., the aerial parts (plant's part above the ground level or shoot, including stem, leaves, and flowers) and below-ground (plant's part below the ground level or roots) biomass, finely ground using an electric grinder, and kept in an airtight container in -20°C until further use. The ground samples were considered crude powder.

In vitro antifungal assays

Antifungal activity of selected weed's crude powder against Ganoderma boninense

Crude powder of aerial and below-ground parts from selected weed species were tested for their in vitro antifungal activity against *G. boninense*. An authenticated *G. boninense* isolate, previously recovered from a basal stem rot-infected oil palm and identified via Internal Transcribed Spacer (ITS) gene sequencing (Darwana et al. 2023), was sourced from the culture collection of the Faculty of Sustainable Agriculture, Universiti Malaysia Sabah (Malaysia). The in vitro antifungal activity assay was conducted using the poisoned agar method (Kiran et al. 2023; Go et al. 2025), where potato dextrose agar (PDA) with standard preparation (39 g L^{-1}) was mixed with crude weed powder at the concentrations of 0.5%, 1.0%, 1.5%, and 2.0% (w/v). The PDA without the crude weed powder was used as a control. The mixtures of crude powder and PDA were autoclaved for 15 min. at 121°C. The mixture was then poured into a 90 mm diameter Petri dish, solidified, and then inoculated with a 5 mm mycelium plug from a 7-day-old active culture at the edge of the Petri dish. Each concentration consisted of five replications, with two parts, resulting in a total of 50 experimental units. The experimental unit was then incubated for 14 days at 30°C temperature in an incubator with a Completely Randomised Design (CRD) experimental layout. The radial extension of *G. boninense* on each plate was measured from the edge of the mycelium plug to the advancing margin of the colony, and the percentage inhibition relative to the control was calculated using the equation below (Srivastava and Singh 2011). The experiment was repeated twice to ensure reproducibility.

$$\text{Percentage of inhibition (\%)} = 100 - \frac{\text{Fungal radial growth on treated plate}}{\text{Fungal radial growth on control}} \times 100$$

Antifungal activity of Polygala paniculata *crude extracts against* Ganoderma boninense

Polygala paniculata (W19) was selected for subsequent assessment of its crude extracts against *G. boninense* based on its inhibitory potential observed in the crude powder assay. Crude powders (aerial and below-ground parts) *P. paniculata* was extracted using solid-liquid and liquid-solid phase Soxhlet extraction (Bitwell et al. 2023). Solvents of increasing polarity, namely hexane (0.009), ethyl acetate (0.228), chloroform (0.259), and methanol (0.762), were used for extraction (Jibhkate et al. 2023). Firstly, the sample underwent a solid-liquid phase, where 10 g of *P. paniculata* crude powder was added with 250 mL of solvent, stirred using a magnetic stirrer at 180 rpm for six hours, and filtered to collect the liquid extract. After that, the filtered liquid undergoes the liquid-solid phase, where the liquid extract was heated using a rotary evaporator at 48°C temperature and a flask rotating at 150 rpm until the solvent evaporates.

The growth-inhibitory effect of *P. paniculata* crude extracts against *G. boninense* were tested using the poisoned agar method stated earlier. The crude extract was added with 1% v/v dimethyl sulfoxide (DMSO) as co-solvent to dissolve the extract (Aziz et al. 2019), then incorporated into molten PDA (maintained below 50°C to prevent degradation of bioactive compounds) at concentrations of 0% (control), 0.02%, 0.04%, 0.06%, 0.08%, and 0.10% (w/v). The mixture was homogenized, and poured into a 90 mm diameter Petri dish, and solidified before used. The control PDA plate contains DMSO without any extract. A 7-day-old active mycelium plug (5 mm) of *G. boninense* was placed onto the PDA at the center. Each treatment has five replications, with two parts and four types of solvents, resulting in a total of 240 experimental units arranged in CRD. The experimental units were incubated for seven days at 30°C temperature in an incubator, and then the percentage of inhibition was calculated based on the diameter growth of *G. boninense* using a similar equation as described previously. The experiment was repeated twice to ensure reproducibility.

Data analysis

Analysis of Variance (ANOVA) was performed with the assumptions of data normality, variance homogeneity, observations independence. The means were compared using Tukey's test at a significance level of 5% ($p \leq 0.05$). The data were subjected to regression analysis to evaluate the trend across the weed's crude extract concentration, where the trends were fitted to either a linear or quadratic model (whichever fit the most). Quantitative data on weed species abundance, expressed as frequency and density, were subjected to Chi-square test of independence for association to determine whether the distribution of weed species differed significantly between areas adjacent to healthy and *Ganoderma*-infected oil palm trees. All statistical analysis was performed using Statistical Analysis System Version 9.4 (SAS 9.4).

RESULTS AND DISCUSSION

Weed selection and quantitative analysis

Twenty weed plants were identified (Figure 1), and quantitative analysis of the plants' distribution adjacent to the healthy and *Ganoderma*-infected oil palm trees, based on their frequency, relative frequency, density, and relative density, were presented in Table 1. Among the weed plants, 12 species were growing adjacent to the surroundings of both healthy and *Ganoderma*-infected oil palm trees. In the healthy oil palm areas, *Axonopus compressus* (W03) (23.19%) and *Cleome rutidosperma* (W12) (15.22%) dominate, followed by *Spermacoce latifolia* (W01) (9.60%) and *P. paniculata* (W19) (9.84%), whereas, in the *Ganoderma*-infected areas, *A. compressus* (W03) (28.53%) was the most dominant weed, followed by *S. latifolia* (W01) (13.26%) and *Ageratum conyzoides* (W02) (8.36%). *Plectranthus* species (W06), *Clidemia hirta* (W08), *Eleutheranthera ruderalis* (W09), *Spermacoce remota* (W13), and *Melastoma malabathricum* (W15) were found exclusively distributed adjacent to *Ganoderma*-infected oil palm trees. Meanwhile, three weed plant species, namely *Hoya carnosa* (W16), *Ischaemum muticum* (W18), and *P. paniculata* (W19), were exclusively distributed adjacent to the healthy oil palm trees.

Figure 1. Twenty weed plants species found in an oil palm plantation at the study area in Sabah, Malaysia

The weed *P. paniculata* showed the highest occurrence adjacent to the healthy oil palm trees with relative frequency and relative density of 10.91% and 9.84%, respectively. A Chi-square test of association revealed a highly significant difference in weed species abundance between areas adjacent to healthy and *Ganoderma*-infected oil palm trees ($p \leq 0.001$). This indicates that the distribution and abundance of weed species were not random but were significantly influenced by the health status of the oil palm, with certain species showing site-specific presence as illustrated in the Venn diagram in Figure 2. Therefore, *H. carnosa*, *I. muticum*, and *P. paniculata* were further evaluated for their antifungal activity against *G. boninense*.

Table 1. Quantitative analysis of weed plants distribution adjacent to the healthy and *Ganoderma*-infected oil palm trees

Plant	Frequency (%)		Relative frequency (%)		Density (Individuals per m^2)		Relative density (%)	
	Healthy oil palm	*Ganoderma*-infected oil palm	Healthy oil palm	*Ganoderma*-infected oil palm	Healthy oil palm	*Ganoderma*-infected oil palm	Healthy oil palm	*Ganoderma*-infected oil palm
Spermacoce latifolia (W01)	50.00	40.00	9.09	7.84	1.30	1.46	9.60	13.26
Ageratum conyzoides (W02)	20.00	20.00	3.64	3.92	0.35	0.92	2.58	8.36
Axonopus compressus (W03)	60.00	80.00	10.91	15.69	3.15	3.15	23.19	28.53
Peperomia pellucida (W04)	30.00	10.00	5.45	1.96	0.13	0.29	0.94	2.59
Mikania micrantha (W05)	20.00	30.00	3.64	5.88	0.13	0.29	0.94	2.59
Coleus sp. (W06)	0.00	20.00	0.00	3.92	0.00	0.41	0.00	3.75
Oxalis barrelieri (W07)	40.00	30.00	7.27	5.88	1.08	0.38	7.96	3.46
Clidemia hirta (W08)	0.00	10.00	0.00	1.96	0.00	0.03	0.00	0.29
Eleutheranthera ruderalis (W09)	0.00	10.00	0.00	1.96	0.00	0.06	0.00	0.58
Oplismenus hirtellus (W10)	20.00	20.00	3.64	3.92	0.16	0.48	1.17	4.32
Hyptis capitata (W11)	40.00	20.00	7.27	3.92	0.35	0.10	2.58	0.86
Cleome rutidosperma (W12)	50.00	40.00	9.09	7.84	2.07	0.57	15.22	5.19
Spermacoce remota (W13)	0.00	20.00	0.00	3.92	0.00	0.16	0.00	1.44
Phyllanthus urinaria (W14)	20.00	10.00	3.64	1.96	0.54	0.03	3.98	0.29
Melastoma malabathricum (W15)	0.00	10.00	0.00	1.96	0.00	0.03	0.00	0.29
**Hoya carnosa* (W16)	20.00	0.00	3.64	0.00	0.38	0.00	2.81	0.00
Spermacoce exilis (W17)	20.00	20.00	3.64	3.92	1.05	0.32	7.73	2.88
**Ischaemum muticum* (W18)	10.00	0.00	1.82	0.00	0.03	0.00	0.23	0.00
**Polygala paniculata* (W19)	60.00	0.00	10.91	0.00	1.34	0.00	9.84	0.00
Davallia divaricata (W20)	20.00	20.00	3.64	3.92	0.13	0.19	0.94	1.73

Note: *: Weed plant species exclusively occurred adjacent to the healthy oil palm area

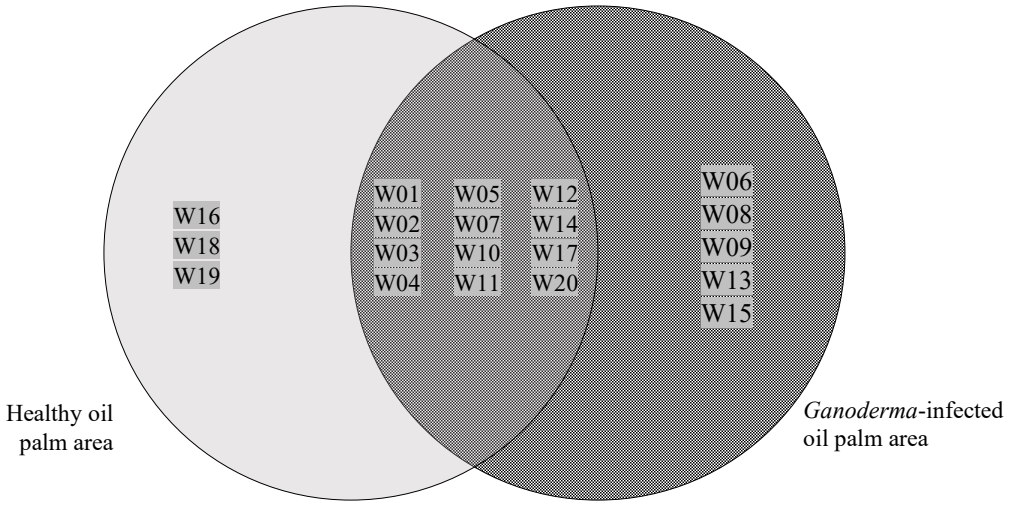

Figure 2. Venn diagram showing the distribution of weed plants species near the healthy and *Ganoderma*-infected oil palm trees

In vitro antifungal activity of weed plant species exclusively occurred near the healthy oil palm trees against *G. boninense*

Three weed plant species, *H. carnosa* (W16), *I. muticum* (W18), and *P. paniculata* (W19) were screened for their antifungal inhibition against *G. boninense* using different crude powder concentrations from their aerial parts and below-ground biomass (Table 2). The negative values indicate the plant promotes *G. boninense* growth rather than inhibition. The crude powder extracts of *P. paniculata* shoots and roots exhibited forceful antifungal activity at all concentrations (97.42-100% inhibition), with no noticeable *G. boninense* mycelial growth (Figure 3), indicating the lowest concentration of *P. paniculata* (W19) crude powder at 0.5% was sufficient to be highly effective in inhibiting *G. boninense*. The aerial part of *I. muticum* (W18) promoted fungal growth, where stronger effects were recorded at higher concentrations. In contrast, the below-ground part of *I. muticum* (W18) showed minimal inhibition at higher concentrations (up to 9.30%) and promoted *G. boninense* growth at 0.5%, indicating very weak antifungal potential. *Hoya carnosa* consistently promoted the fungal growth across all concentrations in both aerial and below-ground parts, indicating no antifungal activity within the tested concentration. Therefore, *P. paniculata* (W19) was selected for further evaluation of its crude extracts on antifungal effectiveness against *G. boninense*.

In vitro antifungal activity of *P. paniculata* extracts against *G. boninense*

In vitro antifungal activity of crude extracts derived from the aerial and below-ground parts of *P. paniculata*, respectively, against *G. boninense* across a gradient of concentrations, using four different solvents of increasing polarity i.e., hexane, ethyl acetate, chloroform, and methanol, corroborated by regression analysis is shown in Figures 4 and 5. Among the aerial parts, methanol and chloroform extracts exhibited potential antifungal activity, with inhibition increasing gradually from 27.69% at 0.02% to 60.77% at 0.10%, and 20.00% at 0.02% to 69.23% at 0.10%, respectively. In addition, the linear regression equation of methanol extract ($y = 655.94x$, $R^2 = 0.9588$) and the quadratic regression equation of chloroform extract ($y = -6832.3x^2 + 1362.9x$, $R^2 = 0.9854$) confirm a strong positive correlation between extract concentration and inhibitory activity. In contrast, hexane and ethyl acetate extracts exhibited effects, with a maximum inhibition of 50.77% and 30.77%, respectively, at the highest concentration. Furthermore, the potential of the extracts was compared based on the EC_{50} values (effective concentration to inhibit 50% of *G. boninense* growth), which was estimated based on the linear regression equations. Lower EC_{50} values indicate higher potential. The EC_{50} of chloroform extract was 0.048%, followed by methanol (0.076%), hexene (0.103%), and ethyl acetate (0.174%).

The below-ground part extracts exhibited a distinctly different profile. The methanol extract of *P. paniculata* showed the highest inhibition against *G. boninense*, reaching 75.77% at 0.10%. Ethyl acetate and hexane extracts followed closely, both showing considerable inhibition of 61.54% and 60.77%, respectively, at the highest concentration. Interestingly, unlike in the aerial parts, hexane extract from below-ground performed better. The chloroform extract from below-ground, in contrast, showed the weakest activity (25.00%) as compared to the aerial part (69.23%), at the highest concentration. Similarly, a strong positive correlation between the extract concentration of the below-ground part and inhibitory activity was observed, with a correlation coefficient, R^2, ranging between 0.9079 to 0.9724. The EC_{50} values from the below-ground methanol extract were 0.028%, followed by ethyl acetate (0.070%), hexane (0.072%), and chloroform (0.175%).

Overall, methanol extract was identified as the most effective extraction solvent for both aerial and below-ground parts, consistently yielding the relatively lower EC_{50} and higher inhibition percentage (Figure 6). The root extracts showed generally higher inhibition across all solvents, especially for methanol, indicating a higher concentration or diversity of antifungal compounds in the roots compared to the shoots.

Table 2. In vitro inhibition activity of selected weed plants' crude powder against *Ganoderma boninense*

Plant and partition	Crude powder concentration, % (w/v)				p-value
	0.5	1.0	1.5	2.0	
H. carnosa (W16), aerial	-19.53±1.47^{abc}	-23.04±3.65^{abc}	-27.91±0.51^{cc}	-28.04±0.73^{cd}	.0234
I. muticum (W18), aerial	-14.41±1.03^{ab}	-17.19±2.32^{ac}	-28.85±2.54^{bc}	-33.43±1.99^{bd}	<.0001
P. paniculata (W19), aerial	97.42±0.35^{ba}	100.00±0.00^{aa}	100.00±0.00^{aa}	100.00±0.00^{aa}	<.0001
H. carnosa (W16), below-ground	-27.10±3.67^{ac}	-26.63±4.11^{ac}	-19.76±4.72^{ac}	-16.22±2.70^{ac}	.1713
I. muticum (W18), below-ground	-11.06±3.41^{bb}	2.14±3.90^{abb}	6.18±3.46^{cb}	9.30±3.21^{cb}	.0043
P. paniculata (W19), below-ground	100.00±0.00^{aa}	100.00 ±0.00^{aa}	100.00±0.00^{aa}	100.00±0.00^{aa}	-
p-value	<.0001	<.0001	<.0001	<.0001	

Note: Negative values indicating the weed's crude powder promoted the growth of the fungi, *G. boninense*. Means (± Standard error) followed with different uppercase within a row, and lowercase within a column were significantly different at $p \leq 0.05$ by Tukey's test

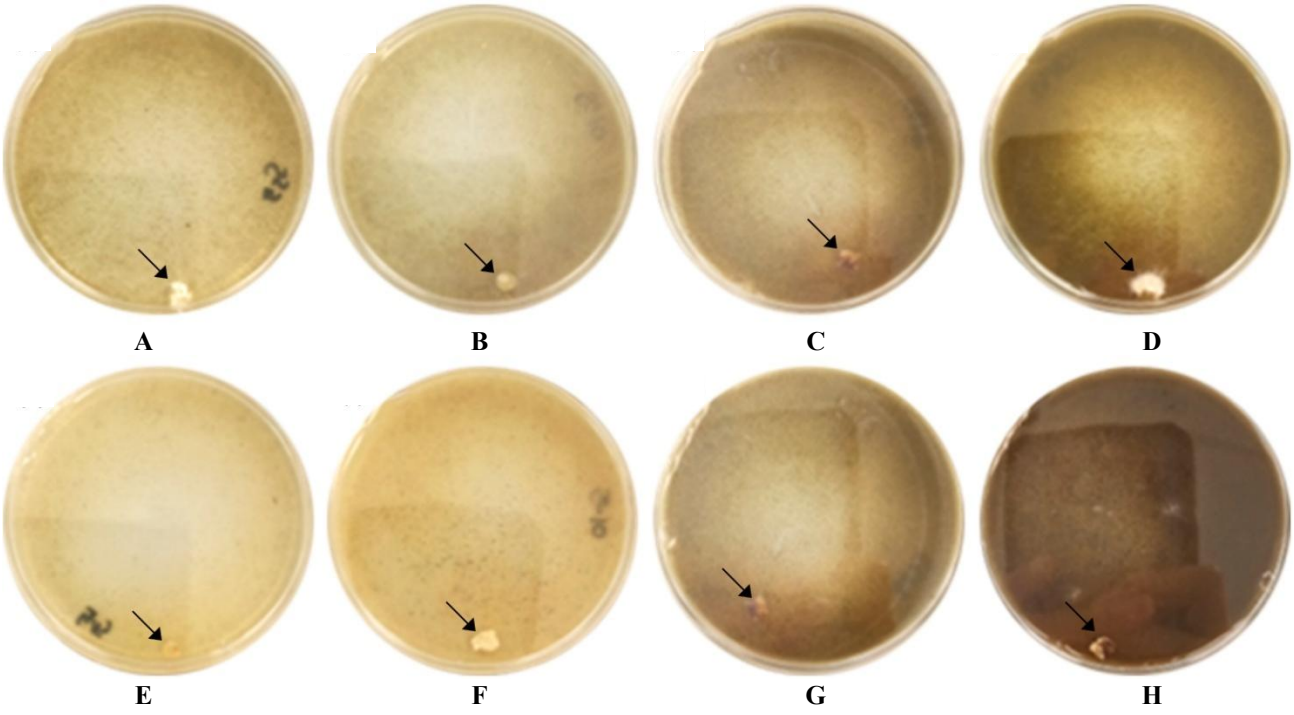

Figure 3. In vitro inhibition of *Polygala paniculata* crude powder across the concentrations against *Ganoderma boninense.* Aerial part of *P. paniculata* at A. 0.5%, B. 1.0%, C. 1.5%, and D. 2.0%. Below-ground part of *P.* paniculata at E. 0.5%, F. 1.0%, G. 1.5%, and H. 2.0%. Note that the colour variations were due to the amount of plant's crude powder added into the agar media, and the arrow indicating the mycelium plug of *G. boninense*

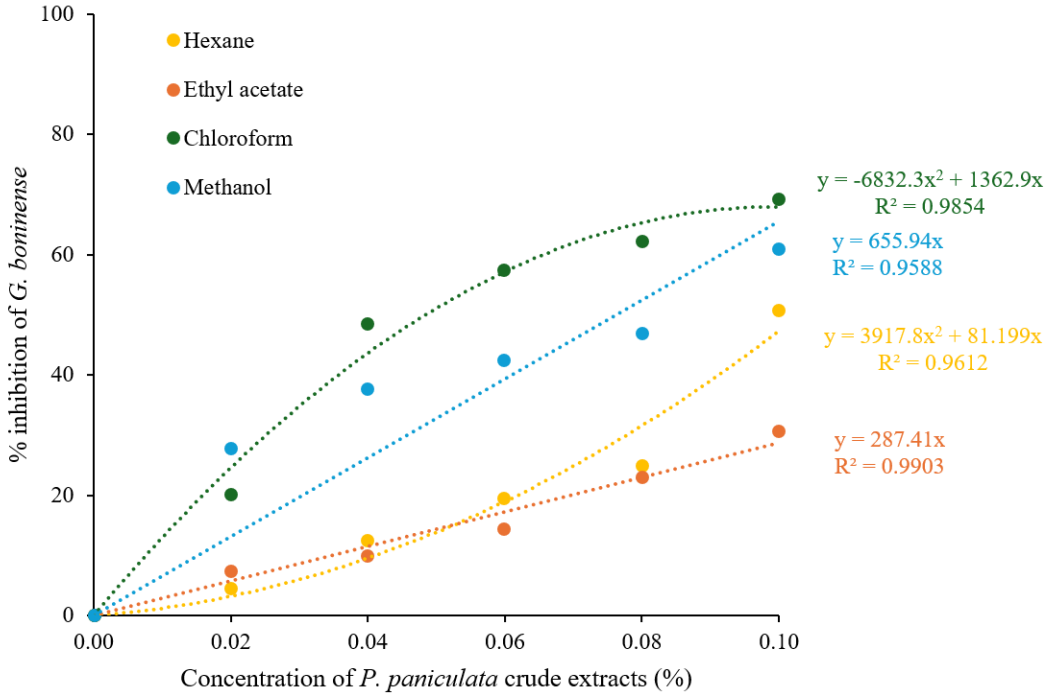

Figure 4. In vitro inhibition activity of *Polygala paniculata* aerial part against *Ganoderma boninense* using different concentrations of crude extracts and solvents

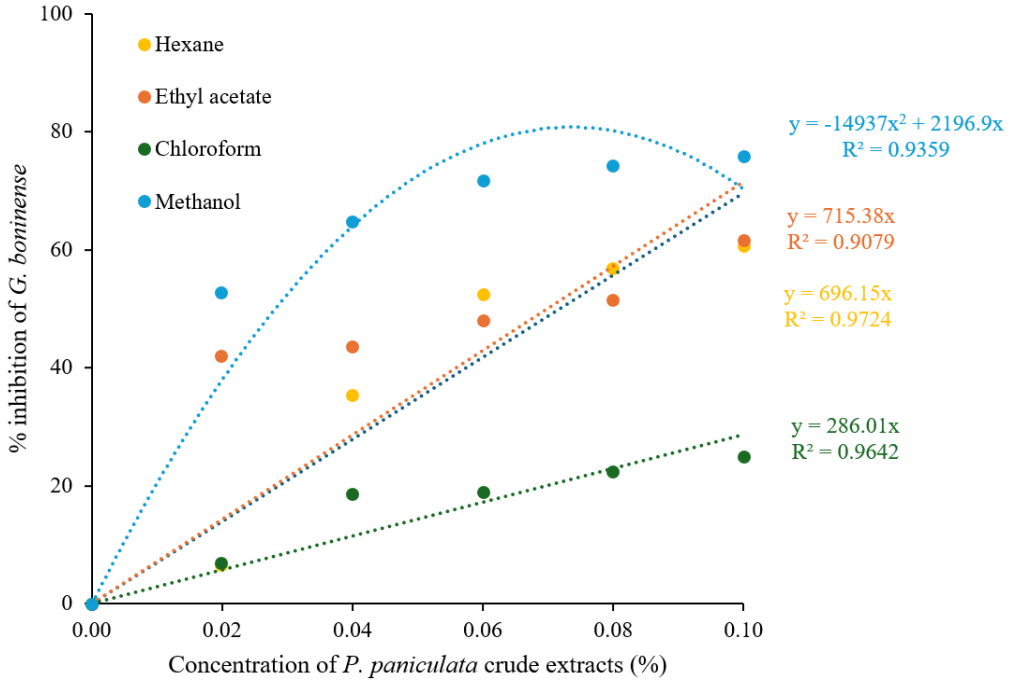

Figure 5. In vitro inhibition activity of *Polygala paniculata* below-ground part against *Ganoderma boninense* using different concentrations of crude extracts and solvents

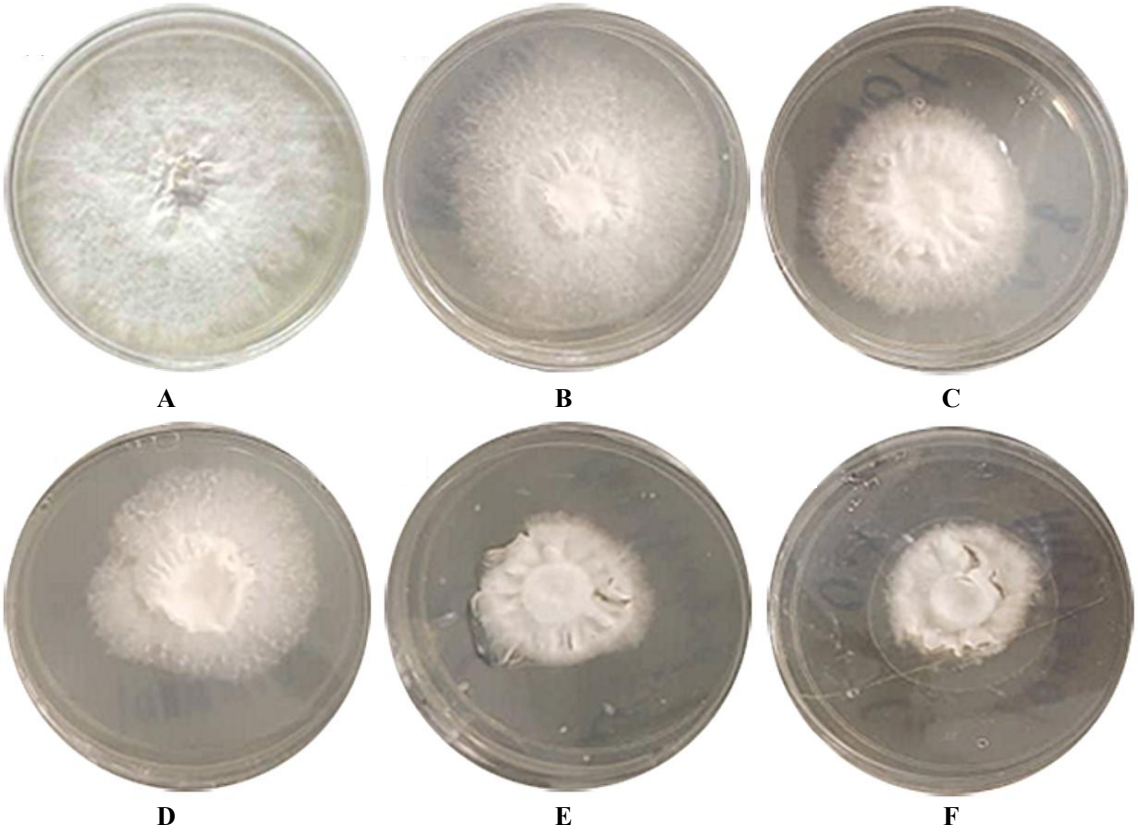

Figure 6. In vitro inhibition of *Polygala paniculata* (aerial part) methanol extract against *Ganoderma boninense* across the concentrations, A. Control, B. 0.02%, C. 0.04%, D. 0.06%, E. 0.08%, and F. 0.10%

Discussion

The significant association between weed species abundance and oil palm health status suggests that biotic stress caused by *G. boninense* infection alters the surrounding microenvironment, influencing weed establishment and composition. Disease-induced changes such as increased soil moisture, nutrient leaching, and altered microbial activity can create ecological niches favourable to specific weed taxa (Lisnawita et al. 2019; Yurnaliza et al. 2019). For instance, *M. malabathricum* and *C. hirta* are often reported as pioneer or disturbance-tolerant species capable of colonizing degraded or pathogen-affected soils. *Melastoma malabathricum*'s tolerance to various soil conditions and its phytoremediation capabilities, along with *C. hirta*'s invasive nature and adaptability to disturbed environments, support the statement effectively (Saberi et al. 2024). The dominance of *P. paniculata* adjacent to the healthy oil palm area could be linked to its ability to produce various bioactive compounds, which may help in creating a nutrient-balanced and microbially stable microenvironment by suppressing harmful pathogens and promoting beneficial microbial communities (Zhao et al. 2020; Ünlü et al. 2022; Tang et al. 2024). The variation in weed distribution reflects common ecological patterns, where interactions between plants and pathogens can indirectly influence the growth of surrounding vegetation by changing soil conditions, root secretions, or microbial communities. Thus, the differences observed in weed composition may result from both biotic interactions such as competition or allelopathy, and abiotic changes such as shifts in soil nutrients and moisture (Wu et al. 2017; Aziz et al. 2021).

In this study, *P. paniculata* showed antifungal activity against *G. boninense* in this study, proving that extractable phytochemicals from weed plants associated with healthy oil palm trees contained antifungal compounds with potential interest in agricultural application, especially in the management of basal stem rot in oil palm. The in vitro inhibition of *P. paniculata* against *G. boninense* markedly outperformed previously reported plant species. In this study, the EC_{50} values of methanolic extracts from the aerial and below-ground parts of *P. paniculata* were determined to be 0.076% and 0.028%, respectively, indicating strong inhibitory potency at low concentrations. By comparison, Wong et al. (2020) reported that substantially higher concentrations of methanolic extracts were required to achieve comparable inhibition, with 5% extracts of mistletoe fig (*Ficus deltoidea*) and God's crown (*Phaleria macrocarpa*), and 15% extract of betel (*Piper betle*) necessary to suppress at least 50% of *G. boninense* mycelial growth in vitro. In addition, Suwandi et al. (2022) reported less than 25% inhibition by 0.1% ethyl root exudates from tannia (*Xanthosoma sagittifolium*) and Indian shot (*Canna indica*).

To date, no study has reported the efficacy of *Polygala* species against *G. boninense*. Nevertheless, several members of the genus, including *P. paniculata*, *P. sabulosa*, *P. campestris*, *P. pulchella*, and *P. cyparissias*, have demonstrated antifungal activity against various fungal species (i.e. *Candida* species, *Cryptococcus gattii* and *Sporothrix schenckii*), with Minimum Inhibitory Concentrations (MICs) ranging from as low as 0.003% to over 0.1% (Johann et al. 2011). Consistently, Yeo et al. (2022) reported that extracts of *P. paniculata* exhibited potent in vitro inhibition against *Fusarium solani*, *Colletotrichum musae*, and *Pyricularia oryzae*, with effective concentrations between 0.005% and 0.01%. These findings reinforce the present study, and highlighting the broad-spectrum antifungal potential of *P. paniculata* for management of fungal diseases. The antifungal potential of *Polygala* species has been largely attributed to their rich repertoire of phytochemicals, including methyl salicylate, alkaloids, xanthones, saponins, oligosaccharides, coumarins, styryl-pyrones, phenolics, flavonoids, and lignans (Dubois et al. 2020; Cruz et al. 2021).

The efficacy of *P. paniculata* extracts as antifungal properties was associated with the solvent's polarity and the plant's part. The superior antifungal performance of methanolic extracts from both aerial and below-ground parts observed in this study is consistent with the findings of Aziz et al. (2019) and Yeo et al. (2022), and can be attributed to the high polarity and extraction efficiency of methanol. As a polar solvent, methanol effectively dissolve more polar bioactive antifungal phytochemicals, particularly phenolics, flavonoids, and glycosides (Aziz et al. 2021; Chatepa et al. 2024). Dhawan and Gupta (2017) also supported that methanol is the most effective solvent for extracting various phytochemicals, such as phenolics and flavonoids. Meanwhile, ethyl acetate with moderate polarity and hexane as a non-polar solvent were either less effective at extracting the phytochemicals from the aerial part of *P. paniculata*, or non-polar compounds are less abundant in the part. In contrast, ethyl acetate and hexane performed better in extracting phytochemicals with antifungal properties from the below-ground part of the plant, suggesting that phytochemicals localised in the below-ground part include a substantial fraction of non-polar and moderately polar compounds such as terpenoids or polyacetylenes, which are known to concentrate in root tissues of many plant species and to exhibit strong antifungal activity to protect the underground plant's organ from soil pathogens (Zhang et al. 2020). Moreover, Nogueira et al. (2005) highlighted methyl salicylate, a non-polar molecule, as the main volatile compound found in *P. paniculata*, contributing about 89% of the total volatile compound in the root. Interestingly, the shift in effectiveness of chloroform extracts between the two plant parts may reflect tissue-specific distribution of antifungal metabolites with different polarities. Non-polar compounds appeared to be more concentrated or active in the root system, while moderately polar and polar compounds predominated in the shoot system.

The strong antifungal activity of *P. paniculata* against *G. boninense* observed in this study can be linked to its diverse group of secondary metabolites, mainly phenolics and flavonoids, given their well-established antifungal mechanisms. The antifungal activity of phenolics and flavonoids are mediated by multiple synergistic mechanisms that interfere with the fungal cell wall

integrity, interfere with ergosterol synthesis, inhibit fungal enzymes, or induce oxidative damage of cellular components through generation of reactive oxygen species (ROS). One of their primary antifungal mechanisms is their ability to disrupt the lipid bilayer of fungal cell membranes, which further increases membrane permeability, resulting in the leakage of intracellular contents, such as electrolytes, nucleotides, and proteins, leading to cell lysis (Lv et al. 2024). Phenolics in *Polygala* species such as gallic acid and ferulic acid interact with ergosterol (a major sterol component of the fungal cell membrane that can combine with phospholipids to maintain stability and cell function) in fungal membranes, disturbing membrane integrity and inhibiting fungal growth (Yan et al. 2023). Additionally, flavonoids disrupt ergosterol biosynthesis by targeting lanosterol 14α-demethylase, which is critical in fungal development and pathogenicity, and also inhibit cytochrome-dependent enzymes, vital for ergosterol biosynthesis (Song et al. 2025). Fungal enzymes, particularly those involved in cell wall biosynthesis such as chitin synthase and β-glucan synthase, and enzymes for energy metabolism such as mitochondrial ATPases could be inhibited by phenolic compounds, which inhibit fungal growth and reproduction (Tian et al. 2022). Phenolic and flavonoid compounds can induce oxidative stress in fungal cells by stimulating the production of ROS such as superoxide anions and hydrogen peroxide. Accumulation of ROS leads to oxidative damage of cellular components, including DNA, lipids, and proteins (Zahra et al. 2024; Jomova et al. 2025). The antifungal mechanisms of terpenoids and polyacetylenes are similar to those of the phenolics and flavonoids discussed earlier, which involve the disruption of fungal cell membranes and ergosterol synthesis, the inhibition of fungal enzymes, or the induction of oxidative damage of cellular components (Freiesleben and Jäger 2014; Câmara et al. 2024; Pedroso et al. 2024). In addition, coumarins and xanthones found in *Polygala* species are known to inhibit fungal enzymes involved in cell wall biosynthesis, such as β-glucan synthase and chitin synthase, thereby preventing hyphal growth and pathogenic invasion (Tian et al. 2022; Jomova et al. 2025). Coumarins also act as photosensitisers, while xanthones contribute to synergistic stress on fungal cellular machinery. Moreover, methyl salicylate, another metabolite present in *P. paniculata*, has been implicated in plant defense signaling and direct antimicrobial action through disruption of pathogen cell walls and membranes (Nogueira et al. 2005).

In conclusion, three weed species, namely *H. carnosa* (W16), *I. muticum* (W18), and *P. paniculata* (W19) were found to be exclusively associated with healthy oil palm trees. Among them, *P. paniculata* demonstrated remarkable antifungal potential against *G. boninense*, with both aerial and below-ground parts of its crude powder achieving inhibition levels of 97.42-100% at concentrations as low as 0.5%. Methanolic extracts further confirmed its potency, with the aerial and below-ground (root) tissues exhibiting concentration-dependent inhibition ranging from 27.69 to 60.70% and 52.69 to 75.77%, respectively, and EC_{50} values of 0.076% (aerial) and 0.028% (below-ground). The present study provides preliminary in vitro evidence that *P. paniculata* exhibits notable antifungal activity against *G. boninense*, and highlights the significance of solvent polarity, tissue selection, and extract concentration in maximizing antifungal efficacy. The species thus represents a promising and sustainable source of natural antifungal agents with potential for bio-fungicide development, offering an eco-friendly and cost-effective approach for managing *G. boninense* in oil palm plantations. Future studies should encompass both qualitative and quantitative phytochemical profiling using techniques such as Thin-Layer Chromatography (TLC), Fourier Transform Infrared Spectroscopy (FTIR), liquid chromatography-mass spectrometry (LC-MS), and High-Performance Liquid Chromatography (HPLC) to identify and quantify the bioactive constituents responsible for antifungal activity. Additionally, in planta trials and field-level validation under nursery and plantation conditions are crucial to evaluate efficacy, phytotoxicity, and formulation stability, thereby supporting the development of safe, effective, and commercially viable plant-based fungicidal formulations.

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