SHORT COMMUNICATION

Antifungal Properties of Selected Medicinal Plant Species Against *Fusarium* spp. – A Preliminary Study

IZZA NADIRA ABU BAKAR, ABDUL RAFFAR ABDUL RAZAK, MUHAMMAD NUR HAKIM ZULKIFLE, NUR ALIAH ROSLI & YEO FREDDY KUOK SAN*

Faculty of Resource Science and Technology, Universiti Malaysia Sarawak,
94300 Kota Samarahan, Sarawak, Malaysia
*Corresponding author: yksfreddy@unimas.my

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ABSTRACT

Usage of synthetic fungicides has inevitably been one of the agricultural practices in combating crop pathogens and maintaining the quality of production. Although fungicides have been proven to be profoundly effective, excessive and frequent reliance on these synthetic fungicides have caused negative impacts to the environment and human health. Besides that, indiscriminate use of fungicides may lead to the development of resistant strains of pathogenic fungi. The need to find an alternative solution to synthetic fungicides has led to the interest in finding plant-based fungicides. This study aimed to test the antifungal properties of plant extracts from 13 different medicinal plant species towards plant pathogenic fungi. Absolute methanol was used as a solvent to extract the secondary metabolites from the different plant species. The effect of methanolic crude extract at different concentrations (500 µg/ml, 250 µg/ml and 100 µg/ml), from different medicinal plant species, were tested on the growth of two *Fusarium* spp., *Fs*B and *Fs*P. The assay showed that the methanolic crude extract from six plant species *viz*. *Alpinia galanga*, *Annona muricata*, *Archidendron jiringa*, *Nephelium lappaceum*, *Polygonum minus* and *Artocarpus* hybrid (Nanchem) had successfully inhibit the radial mycelial growth of either *Fs*B or *Fs*P, or both. The assay suggested that the six plant species have antifungal properties towards the crop pathogenic fungi tested.

Keywords: antimicrobial, *Fusarium*, plant extracts, methanolic extracts, biofungicides

Combating plant pathogens has always been a constant struggle for farmers to obtain high yield with good quality produce. Agricultural practices such as application of synthetic fungicides are commonly used to reduce diseases (Brent & Holloman, 2007). However, the excessive usage and frequent reliance of synthetic fungicides, have led to several other problems such as toxicity towards the environment due to the residues of synthetic fungicides (Wightwick *et al.*, 2010), threatening the functionality of human reproductive system by the active compounds (Peraica *et al.*, 1999; Hossain *et al*., 2010) and emergence of resistant strains. These may cause more severe diseases and outbreaks (Francis & Keinath, 2010). Hence, there is a need to produce a safer and biodegradable fungicides derived from natural resources (Brito-Argaez *et al.*, 2009).

Plant-based compounds are one of the potential resources for production of safer and biodegradable fungicides. Secondary metabolites from plants such as alkaloids, terpenoids, cyanogenic, glucosides, and phenolics are groups of compounds known to be involved in plant defence mechanisms (Bennett & Wallsgrove, 1994; Bravo, 1998; Balasundram *et al*., 2006). These compounds are the possible candidates for developing biofungicides. For instance, phenolic compounds such as luteolin-7-glucoside, oleuropein, rutin, and tyrosol in olive plants were reported to possess antimicrobial effect against selected pathogens (Báidez *et al*., 2007; Pereira *et al*., 2007). The present study aimed to test the antifungal properties of methanolic crude extracts from 13 plant species against two *Fusarium* spp., a common plant pathogenic fungus. Table 1 shows the list of

Table 1. The plant parts used for extraction in this study for the 13 medicinal plant species.

Plant parts	Plant species	Common name
Leaves	*Annona muricata* (L.)	Durian Belanda
	Archidendron jiringa (Jack)	Jering
	Artocarpus sp. hybrid (Nanchem)	Cempedak
	Centella asiatica (L.)	Pegaga
	Cymbopogon citratus (D.C.) Stapf	Serai
	Manihot esculenta (Crantz)	Ubi kayu
	Mentha piperita (L.)	Pudina
	Nephelium lappaceum (L.)	Rambutan
	Oenanthe javanica (Blume)	Selom
	Polygonum minus (Huds)	Kesum
	Piper betle (L.)	Sireh
Rhizome	*Alpinia galanga* (L.) Willd.	Lengkuas
	Zingiber officinale Rosc.	Halia

plant species and the plant parts used in this study.

Fresh and disease-free leaves from 11 medicinal plant species were collected from different residential areas or were bought from Medan Niaga Satok, Sarawak. The leaves were rinsed with tap water to remove contaminants. The leaves were patted dry and air dried for seven days without direct sunlight, at room temperature. Fresh and disease-free rhizomes from the other two medicinal plant species (*Alpinia galanga* and *Zingiber officinale*) were also rinsed with tap water and patted dry. They were then cut into smaller pieces and air dried for two weeks without direct sunlight at room temperature.

The air-dried samples were homogenized into powder form using a blender. A total of 150 grams of the powdered samples were soaked in pure methanol and shaken overnight at room temperature. The extracts were filtered using Whatman filter paper No. 1 the following day. The methanolic extract from each plant species was then concentrated using a rotary evaporator. The water bath of rotary evaporator was set at 45°C ± 2.0. The concentrated crude extract was weighed and the yield of crude extract was calculated. In average, the yield of methanolic crude extract from the 13 medicinal plant species was between 4 – 14%.

The antifungal assay was adapted and modified from CSLI (2012). Concentrated crude extract was dissolved with Dimethyl sulfoxide (DMSO) and filter sterilized using a micro filter (0.22 μm). Media was prepared by mixing equal volume of methanol crude extract (from different stock concentrations) into PDA (Sigma-Aldrich) to obtain final concentrations of 500 μg/ml, 250 μg/ml, and 100 μg/ml. Control was prepared by mixing DMSO (same volume as the crude extract) into PDA. The concentration of DMSO in the media was kept at 1%.

Two fungal species were selected for this study, namely *Fusarium* sp. isolated from banana (*Fs*B) and *Fusarium* sp. isolated from black pepper (*Fs*P). The pathogenic fungi were provided by Semongok, Agriculture Research Centre (ARC). Plugs of fungi (6 mm Ø) used in the antifungal assay were obtained from pure cultures of six to seven days old. The antifungal assay had ten replicates per treatment for each plant-fungal isolate pair. All plates were incubated at room temperature. The radial mycelial growth of the fungus was scored daily by measuring the diameter of the mycelia at

the back of Petri dish. The average fungal growth rate was calculated. One-way ANOVA was used to analyse the data. Methanolic crude extract from six plant species showed significant effects on the growth rate of *Fusarium* spp. tested. Methanolic crude extract of *Annona muricata* (L.), *Alpinia galanga* (L.) Willd., and *Nephelium lappaceum* (L.) retarded the growth of both *Fs*B and *Fs*P, whereas methanolic crude extract from *Archidendron jiringa* (Jack), *Artocarpus* sp. hybrid (Nanchem), and *Polygonum minus* (Huds) had slowed down the growth of either *Fs*B or *Fs*P, while the methanolic crude extract of the other medicinal plant species had no effect towards the growth rate of *Fusarium* spp. tested (Table 2).

Table 2. Average growth rate (cm/day) of the two *Fusarium* spp. on different concentrations of methanol crude extract from the 13 plant species.

Plant species	Fungal isolate	Growth rate (cm/day)*			
		Control	100 µg/ml	250 µg/ml	500 µg/ml
A. galanga	*Fs*B	0.36[a]	0.37[a]	0.32[b]	0.28[c]
	*Fs*P	0.48[a]	0.46[a]	0.45[a]	0.40[b]
A. muricata	*Fs*B	0.70[a]	0.66[a]	0.65[b]	0.68[c]
	*Fs*P	0.82[a]	0.73[b]	0.71[c]	0.68[d]
A. jiringa	*Fs*B	0.64[a]	0.69[a]	0.66[a]	0.59[a]
	*Fs*P	0.74[a]	0.75[b]	0.76[c]	0.66[d]
Artocarpus sp. hybrid (Nanchem)	*Fs*B	0.61[a]	0.60[a]	0.59[a]	0.55[b]
	*Fs*P	0.84[a]	0.89[a]	0.85[a]	0.82[a]
C. asiatica	*Fs*B	0.40[a]	0.40[a]	0.40[a]	0.39[a]
	*Fs*P	0.33[a]	0.37[a]	0.37[a]	0.41[a]
C. citratus	*Fs*B	0.77[a]	0.87[a]	0.79[a]	0.79[a]
	*Fs*P	1.14[a]	1.06[a]	1.10[a]	1.13[a]
M. esculenta	*Fs*B	0.94[a]	1.06[a]	1.09[a]	0.92[a]
	*Fs*P	2.06[a]	2.23[a]	2.28[a]	2.12[a]
M. piperita	*Fs*B	0.73[a]	0.75[a]	0.78[a]	0.77[a]
	*Fs*P	1.45[a]	1.50[a]	1.60[a]	1.61[a]
N. lappaceum	*Fs*B	0.42[a]	0.38[a]	0.34[b]	0.32[c]
	*Fs*P	0.45[a]	0.43[b]	0.40[c]	0.37[d]
O. javanica	*Fs*B	0.80[a]	0.98[a]	0.94[a]	0.87[a]
	*Fs*P	0.66[a]	0.89[a]	0.87[a]	0.83[a]
P. minus	*Fs*B	0.38[a]	0.39[a]	0.48[a]	0.39[a]
	*Fs*P	0.39[a]	0.41[a]	0.39[a]	0.35[b]
P. betle	*Fs*B	0.60[a]	0.55[a]	0.61[a]	0.59[a]
	*Fs*P	0.87[a]	0.89[a]	1.09[a]	0.85[a]
Z. officinale	*Fs*B	0.75[a]	0.71[a]	0.73[a]	0.78[a]
	*Fs*P	0.74[a]	0.93[a]	0.69[a]	0.67[a]

*Different letters indicate significant difference of fungal growth rate on media infused with different concentrations of crude extract ($p<0.05$).

In general, the methanolic crude extract from the six plant species managed to retard the fungal growth by a delay of one to three days to reach full plate (8 cm Ø) as compared to the corresponding control plates (Table 3).

The inhibition percentage of the fungal growth by the different concentrations of methanolic crude extract were small for both *Fusarium* spp. and negligible, therefore it is not presented.

Table 3. The significant fungal growth delay on the plates containing methanolic crude extract from the six medicinal plant species having antifungal properties.

Plant species	Fungal isolates	Effective concentrations	Number of days
A. galanga	*Fs*B	500 µg/ml	2-3 days
		250 µg/ml	1 day
	*Fs*P	500 µg/ml	2-3 days
A. muricata	*Fs*B	250 µg/ml	1 day
	*Fs*P	ALL	1 day
A. jiringa	*Fs*P	500 µg/ml	1 day
N. lappaceum	*Fs*B	500 µg/ml	2-3 days
		250 µg/ml	2-3 days
	*Fs*P	500 µg/ml	2-3 days
		250 µg/ml	2-3 days
		100 µg/ml	1 day
P. minus	*Fs*P	500 µg/ml	1 day
Artocarpus sp. hybrid (Nanchem)	*Fs*B	500 µg/ml	1 day

Although the inhibition effect may be negligible, the success of retarding the fungal growth is an indication for the presence of antifungal compounds in the plant methanolic crude extract against *Fs*B and *Fs*P. Previous phytochemical studies on methanolic extracts of *A. galanga*, *A. muricata*, *A. jiringa*, *N. lappaceum*, and *P. minus* reported potential compounds in the plant extract that possess antimicrobial properties in the respective studies (Table 4).

There is a possibility that the compounds reported (Table 4) is also present in the crude extract of the current study. Against *Fs*B and *Fs*P, these compounds may not have strong effect but only manage to slow down the fungal growth. So far, there is no phytochemical studies on Nanchem. It is possible that the methanolic crude extract of Nanchem contain polar compounds (Cowan, 1999) with antifungal properties.

Table 4. Class of compounds detected in the methanolic extract of the six medicinal plant species having antifungal properties identified.

Plant species	Classes of compounds	References
A. galanga	Phenols Flavonoids ie. 1'- Acetoxychavicol acetate (ACA) and galangin	Seo *et al*. (2013) Nuttaporn (2007)
A. muricata	Alkaloids Flavonoids Tannins Terpenoids Polyphenols	Chauhan & Mittu (2015) George *et al*. (2015)
A. jiringa	Terpenoids Tannins Polyphenols	Cowan (1999)
N. lappaceum	Phenols	Thitilertdecha *et al*. (2008)
P. minus	Phenols Flavonoids	Qader *et al*. (2012)
Artocarpus sp. hybrid (Nanchem)	N/A	-

To conclude, this preliminary study showed that six of the 13 medicinal plant species used in this study *viz*. *A. muricata*, *A. jiringa*, *A. galanga*, Nanchem, *N. lappaceum* and *P. minus*, have antifungal property against *Fs*B and *Fs*P. The methanolic crude extract, at high concentration, managed to retard the fungal growth. The inhibition percentage was, however, negligible. Future studies can be conducted by using different parts of the plant species to screen for antifungal properties.

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