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Antifungal effect of nine selected medicinal plants against crop pathogenic fungi

Freddy Kuok San Yeo[1]*, Siew Ting Ling[1], S. Uvanappria Sathasivam[1], Mohd Razip Asaruddin[1], Hashimatul Fatma Hashim[1] and Lee San Lai[2]

[1]Faculty of Resource Science and Technology, Universiti Malaysia Sarawak, 94300, Kota Samarahan, Sarawak, Malaysia.
[2]Agriculture Research Centre, Semongok, 12th Mile, Borneo Heights Road, Kuching-Serian Road, 93250 Kuching, Sarawak, Malaysia.
Email: yksfreddy@unimas.my

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ABSTRACT

Aims: Plant diseases caused by pathogens are threatening crop yield. Agrochemicals are used extensively to curb pathogens. Efforts to reduce the usage of agrochemicals are needed for sustainable agriculture. This study was aimed to screen medicinal plants possessing antifungal properties against crop pathogenic fungi.
Methodology and results: Sequential extraction using absolute n-hexane, ethyl acetate and methanol was performed on nine selected medicinal plants to obtain crude extract. An antifungal assay using these crude extracts was performed on *Fusarium solani*, *Collectotrichum musae* and two isolates of *Pyricularia oryzae*. The assay showed that medicinal plant species with all three types of crude extract inhibited the growth of all three pathogenic fungal species tested. The inhibitory effects of crude extracts were not only fungal species dependent but also isolate dependent.
Conclusion, significance and impact of study: Antifungal effect of nine selected medicinal plant species was observed against the three tested fungal pathogens. These research findings suggest that the selected medicinal plant species may serve as a potential source for the development of new biofungicide products.

Keywords: Antifungal, crop pathogens, fungi, medicinal plants

INTRODUCTION

Agriculture is one of the driving forces for increasing economic growth in developing countries such as Malaysia. Crop pathogenic fungi are a major threat to agricultural production. Agrochemicals are usually used in curbing the spread of crop pathogenic fungi in a crop field to a level that inflicts minimum economic injury to the quantity and quality of production. Undeniably, agrochemicals are effective most of the time. Over-application of agrochemicals, however, may lead to the development of fungicide resistant pathogens (Fillinger *et al.*, 2008; Leroch *et al.*, 2011), as well as an adverse effect on human health and the environment (Bernardes *et al.*, 2015; Nicolopoulou-Stamati *et al.*, 2016). Alternative to agrochemicals is desirable. Biofungicide based on plant extracts is an alternative that is biodegradable and has low toxicity to humans and the environment (Martínez, 2012). Several plant extracts have been reported to exhibit fungicidal properties in slowing the growth of the targeted plant pathogens (Dellavalle *et al.*, 2011; Al-Samarrai *et al.*, 2012).

Most of the higher plants contain secondary plant metabolites like phenols, flavonoids, quinones, tannins, alkaloids, saponins, sterols and terpenoids, which are responsible for playing a role in the plant defence system. These natural products may be exploited to substitute agrochemicals for plant disease control (Morales *et al.*, 2008; Ashmawy *et al.*, 2020). These active compounds can be acquired from various parts of plants, such as roots, stems, leaves, seeds, flowers and fruits (Geetha *et al.*, 2011; Joaquín-Ramos *et al.*, 2020; Ahmadu *et al.*, 2021).

The objective of the present study was to test the antifungal effect of different crude extracts from nine selected medicinal plant species against three crop pathogenic fungal species (*Fusarium solani*, *Colletotrichum musae* and *Pyricularia oryzae*). *Fusarium solani* is a phytopathogenic fungus that causes crop diseases such as *Fusarium* root rot and dry rot of crops (Mohamed Zubi *et al.*, 2021). *Colletotrichum musae* is a plant pathogenic fungus that affects the genus *Musa* (Zakaria *et al.*, 2009). It is associated with anthracnose that causes dark, sunken and necrotic leaves, flowers, and fruits (Wijekoon *et al.*, 2008). *Pyricularia oryzae* causes rice blast. Infection of *P. oryzae* causes lesions on the leaf, leaf collar, panicle, culm and culm nodes (Wilson and Talbot, 2009; Lai *et al.*, 2019; Hussin *et al.*, 2020).

*Corresponding author

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MATERIALS AND METHODS

Collection and preparation of plant materials

Nine medicinal plant species were used in this study (Table 1). Mature and disease-free leaves from the nine medicinal plants were collected from different locations. The leaves were washed with clean running tap water to remove contaminants, patted dry and air-dried at room temperature. The air-dried leaves were ground and used for phytochemical extraction.

Preparation of plant extracts

Sequential extraction was performed using absolute n-hexane, ethyl acetate and methanol. A total of 150 g of ground samples was soaked in 750 mL of absolute n-hexane and shaken for 72 h in a conical flask covered with aluminium foil at room temperature. The solvent containing extract was filtered by using Whatman No. 1 filter paper. The residue obtained after filtration was then used to continue extraction with absolute ethyl acetate and absolute methanol orderly (Figure 1). All three different solvent-filtrates were evaporated under reduced pressure by using a rotary evaporator with a water bath at 50°C to obtain crude extracts. The concentrated crude extracts were weighed, and the yield was calculated.

Preparation of crop pathogenic fungi

There were three crop pathogenic fungal species used in this study, namely *F. solani* isolated from black pepper (provided by Agriculture Research Centre, Semongok, Department of Agriculture Sarawak), *C. musae* (from Kong and Vu, 2019) and *P. oryzae* isolate POSA1 and POSA2 (from Hussin *et al.*, 2020). *Fusarium solani* and *C. musae* were cultured on Potato Dextrose Agar (PDA; Hilmedia). The fungi were incubated at room temperature. Whereas *P. oryzae* isolates were cultured on oatmeal agar (OMA) and incubated as described by Hussin *et al.* (2020).

Antifungal assay

The antifungal reported by Abu Bakar *et al*. (2018) was used with some modifications. The concentrated crude

Table 1: Name and source of the nine medicinal plant species used in this study.

Plant species	Families	Common Malay name	Source of plant materials
Clinacanthus nutans (Burm.f.) Lindau	Acanthaceae	Belalai gajah	Lutong, Miri; Residential area
Elephantopus scaber L.	Asteraceae	Tutup bumi	Lutong, Miri; Residential area
Tridax procumbens L.	Asteraceae	Kanching baju	Universiti Malaysia Sarawak Campus; Kota Samaharan
Garcinia mangostana L.	Clusiaceae	Pokok manggis	Universiti Malaysia Sarawak Campus; Kota Samaharan
Moringa oleifera Lam.	Moringaceae	Pokok kelor	Lutong, Miri; Residential area
Pandanus amaryllifolius Roxb.	Pandanaceae	Pandan	Lutong, Miri; Residential area
Cymbopogon citratus (DC.) Stapf	Poaceae	Serai	Unisquare, Kota Samarahan; Residential area
Triticum aestivum L.	Poaceae	Wheatgrass	Lutong, Miri; Residential area
Polygala paniculata L.	Polygalaceae	Kayu putih	Universiti Malaysia Sarawak Campus; Kota Samaharan

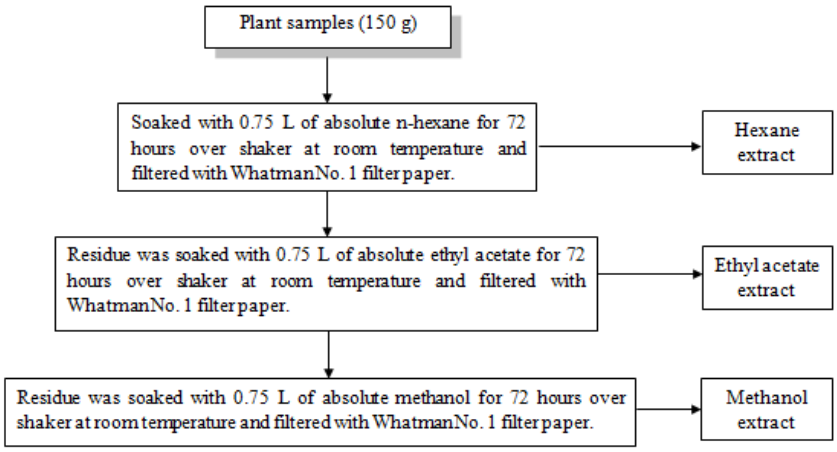

Figure 1: General procedure of sequential extraction.

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extracts obtained from different solvents were dissolved with dimethyl sulfoxide (DMSO) and filtered by a Whatman®Puradisc Syringe Filters (0.2 µm). The media was prepared as described by Abu Bakar *et al*. (2018). The assay was performed using three different concentrations (100 µg/mL, 50 µg/mL and 25 µg/mL) of crude extract together with control (1% DMSO), with eight replications and repeated twice. Using five to six days old fungal culture, mycelium plugs (6 mm) were harvested from the colony's edge and cultured onto the center of agar plates (ca. 20 mL of medium) of the different treatments. All agar plates cultured with the different fungi were incubated as described above. The colony growth was measured daily until a colony from any treatments reached full plate (8.0 cm). The average growth rate was then calculated. Analysis of variance ($p \leq 0.05$) followed by Tukey post hoc test ($p \leq 0.05$) was used to compare the difference in the growth rate of fungal colonies treated with different concentrations of crude extract from different solvents and medicinal plant species (SPSS ver. 20).

RESULTS

Extraction yield of the different solvents and selected medicinal plant species

Figure 2 shows the yield percentage of the 27 crude extracts from nine medicinal plant species (three crude extracts per plant species). In general, methanol had a higher extraction yield than other solvents for all selected medicinal plant species except for *Moringa oleifera*. The yield of the methanol extraction ranged from 3 to 15%. For hexane and ethyl acetate, the extraction yield ranged from 0.5 to 4.7% and 0.8 to 9.0%, respectively.

Table 2 shows that 17 to 21 crude extracts (out of 27) showed an inhibition effect on the growth of *F. solani*, *C. musae* and *P. oryzae*. Against *F. solani*, 18 crude extracts (six crude extracts from each solvent) significantly slowed the growth of the *F. solani*. For *C. musae*, four hexane crude extracts, six ethyl acetate crude extracts and seven methanolic crude extracts showed a significant effect on the growth of *C. musae*. Twenty-one crude extracts significantly affected the growth of *P. oryzae* isolate POSA1, but 17 for isolate POSA2.

Out of the 27 crude extracts, eight crude extracts were found to be significantly affecting the growth of all tested crop pathogenic fungi. There were 16 crude extracts that significantly slowed the growth of at least one crop pathogenic fungus. The remaining three crude extracts, hexane crude extract of *M. oleifera*, methanolic crude extract of *P. amaryllifolius* and hexane crude extract of *T. aestivum* had no effect on either one of the tested crop pathogenic fungi.

The effect of crude extracts on the growth of *Fusarium solani*

There were five medicinal plants; *Clinacanthus nutans*, *Elepahntopus scaber*, *Tridax procumbens*, *Cymbopogon citratus* and *Polygala paniculata*, which had all three of their crude extracts significantly inhibited the growth of *F. solani.* For crude extracts from *M. oleifera* and *Pandanus amaryllifolius*, there was at least one of the crude extracts showed a significant inhibitory effect on *F. solani*. All crude extracts of *Garcinia mangostana* and *Triticum aestivum* had no inhibitory effect on the growth of *F. solani* (Table 3).

Among the crude extracts showing a growth inhibitory effect, it was observed that the growth of *F. solani* was slowed down by 0.04 to 1.8 days, depending on the type and concentration of the crude extract (Table 4). Five crude extracts (hexane crude extract of *C. nutans*, ethyl acetate crude extract of *C. nutans* and *C. citratus*, and methanolic crude extracts of *C. nutans* and *M. oleifera*) showed an inhibitory effect at the lowest concentration (25 µg/mL). Only *C. nutans* had all three types of crude extract (Supplementary Figure S1) showed an inhibitory effect with delay of fungal growth by 0.04 to 0.06 day at the lowest concentration (25 µg/mL). Five crude extracts

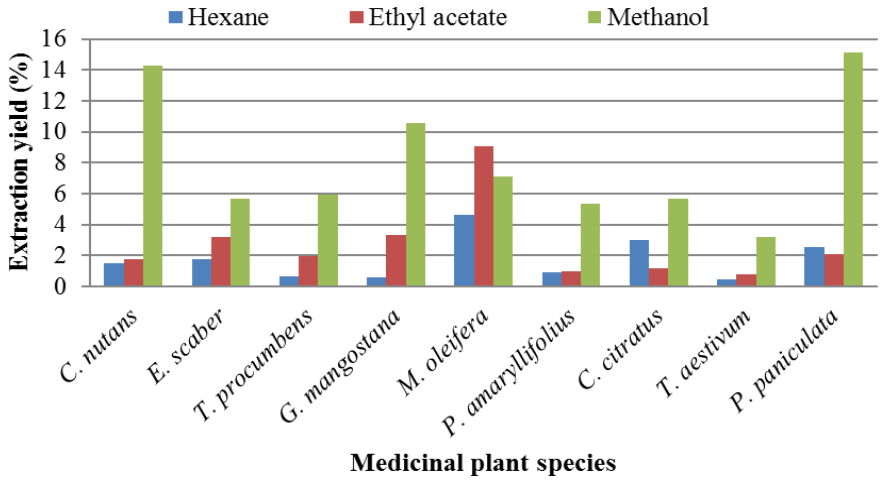

Figure 2: Yield of crude extracts from nine selected medicinal plant species.

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Table 2: Summary of antifungal effect of the 27 crude extracts from the selected medicinal plant species against tested crop pathogenic fungi.

Plant species	Crude extract	Crop pathogenic fungi*			
		F. solani	*C. musae*	*P. oryzae* (POSA1)	*P. oryzae* (POSA2)
C. nutans	Hexane	√		√	√
	Ethyl acetate	√	√	√	√
	Methanol	√	√	√	√
E. scaber	Hexane	√		√	
	Ethyl acetate	√	√	√	√
	Methanol	√			√
T. procumbens	Hexane	√		√	√
	Ethyl acetate	√	√		√
	Methanol	√	√	√	√
G. mangostana	Hexane		√	√	√
	Ethyl acetate		√	√	√
	Methanol		√	√	√
M. oleifera	Hexane				
	Ethyl acetate			√	
	Methanol	√	√		
P. amaryllifolius	Hexane	√	√	√	
	Ethyl acetate	√	√	√	
	Methanol				
C. citratus	Hexane	√	√	√	√
	Ethyl acetate	√		√	√
	Methanol	√	√	√	√
T. aestivum	Hexane				
	Ethyl acetate			√	
	Methanol		√	√	√
P. paniculata	Hexane	√	√	√	√
	Ethyl acetate	√	√	√	√
	Methanol	√	√	√	
Total crude extracts from each solvent having significant growth inhibition effect	Hexane	6	4	7	5
	Ethyl acetate	6	6	8	6
	Methanol	6	7	6	6
Total crude extracts having significant growth inhibition effect		18	17	21	17

* "√" means the tested crop pathogenic fungi was significantly inhibited by at least one of the concentrations of crude extract from a medicinal plant species (Representative of positive antifungal activity is represented in Supplementary Figures S1, S2, S3 and S4; $p<0.05$).

(hexane crude extract of *P. amaryllifolius*, ethyl acetate crude extracts of *P. amaryllifolius* and *P. paniculata*, and methanolic crude extracts of *C. citratus* and *T. aestivum*) showed inhibitory effect (delay of fungal growth by 0.3 to 0.9 day) only at the highest concentration (100 µg/mL).

The effect of crude extracts on the growth of *Colletotrichum musae*

All three crude extracts of two medicinal plant species, *G. mangostana* and *P. paniculata* significantly inhibited the growth of *C. musae.* For crude extracts from *C. nutans*, *T. procumbens*, *P. amaryllifolius* and *C. citratus*, there were at least two of the three crude extracts that showed inhibitory effect on *C. musae*. For crude extracts from *E. scaber*, *M. oleifera* and *T. aestivum*, at least one of the three crude extracts inhibited the growth of *C. musae* (Table 5).

Among the crude extracts showing a growth inhibitory effect, it was observed that the growth of *C. musae* was delayed by 0.08 to 3.15 days, depending on the type and concentration of the crude extract (Table 6). Four crude extracts (hexane crude extracts of *G. mangostana* and *P. amaryllifolius*, ethyl acetate and methanolic crude extract

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Table 3: Average growth rate (cm/day) of *F. solani* on different concentrations of different crude extracts from nine plant species.

Plant species	Extract	Growth rate (cm/day)*			
		Control	100 µg/mL	50 µg/mL	25 µg/mL
C. nutans[!]	Hexane	0.399 ± 0.012[a]	0.315 ± 0.010[c]	0.326 ± 0.016[c]	0.352 ± 0 .015[b]
	Ethyl acetate	0.357 ± 0.012[a]	0.312 ± 0.006[c]	0.335 ± 0.019[b]	0.337 ± 0.029[b]
	Methanol	0.357 ± 0.011[a]	0.306 ± 0.010[c]	0.308 ± 0.004[c]	0.338 ± 0.017[b]
E. scaber[!]	Hexane	0.387 ± 0.006[a]	0.326 ± 0.010[c]	0.346 ± 0.005[b]	N/A
	Ethyl acetate	0.386 ± 0.003[a]	0.373 ± 0.009[b]	0.374 ± 0.010[b]	0.386 ± 0.005[a]
	Methanol	0.381 ± 0.004[a]	0.371 ± 0.004[c]	0.373 ± 0.004[bc]	0.376 ± 0.003[b]
T. procumbens[!]	Hexane	0.330 ± 0.012[a]	0.297 ± 0.010[b]	0.301 ± 0.011[b]	0.331 ± 0.010[a]
	Ethyl acetate	0.340 ± 0.011[a]	0.322 ± 0.009[b]	0.323 ± 0.012[b]	0.335 ± 0.011[a]
	Methanol	0.340 ± 0.007[a]	0.332 ± 0.013[b]	0.335 ± 0.007[ab]	0.337 ± 0.007[ab]
G. mangostana	Hexane	0.379 ± 0.018[a]	0.368 ± 0.022[a]	0.369 ± 0.028[a]	0.375 ± 0.022[a]
	Ethyl acetate	0.358 ± 0.010[a]	0.352 ± 0.011[a]	0.353 ± 0.012[a]	0.356 ± 0.011[a]
	Methanol	0.420 ± 0.063[a]	0.400 ± 0.069[a]	0.411 ± 0.067[a]	0.415 ± 0.069[a]
M. oleifera	Hexane	0.412 ± 0.041[a]	0.406 ± 0.045[a]	0.406 ± 0.043[a]	0.407 ± 0.038[a]
	Ethyl acetate	0.409 ± 0.039[a]	0.405 ± 0.039[a]	0.405 ± 0.042[a]	0.407 ± 0.040[a]
	Methanol	0.406 ± 0.037[a]	0.367 ± 0.002[b]	0.370 ± 0.004[b]	0.374 ± 0.005[b]
P. amaryllifolius	Hexane	0.388 ± 0.016[a]	0.373 ± 0.004[b]	0.380 ± 0.004[ab]	0.383 ± 0.006[a]
	Ethyl acetate	0.378 ± 0.008[a]	0.361 ± 0.005[b]	0.372 ± 0.007[a]	0.376 ± 0.004[a]
	Methanol	0.367 ± 0.018[a]	0.362 ± 0.005[a]	0.363 ± 0.006[a]	0.366 ± 0.005[a]
C. citratus[!]	Hexane	0.375 ± 0.013[a]	0.341 ± 0.017[c]	0.354 ± 0.009[b]	0.367 ± 0.007[a]
	Ethyl acetate	0.366 ± 0.003[a]	0.358 ± 0.004[b]	0.359 ± 0.003[b]	0.361 ± 0.008[b]
	Methanol	0.364 ± 0.006[a]	0.332 ± 0.020[b]	0.361 ± 0.010[a]	0.362 ± 0.011[a]
T. aestivum	Hexane	0.429 ± 0.075[a]	0.390 ± 0.040[a]	0.411 ± 0.093[a]	0.426 ± 0.078[a]
	Ethyl acetate	0.416 ± 0.108[a]	0.399 ± 0.091[a]	0.404 ± 0.099[a]	0.404 ± 0.079[a]
	Methanol	0.407 ± 0.072[a]	0.402 ± 0.100[a]	0.404 ± 0.100[a]	0.407 ± 0.102[a]
P. paniculata[!]	Hexane	0.384 ± 0.017[a]	0.366 ± 0.007[c]	0.372 ± 0.006[bc]	0.382 ± 0.014[ab]
	Ethyl acetate	0.374 ± 0.010[a]	0.357 ± 0.011[b]	0.369 ± 0.006[a]	0.372 ± 0.007[a]
	Methanol	0.377 ± 0.017[a]	0.363 ± 0.008[b]	0.366 ± 0.011[b]	0.368 ± 0.008[ab]

*Different letters indicate a significant difference in fungal growth rate on media infused with different concentrations of crude extract ($p \leq 0.05$); [!]All crude extracts from the plant species slowed the growth rate of *F. solani* at a certain concentration.

Table 4: Significantly inhibited fungal colony growth of *F. solani* treated with different crude extracts from nine plant species.

Plant species	Extract	Effective concentrations	Additional days for *F. solani* to reach full plate*
C. nutans	Hexane	100 µg/mL	More than 1 day[ʃ]
		50 µg/mL and 25 µg/mL	Less than 1 day
	Ethyl acetate	100 µg/mL	More than 1 day[ʃ]
		50 µg/mL and 25 µg/mL	Less than 1 day
	Methanol	100 µg/mL and 50 µg/mL	More than 1 day[ʃ]
		25 µg/mL	Less than 1 day
E. scaber	Hexane	100 µg/mL	More than 1 day[ʃ]
		50 µg/mL	Less than 1 day
	Ethyl acetate	100 µg/mL and 50 µg/mL	Less than 1 day
	Methanol	All concentrations	Less than 1 day
T. procumbens	Hexane	100 µg/mL and 50 µg/mL	Less than 1 day
	Ethyl acetate	100 µg/mL and 50 µg/mL	Less than 1 day
	Methanol	100 µg/mL	Less than 1 day
G. mangostana	Hexane	-	-
	Ethyl acetate	-	-
	Methanol	-	-
M. oleifera	Hexane	-	-
	Ethyl acetate	-	-
	Methanol	All concentrations	Less than 1 day
P. amaryllifolius	Hexane	100 µg/mL and 50 µg/mL	Less than 1 day
	Ethyl acetate	100 µg/mL	Less than 1 day
	Methanol	-	-

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(Continued)

C. citratus	Hexane	100 µg/mL and 50 µg/mL	Less than 1 day
	Ethyl acetate	All concentrations	Less than 1 day
	Methanol	100 µg/mL	Less than 1 day
T. aestivum	Hexane	-	-
	Ethyl acetate	-	-
	Methanol	-	-
P. paniculata	Hexane	100 µg/mL and 50 µg/mL	Less than 1 day
	Ethyl acetate	100 µg/mL	Less than 1 day
	Methanol	100 µg/mL and 50 µg/mL	Less than 1 day

*The number of additional days needed to reach full plate in comparison to control plate; ⸸The number of additional days needed to reach the entire plate in comparison to the control plate was more than one day but less than 2 days.

Table 5: Average growth rate (cm/day) of *C. musae* on different concentrations of different extracts from nine plant species.

Plant species	Extract	Growth rate (cm/day)*			
		Control	100 µg/mL	50 µg/mL	25 µg/mL
C. nutans	Hexane	0.697 ± 0.115^{a}	0.621 ± 0.042^{a}	0.688 ± 0.107^{a}	0.691 ± 0.117^{a}
	Ethyl acetate	0.618 ± 0.048^{a}	0.574 ± 0.049^{b}	0.608 ± 0.041^{ab}	0.611 ± 0.048^{ab}
	Methanol	0.603 ± 0.026^{a}	0.518 ± 0.050^{b}	0.525 ± 0.056^{b}	0.606 ± 0.015^{a}
E. scaber	Hexane	0.501 ± 0.123^{a}	0.475 ± 0.106^{a}	0.495 ± 0.121^{a}	0.499 ± 0.125^{a}
	Ethyl acetate	0.428 ± 0.047^{a}	0.365 ± 0.021^{b}	0.415 ± 0.040^{a}	0.424 ± 0.050^{a}
	Methanol	0.521 ± 0.150^{a}	0.442 ± 0.086^{a}	0.512 ± 0.140^{a}	0.520 ± 0.151^{a}
T. procumbens	Hexane	0.516 ± 0.101^{a}	0.436 ± 0.135^{a}	0.492 ± 0.113^{a}	0.503 ± 0.100^{a}
	Ethyl acetate	0.609 ± 0.056^{a}	0.513 ± 0.105^{b}	0.535 ± 0.062^{b}	0.604 ± 0.059^{a}
	Methanol	0.539 ± 0.064^{a}	0.422 ± 0.153^{b}	0.455 ± 0.121^{ab}	0.533 ± 0.053^{a}
G. mangostana[!]	Hexane	0.577 ± 0.025^{a}	0.433 ± 0.013^{c}	0.449 ± 0.027^{c}	0.488 ± 0.025^{b}
	Ethyl acetate	0.596 ± 0.028^{a}	0.400 ± 0.006^{d}	0.523 ± 0.012^{c}	0.574 ± 0.016^{b}
	Methanol	0.646 ± 0.027^{a}	0.503 ± 0.016^{d}	0.551 ± 0.014^{c}	0.608b ± 0.013^{b}
M. oleifera	Hexane	0.554 ± 0.038^{a}	0.507 ± 0.079^{a}	0.521 ± 0.069^{a}	0.554 ± 0.036^{a}
	Ethyl acetate	0.540 ± 0.062^{a}	0.474 ± 0.116^{a}	0.491 ± 0.098^{a}	0.490 ± 0.103^{a}
	Methanol	0.576 ± 0.042^{a}	0.514 ± 0.086^{b}	0.566 ± 0.020^{a}	0.562 ± 0.030^{a}
P. amaryllifolius	Hexane	0.564 ± 0.039^{a}	0.427 ± 0.050^{b}	0.434 ± 0.016^{b}	0.445 ± 0.027^{b}
	Ethyl acetate	0.512 ± 0.065^{a}	0.377 ± 0.086^{b}	0.460 ± 0.015^{a}	0.508 ± 0.062^{a}
	Methanol	0.465 ± 0.068^{a}	0.431 ± 0.082^{a}	0.453 ± 0.072^{a}	0.448 ± 0.061^{a}
C. citratus	Hexane	0.605 ± 0.050^{a}	0.500 ± 0.095^{b}	0.545 ± 0.093^{ab}	0.581 ± 0.067^{a}
	Ethyl acetate	0.535 ± 0.117^{a}	0.402 ± 0.168^{a}	0.452 ± 0.171^{a}	0.525 ± 0.106^{a}
	Methanol	0.606 ± 0.046^{a}	0.467 ± 0.080^{c}	0.546 ± 0.045^{b}	0.584 ± 0.077^{ab}
T. aestivum	Hexane	0.637 ± 0.086^{a}	0.576 ± 0.100^{a}	0.611 ± 0.094^{a}	0.622 ± 0.096^{a}
	Ethyl acetate	0.655 ± 0.075^{a}	0.582 ± 0.118^{a}	0.615 ± 0.118^{a}	0.637 ± 0.086^{a}
	Methanol	0.652 ± 0.081^{a}	0.527 ± 0.174^{b}	0.554 ± 0.149^{ab}	0.608 ± 0.095^{ab}
P. paniculata[!]	Hexane	0.640 ± 0.026^{a}	0.489 ± 0.035^{c}	0.539 ± 0.029^{b}	0.632 ± 0.037^{a}
	Ethyl acetate	0.627 ± 0.031^{a}	0.472 ± 0.029^{c}	0.523 ± 0.025^{b}	0.629 ± 0.018^{a}
	Methanol	0.624 ± 0.026^{a}	0.377 ± 0.042^{c}	0.503 ± 0.039^{b}	0.599 ± 0.014^{a}

*Different letters indicate significant difference in fungal growth rate on media infused with different concentrations of crude extract ($p \leq 0.05$); [!]All crude extracts from plant species slowed down the growth rate of *C. musae* at a certain concentration.

of *G. mangostana*) showed inhibitory effect (delay of fungal growth by 0.2 to 1.9 days) at the lowest concentration (25 µg/mL). Only *G. mangostana* had all the types of crude extracts (Supplementary Figure S2) that showed inhibitory effect at the lowest concentration (25 µg/mL) with a delay in fungal growth of *C. musae* by 0.2 to 1 day. Seven crude extracts (hexane crude extract of *C. citratus*, ethyl acetate crude extracts of *C. nutans*, *E. scaber* and *P. amaryllifolius*, and methanolic crude extracts of *G. mangostana*, *T. aestivum* and *T. procumbens*) showed inhibitory effect only at the highest concentration (100 µg/mL) with a delay of fungal growth by 0.08 to 2.4 days.

The effect of crude extracts on the growth of *Pyricularia oryzae*

There were three medicinal plant species, *C. nutans*, *G. mangostana* and *C. citratus*, all of their crude extracts significantly inhibited the growth of isolate POSA1 and POSA2. For crude extracts from *E. scaber*, *T. procumbens*, *T. aestivum* and *P. paniculata*, there was at least one crude extract that showed an inhibitory effect on the isolates of *P. oryzae*. For *M. oleifera* and *P. amaryllifolius*, at least one crude extract inhibited the growth of isolate POSA1 but not isolate POSA2 (Table 7).

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Table 6: Significantly inhibited fungal colony growth of *C. musae* treated with different crude extracts from nine plant species.

Plant species	Extract	Effective concentrations	Additional days for *C. musae* to reach full plate*
C. nutans	Hexane	-	-
	Ethyl acetate	100 µg/mL	Less than 1 day
	Methanol	100 µg/mL and 50 µg/mL	Less than 1 day
E. scaber	Hexane	-	-
	Ethyl acetate	100 µg/mL	Less than 1 day
	Methanol	-	-
T. procumbens	Hexane	-	-
	Ethyl acetate	100 µg/mL	More than 1 day[ɻ]
		50 µg/mL	Less than 1 day
	Methanol	100 µg/mL	More than 1 day[ɻ]
G. mangostana	Hexane	100 µg/mL	2 days
		50 µg/mL and 25 µg/mL	More than 1 day[ɻ]
	Ethyl acetate	100 µg/mL	3 days
		50 µg/mL and 25 µg/mL	Less than 1 day
	Methanol	100 µg/mL	More than 1 day[ɻ]
		50 µg/mL	1 day
		25 µg/mL	Less than 1 day
M. oleifera	Hexane	-	-
	Ethyl acetate	-	-
	Methanol	100 µg/mL	Less than 1 day
P. amaryllifolius	Hexane	100 µg/mL and 50 µg/mL	More than 2 days[♯]
		25 µg/mL	More than 1 day[ɻ]
	Ethyl acetate	100 µg/mL	More than 2 days[♯]
	Methanol	-	-
C. citratus	Hexane	100 µg/mL	More than 1 day[ɻ]
	Ethyl acetate	-	-
	Methanol	100 µg/mL	More than 1 day[ɻ]
		50 µg/mL	Less than 1 day
T. aestivum	Hexane	-	-
	Ethyl acetate	-	-
	Methanol	100 µg/mL	More than 1 day[ɻ]
P. paniculata	Hexane	100 µg/mL	More than 1 day[ɻ]
		50 µg/mL	Less than 1 day
	Ethyl acetate	100 µg/mL and 50 µg/mL	Less than 1 day
	Methanol	100 µg/mL	More than 3 days[♦]
		50 µg/mL	More than 1 day[ɻ]

*The number of additional days needed to reach full plate in comparison to control plate; [ɻ]The number of additional days needed to reach the full plate in comparison to the control plate was more than one day but less than 2 days; [♯]The number of additional days needed to reach the full plate in comparison to the control plate was more than 2 days but less than 3 days; [♦]The number of additional days needed to reach the full plate in comparison to the control plate was more than 3 days but less than 4 days.

Among the crude extracts showing a growth inhibitory effect, it was observed that the growth of isolate POSA1 was slowed down by 0.5 to 4.15 days while isolate POSA2 by 0.75 to 2.5 days, depending on the type and concentration of the crude extract (Table 8). For isolate POSA1, eight crude extracts (hexane crude extracts of *C. nutans*, *C. citratus* and *G. mangostana*, ethyl acetate crude extract of *C. nutans*, *C. citratus* and *G. mangostana*, and methanolic crude extracts of *C. citratus* and *G. mangostana*) showed inhibitory effect (delay of fungal growth by 0.5 to 2.1 days) at the lowest concentration (25 µg/mL). Four crude extracts (hexane crude extracts of *E. scaber*, *P. amaryllifolius* and *P. paniculata*, and methanolic crude extract of *C. nutans*) showed inhibitory effect (delay of fungal growth by 0.8 to 4.15 days) only at the highest concentration (100 µg/mL). For isolate POSA2, six crude extracts (hexane crude extract of *C. citratus*, ethyl acetate crude extracts of *C. citratus*, *E. scaber* and *P. paniculata*, and methanolic crude extracts of *C. citratus* and *E. scaber*) showed inhibitory effect (delay of fungal growth by 0.75 to 2.5 days) at the lowest concentration (25 µg/mL). Methanolic crude extracts of *C. nutans* and *T. aestivum* showed an inhibitory effect at the highest concentration (100 µg/mL) with a delay of fungal growth by 1.5 to 2.2 days.

DISCUSSION

Antifungal activities of 27 crude extracts from nine medicinal plant species against three species of crop

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Table 7: Average growth rate (cm/day) of *P. oryzae* on different concentrations of different extracts from nine plant species.

Plant species	Isolate	Extract	Growth rate (cm/day)*			
			Control	100 µg/mL	50 µg/mL	25 µg/mL
C. nutans[!]	POSA1	Hexane	0.231 ± 0.007^{a}	0.207 ± 0.006^{c}	0.209 ± 0.008^{bc}	0.214 ± 0.006^{b}
		Ethyl acetate	0.219 ± 0.007^{a}	0.198 ± 0.009^{c}	0.203 ± 0.011^{bc}	0.208 ± 0.006^{b}
		Methanol	0.214 ± 0.007^{a}	0.204 ± 0.005^{b}	0.209 ± 0.007^{ab}	0.209 ± 0.009^{ab}
	POSA2	Hexane	0.258 ± 0.014^{a}	0.226 ± 0.016^{b}	0.231 ± 0.025^{b}	0.241 ± 0.022^{ab}
		Ethyl acetate	0.249 ± 0.013^{a}	0.224 ± 0.020^{b}	0.224 ± 0.030^{b}	0.239 ± 0.021^{ab}
		Methanol	0.249 ± 0.011^{a}	0.225 ± 0.013^{b}	0.230 ± 0.024^{ab}	0.238 ± 0.029^{ab}
E. scaber	POSA1	Hexane	0.205 ± 0.013^{a}	0.167 ± 0.028^{b}	0.191 ± 0.010^{a}	0.198 ± 0.015^{a}
		Ethyl acetate	0.201 ± 0.011^{a}	0.178 ± 0.011^{b}	0.180 ± 0.016^{b}	0.194 ± 0.014^{a}
		Methanol	0.227 ± 0.020^{a}	0.210 ± 0.012^{a}	0.213 ± 0.020^{a}	0.214 ± 0.021^{a}
	POSA2	Hexane	0.275 ± 0.063^{a}	0.250 ± 0.020^{a}	0.263 ± 0.023^{a}	0.263 ± 0.023^{a}
		Ethyl acetate	0.285 ± 0.040^{a}	0.235 ± 0.016^{b}	0.244 ± 0.010^{b}	0.246 ± 0.017^{b}
		Methanol	0.284 ± 0.049^{a}	0.228 ± 0.014^{b}	0.238 ± 0.031^{b}	0.241 ± 0.048^{b}
T. procumbens	POSA1	Hexane	0.214 ± 0.013^{a}	0.192 ± 0.011^{b}	0.201 ± 0.012^{b}	0.214 ± 0.012^{a}
		Ethyl acetate	0.206 ± 0.007^{a}	0.202 ± 0.012^{a}	0.203 ± 0.012^{a}	0.203 ± 0.006^{a}
		Methanol	0.212 ± 0.009^{a}	0.193 ± 0.007^{b}	0.197 ± 0.009^{b}	0.208 ± 0.013^{a}
	POSA2	Hexane	0.251 ± 0.018^{a}	0.205 ± 0.023^{c}	0.226 ± 0.022^{bc}	0.234 ± 0.029^{ab}
		Ethyl acetate	0.260 ± 0.016^{a}	0.218 ± 0.027^{b}	0.223 ± 0.014^{b}	0.238 ± 0.033^{ab}
		Methanol	0.246 ± 0.012^{a}	0.214 ± 0.020^{c}	0.224 ± 0.030^{bc}	0.238 ± 0.026^{ab}
G. mangostana[!]	POSA1	Hexane	0.269 ± 0.031^{a}	0.214 ± 0.024^{b}	0.218 ± 0.024^{b}	0.227 ± 0.010^{b}
		Ethyl acetate	0.251 ± 0.032^{a}	0.193 ± 0.018^{c}	0.204 ± 0.020^{bc}	0.218 ± 0.017^{b}
		Methanol	0.253 ± 0.042^{a}	0.211 ± 0.013^{b}	0.215 ± 0.016^{b}	0.215 ± 0.021^{b}
	POSA2	Hexane	0.270 ± 0.025^{a}	0.238 ± 0.022^{c}	0.247 ± 0.021^{bc}	0.258 ± 0.014^{ab}
		Ethyl acetate	0.253 ± 0.014^{a}	0.204 ± 0.025^{b}	0.205 ± 0.018^{b}	0.233 ± 0.018^{a}
		Methanol	0.255 ± 0.011^{a}	0.230 ± 0.021^{c}	0.238 ± 0.011^{bc}	0.246 ± 0.015^{ab}
M. oleifera	POSA1	Hexane	0.217 ± 0.012^{a}	0.197 ± 0.032^{a}	0.197 ± 0.029^{a}	0.208 ± 0.017^{a}
		Ethyl acetate	0.221 ± 0.015^{a}	0.194 ± 0.031^{c}	0.200 ± 0.024^{bc}	0.217 ± 0.010^{ab}
		Methanol	0.217 ± 0.017^{a}	0.194 ± 0.033^{a}	0.196 ± 0.036^{a}	0.202 ± 0.032^{a}
	POSA2	Hexane	0.235 ± 0.023^{a}	0.217 ± 0.044^{a}	0.218 ± 0.043^{a}	0.220 ± 0.034^{a}
		Ethyl acetate	0.238 ± 0.022^{a}	0.221 ± 0.040^{a}	0.221 ± 0.031^{a}	0.223 ± 0.036^{a}
		Methanol	0.239 ± 0.021^{a}	0.222 ± 0.031^{a}	0.224 ± 0.031^{a}	0.231 ± 0.024^{a}
P. amaryllifolius	POSA1	Hexane	0.222 ± 0.020^{a}	0.189 ± 0.036^{b}	0.191 ± 0.038^{ab}	0.194 ± 0.035^{ab}
		Ethyl acetate	0.226 ± 0.013^{a}	0.189 ± 0.045^{b}	0.196 ± 0.037^{b}	0.212 ± 0.013^{ab}
		Methanol	0.222 ± 0.019^{a}	0.199 ± 0.027^{a}	0.200 ± 0.036^{a}	0.204 ± 0.025^{a}
	POSA2	Hexane	0.246 ± 0.017^{a}	0.217 ± 0.037^{a}	0.220 ± 0.039^{a}	0.221 ± 0.034^{a}
		Ethyl acetate	0.241 ± 0.017^{a}	0.214 ± 0.051^{a}	0.230 ± 0.032^{a}	0.235 ± 0.027^{a}
		Methanol	0.236 ± 0.023^{a}	0.216 ± 0.027^{a}	0.219 ± 0.039^{a}	0.227 ± 0.030^{a}
C. citratus[!]	POSA1	Hexane	0.212 ± 0.007^{a}	0.178 ± 0.009^{d}	0.191 ± 0.007^{c}	0.200 ± 0.010^{b}
		Ethyl acetate	0.217 ± 0.006^{a}	0.179 ± 0.015^{c}	0.179 ± 0.015^{c}	0.199 ± 0.012^{b}
		Methanol	0.211 ± 0.009^{a}	0.191 ± 0.007^{c}	0.193 ± 0.007^{c}	0.204 ± 0.006^{b}
	POSA2	Hexane	0.236 ± 0.008^{a}	0.206 ± 0.008^{b}	0.214 ± 0.010^{b}	0.214 ± 0.010^{b}
		Ethyl acetate	0.226 ± 0.008^{a}	0.208 ± 0.004^{b}	0.210 ± 0.008^{b}	0.210 ± 0.008^{b}
		Methanol	0.230 ± 0.010^{a}	0.217 ± 0.007^{b}	0.219 ± 0.006^{b}	0.219 ± 0.006^{b}
T. aestivum	POSA1	Hexane	0.225 ± 0.013^{a}	0.211 ± 0.020^{a}	0.212 ± 0.014^{a}	0.217 ± 0.013^{a}
		Ethyl acetate	0.224 ± 0.014^{a}	0.203 ± 0.017^{b}	0.207 ± 0.017^{b}	0.210 ± 0.013^{ab}
		Methanol	0.226 ± 0.016^{a}	0.207 ± 0.021^{b}	0.210 ± 0.016^{b}	0.216 ± 0.009^{ab}
	POSA2	Hexane	0.255 ± 0.043^{a}	0.229 ± 0.038^{a}	0.240 ± 0.033^{a}	0.248 ± 0.035^{a}
		Ethyl acetate	0.260 ± 0.032^{a}	0.235 ± 0.032^{a}	0.240 ± 0.027^{a}	0.248 ± 0.028^{a}
		Methanol	0.263 ± 0.029^{a}	0.229 ± 0.037^{b}	0.244 ± 0.029^{ab}	0.248 ±0.032^{ab}
P. paniculata	POSA1	Hexane	0.249 ± 0.046^{a}	0.211 ± 0.019^{b}	0.229 ± 0.036^{ab}	0.238 ± 0.029^{ab}
		Ethyl acetate	0.247 ± 0.041^{a}	0.202 ± 0.015^{c}	0.220 ± 0.025^{bc}	0.229 ± 0.027^{ab}
		Methanol	0.250 ± 0.038^{a}	0.221 ± 0.024^{b}	0.226 ± 0.020^{ab}	0.240 ± 0.030^{ab}
	POSA2	Hexane	0.276 ± 0.021^{a}	0.224 ± 0.022^{c}	0.244 ± 0.030^{bc}	0.263 ± 0.036^{ab}
		Ethyl acetate	0.280 ± 0.028^{a}	0.226 ± 0.027^{b}	0.240 ± 0.023^{b}	0.252 ± 0.035^{b}
		Methanol	0.277 ± 0.030^{a}	0.248 ± 0.037^{a}	0.252 ± 0.029^{a}	0.267 ± 0.040^{a}

*Different letters indicate significant difference in fungal growth rate on media infused with different concentrations of crude extract ($p \leq 0.05$); [!]All crude extracts from plant species slowed the growth rate of *P. oryzae* at a certain concentration.

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Table 8: Significantly inhibited fungal colony growth of *P. oryzae* treated with different crude extracts from nine plant species.

Plant species	Isolate	Extract	Effective concentrations	Additional days for *P. oryzae* to reach full plate*
C. nutans	POSA1	Hexane	All concentrations	More than 1 day♩
		Ethyl acetate	100 µg/mL and 50 µg/mL	More than 1 day♩
			25 µg/mL	Less than 1 day
		Methanol	100 µg/mL	Less than 1 day
	POSA2	Hexane	All concentrations	More than 1 day♩
		Ethyl acetate	100 µg/mL and 50 µg/mL	More than 1 day♩
		Methanol	100 µg/mL and 50 µg/mL	More than 1 day♩
E. scaber	POSA1	Hexane	100 µg/mL	More than 4 days♫
		Ethyl acetate	100 µg/mL and 50 µg/mL	More than 2 days♯
		Methanol	-	-
	POSA2	Hexane	-	-
		Ethyl acetate	100 µg/mL	More than 2 days♯
			50 µg/mL and 25 µg/mL	More than 1 day♩
		Methanol	All concentrations	More than 2 days♯
T. procumbens	POSA1	Hexane	100 µg/mL	More than 1 day♩
			50 µg/mL	1 day
		Ethyl acetate	-	-
		Methanol	100 µg/mL and 50 µg/mL	More than 1 day♩
	POSA2	Hexane	100 µg/mL	More than 2 days♯
			50 µg/mL	1 day
		Ethyl acetate	100 µg/mL and 50 µg/mL	More than 2 days♯
		Methanol	100 µg/mL	2 days
			50 µg/mL	More than 1 day♩
G. mangostana	POSA1	Hexane	100 µg/mL and 50 µg/mL	More than 3 days♦
			25 µg/mL	More than 2 days♯
		Ethyl acetate	100 µg/mL	More than 3 days♦
			50 µg/mL	More than 2 days♯
			25 µg/mL	More than 1 day♩
		Methanol	100 µg/mL	More than 2 days♯
			50 µg/mL	2 days
			25 µg/mL	More than 1 day♩
	POSA2	Hexane	100 µg/mL and 50 µg/mL	More than 1 day♩
		Ethyl acetate	100 µg/mL and 50 µg/mL	More than 2 days♯
		Methanol	100 µg/mL	More than 1 day♩
			50 µg/mL	Less than 1 day
M. oleifera	POSA1	Hexane	-	-
		Ethyl acetate	100 µg/mL	More than 2 days♯
			50 µg/mL	More than 1 day♩
		Methanol	-	-
	POSA2	Hexane	-	-
		Ethyl acetate	-	-
		Methanol	-	-
P. amaryllifolius	POSA1	Hexane	100 µg/mL	3 days
		Ethyl acetate	100 µg/mL	More than 2 days♯
			50 µg/mL	More than 1 day♩
		Methanol	-	-
	POSA2	Hexane	-	-
		Ethyl acetate	-	-
		Methanol	-	-
C. citratus	POSA1	Hexane	100 µg/mL	More than 2 days♯
			50 µg/mL and 25 µg/mL	More than 1 day♩
		Ethyl acetate	100 µg/mL and 50 µg/mL	More than 3 days♦
			25 µg/mL	More than 1 day♩
		Methanol	100 µg/mL and 50 µg/mL	More than 1 day♩
			25 µg/mL	Less than 1 day

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(Continued)

	POSA2	Hexane	100 µg/mL	More than 2 days♯
			50 µg/mL and 25 µg/mL	More than 1 day♩
		Ethyl acetate	All concentrations	More than 1 day♩
		Methanol	All concentrations	Less than1 day
T. aestivum	POSA1	Hexane	-	-
		Ethyl acetate	100 µg/mL and 50 µg/mL	More than 1 day♩
		Methanol	100 µg/mL and 50 µg/mL	More than 1 day♩
	POSA2	Hexane	-	-
		Ethyl acetate	-	-
		Methanol	100 µg/mL	More than 2 days♯
P. paniculata	POSA1	Hexane	100 µg/mL	More than 2 days♯
		Ethyl acetate	100 µg/mL	More than 2 days♯
			50 µg/mL	More than 1 day♩
		Methanol	100 µg/mL	More than 1 day♩
	POSA2	Hexane	100 µg/mL	More than 2 days♯
			50 µg/mL	More than 1 day♩
		Ethyl acetate	100 µg/mL and 50 µg/mL	More than 2 days♯
			25 µg/mL	More than 1 day♩
		Methanol	-	-

*The number of additional days needed to reach full plate in comparison to control plate; ♩The number of additional days needed to reach the full plate in comparison to the control plate is more than one day but less than 2 days; ♯The number of additional days needed to reach the full plate in comparison to the control plate is more than 2 days but less than 3 days; ♦The number of additional days needed to reach the full plate in comparison to control plate is more than 3 days but less than 4 days; ♫The number of additional days needed to reach the full plate in comparison to the control plate is more than 4 days but less than 5 days.

pathogenic fungi are reported in this study. There were 17 to 21 crude extracts that slowed down (fungistatic) the growth of *F. solani*, *C. musae* and *P. oryzae* (Table 2). The difference in the growth inhibitory effect between the crude extracts is probably due to the different content of compounds in the different medicinal plant species (Ganga Rao *et al*., 2012; Missau *et al*., 2014; Alsultan *et al*., 2016; Lalitha *et al*., 2017; Khoo *et al*., 2018; Pathak *et al*. 2020; Syed *et al*., 2020; Ove *et al*., 2021; Wuryatmo *et al*., 2021). Flavonoids, alkaloids and tannins are frequently reported from the extracts of the nine medicinal plant species (Ganga Rao *et al*., 2012; Salome *et al*., 2012; Alsultan *et al*., 2017; Bhagyasri *et al*., 2017; Johri and Khan, 2017; Ayirezang *et al*., 2020; Syed *et al*., 2020). Among the nine medicinal plant species, crude extract from eight species has been reported before to have antimicrobial activity against bacteria or fungi or both (Johann *et al*., 2011; Rizvi *et al*., 2011; Ganga Rao *et al*., 2012; Salome *et al*., 2012; Missau *et al*., 2014; Ojiako, 2014; Kamble and Moon, 2015; Rajoria *et al*., 2015; Alsultan *et al*., 2017; Bhagyasri *et al*., 2017; Hasbollah, 2017; Lalitha *et al*., 2017; Ayirezang *et al*., 2020; Mncube et al. 2021; Wuryatmo *et al*., 2021). These reported compounds or those that have not yet been identified in the nine medicinal plant species may have a role in the antifungal effect observed in this study.

In this study, the best growth inhibition effect of an extract can delays a fungal colony grow to full plate (8.0 cm) by two to four days. The observed effect may not be significant in controlling postharvest pathogens nor curbing the crop pathogen in a crop field. A high concentration of an extract is needed to observe such effect. Species-dependent inhibition of crude extract was observed in this study, where there were crude extracts that showed the inhibitory effect on the growth of all tested species of crop pathogenic fungi, but also crude extracts which had the inhibitory effect on a certain crop pathogenic fungi species. This suggested species specificity or selective activity of the crude extract, which is also observed in Kuete *et al*. (2008). Isolate dependent growth inhibitory effect was also observed based on the antifungal assay against the two isolates of *P. oryzae* (POSA1 and POSA2). Out of the 27 crude extracts, there were 21 crude extracts had an inhibitory effect against POSA1, while 17 for POSA2. Isolate-dependent inhibitory effect refers to crude extract, which shows effect either to POSA1 or POSA2. For instance, the hexane crude extract of *E. scaber* extract showed inhibition to POSA1 but not against POSA2. Contrastingly, the methanolic crude extract of *E. scaber* showed inhibition to POSA2 but not against POSA1 (Table 7). Isolate-dependent inhibitory effect is also referring to crude extract, which shows the inhibitory effect to both isolates but requires a different concentration to see the inhibition effect. For example, the inhibition effect of ethyl acetate crude extract of *E. scaber* and *G. mangostana*. It is also true for the methanolic crude extract of *T. aestivum* (Table 7). Isolate dependent inhibitory effect of extract suggested there is a need to find a potent antimicrobial agent that is effective not only to broad pathogen species but also significant inhibition against different isolates or genotypes of a pathogen species.

The present study provides important baseline information for the antifungal effect of the crude extracts from nine medicinal plant species. Bio-essay-guided isolation of compound giving the antifungal effect of the crude extracts is the way forward for this study.

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CONCLUSION

In conclusion, the antifungal effect of nine medicinal plant species was exhibited through growth inhibition of fungal colony growth for three crop pathogenic fungi, namely *F. solani*, *C. musae* and *P. oryzae*. The nine medicinal plant species may be a good source of antifungal compounds to control plant diseases caused by the three tested fungal pathogens to major crops (pepper, banana and paddy) in Sarawak, Malaysia. However, further research is required.

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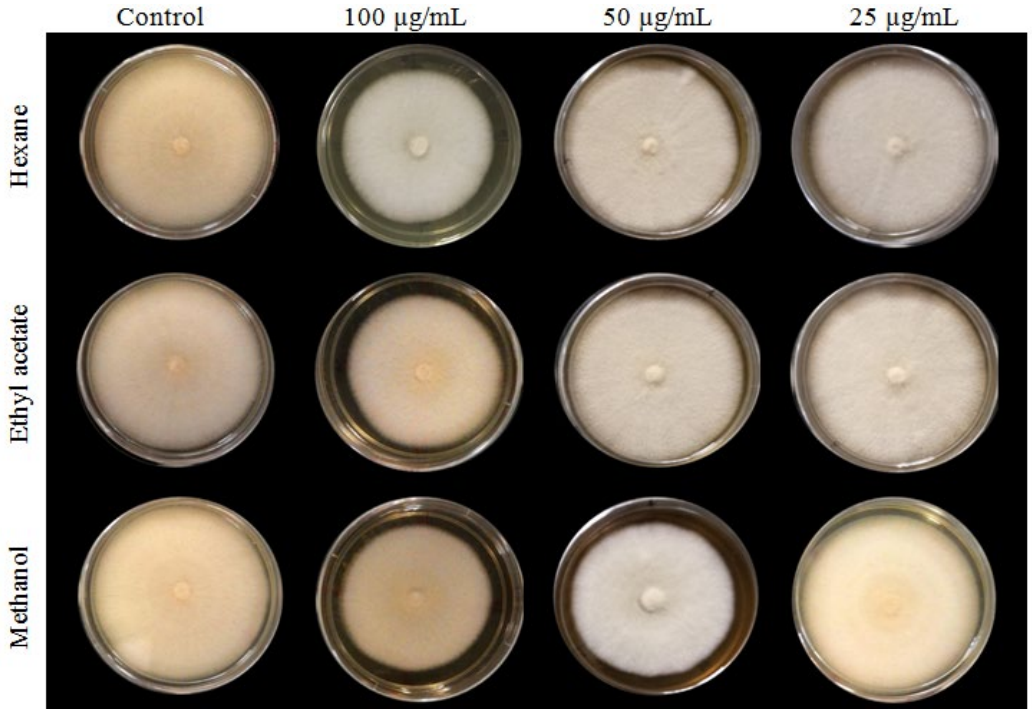
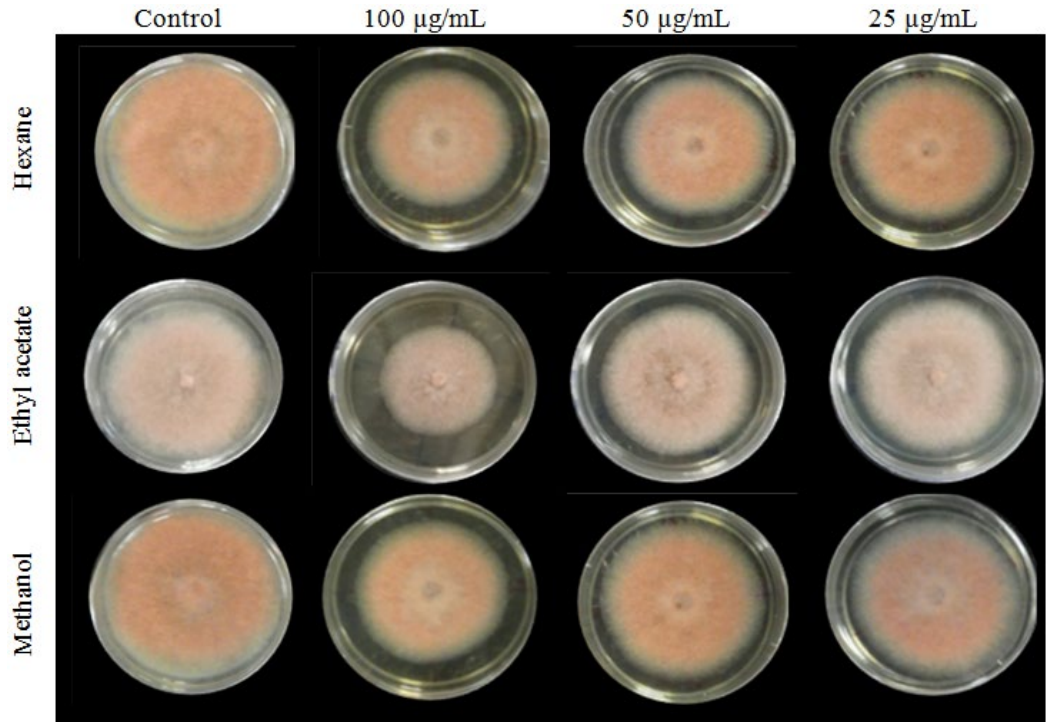
SUPPLEMENTARY INFORMATION



Supplementary Figure S1: Antifungal activity of three leaf crude extracts from *Clinacanthus nutans* against *Fusarium solani* in different concentrations.



Supplementary Figure S2: Antifungal activity of three leaf crude extracts from *Garcinia mangostana* against *Colletotrichum musae* in different concentrations.

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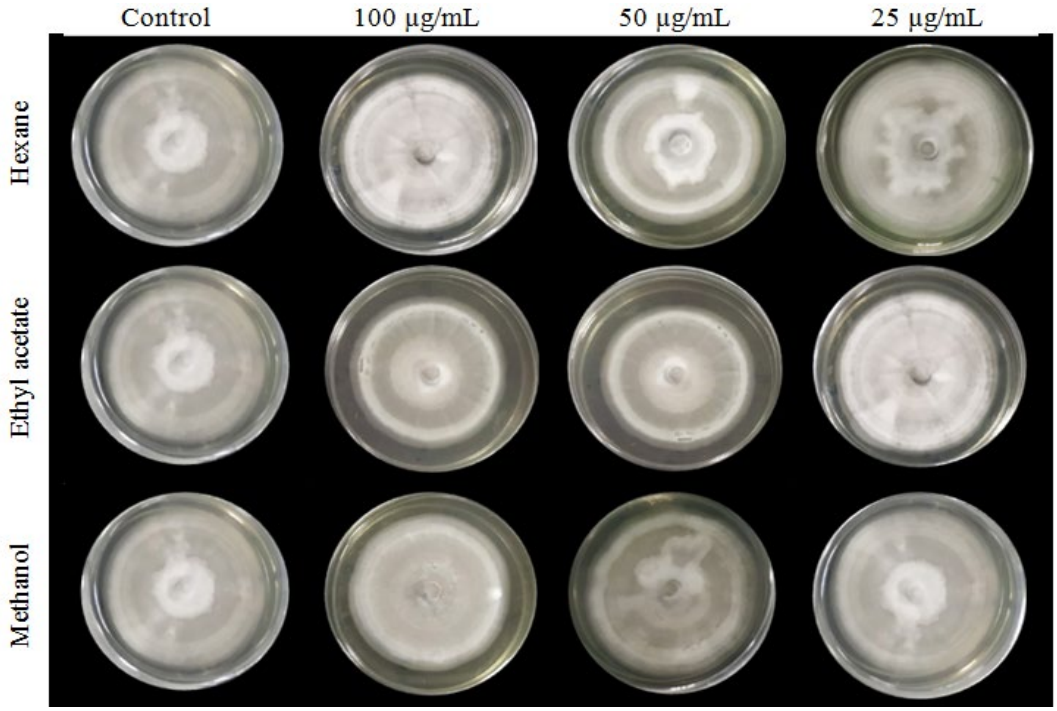
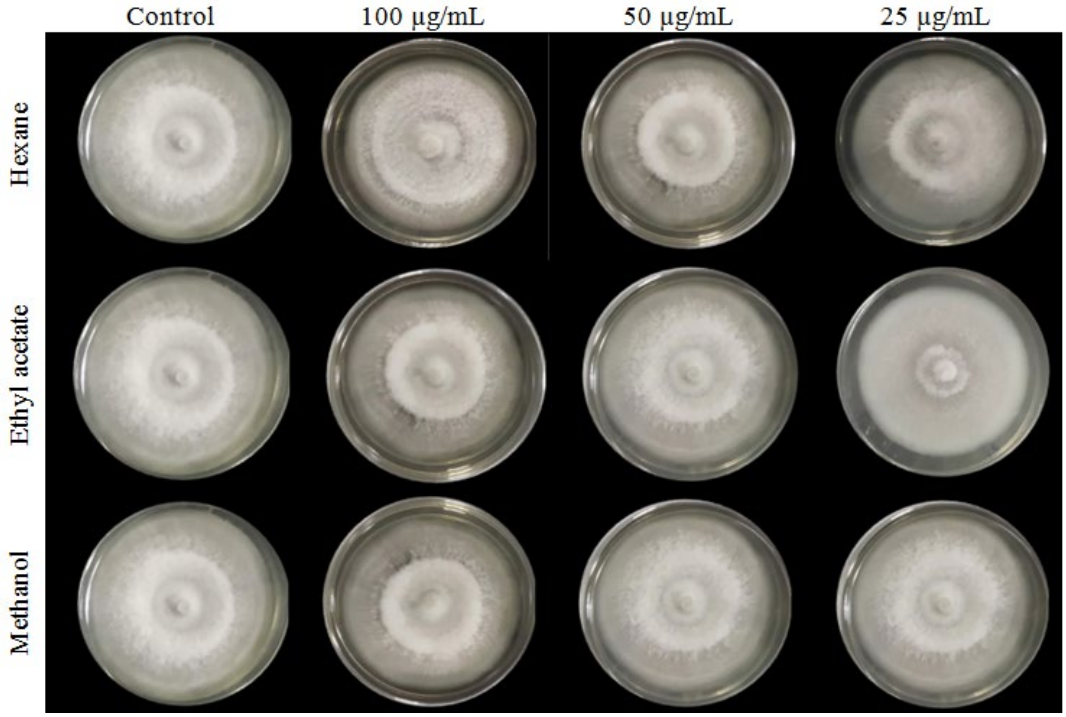


Supplementary Figure S3: Antifungal activity of three leaf crude extracts from *Cymbopogon citratus* against *Pyricularia oryzae* (isolate POSA1) in different concentrations.



Supplementary Figure S4: Antifungal activity of three leaf crude extracts from *Cymbopogon citratus* against *Pyricularia oryzae* (isolate POSA2) in different concentrations.

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