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The effect of herbal plant extracts on the growth and sporulation of *Colletotrichum gloeosporioides*

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ABSTRACT
Objectives: The antifungal activities of the leaf extracts of fifteen selected medicinal plants; *Alpinia galanga* L., *Alstonia spatulata* Blume., *Annona muricata* L., *Blechnum orientale* L., *Blumea balsamifera* L., *Centella asiatica* L., *Dicranopteris linearis, Dillenia suffruticosa, Litsea garciae* Vidal., *Melastoma malabathricum* L., *Momordica charantia* L., *Nephrolepis biserrata* (Sw.)., *Pangium edule* Reinw., *Piper betle* L., and *Polygonum minus* Huds., were evaluated on the plant pathogenic fungus; *C. gloeosporioides* isolated from mango.
Methodology and results: Different antifungal assays were employed, i.e. Agar-Disc Dilution assay as primary screening assay, followed by determination of Minimum Inhibition Concentration (MIC), and the rate of sporulation assay. The antifungal assay was carried out in Potato Dextrose Media in five different treatments, i.e.; distilled water as negative control, crude extract of leaves in methanol, chloroform, acetone and Benomyl as positive control. *A. galanga* extracts were most effective and exhibited highest antifungal activities against *C. gloeosporioides*. Methanol crude extract reduced radial growth of *C. gloeosporioides* by 66.39%, followed by chloroform crude extract 63.26%, and 61.56% for acetone crude extracts. The exact concentrations that have definite potential to fully restrict the growth of *C. gloeosporioides* (MICs) for *A. galanga* is 15.00 mg/mL in methanol, 17.50 mg/mL in chloroform, and 17.50 mg/mL in acetone. The sporulation assay also revealed that *A. galanga* leaves crude extracts showed highest inhibition of spore germination of *C. gloeosporioides* overall at concentration of 10 mg/mL; with 68.89% inhibition by methanol extracts, 64.13% by chloroform extracts, and 62.86% by acetone extracts.
Conclusion and application of findings: Numerous natural products of plant origin are pesticidal and have the potential to control fungal diseases of crops. Thus, considerable effort should be devoted to screening plants in order to develop new natural fungicides as alternative to existing. In this study, the leaf crude extracts of *A. galanga* exhibited effectiveness against *C. gloeosporioides* and should be considered for further evaluation.
Key words: Medicinal plants, crude extracts, anti-fungal growth, *C. gloeosporioides*

INTRODUCTION
Colletotrichum gloeosporioides are one of the most important pathogens affecting the flowers and fruits of mango trees causing anthracnose worldwide. In areas where rain is prevalent during flowering and fruit set, anthracnose can cause destruction of the inflorescences and infection and drop of young fruit where this can obviously lead to serious losses, reaching up to 35% of the harvested fruit (Martinez *et*

al., 2009).Excessive use of benomyl, thiophanate-methyl and thiobendazole as pre- and post-harvest sprays has led to a reduction in effectiveness in certain areas where pathogen resistance to fungicides has been reported (Spalding, 1982). Indiscriminate use of the chemicals is not only hazardous to people but also disrupt the natural ecological balance by killing the beneficial soil microbes (Ansari, 1995). So, alternatives have to be developed to control anthracnose in order to guarantee safe food production as well as reduce environmental pollution. The integration of a number of practices aiming to reduce or eliminate negative side effects caused by chemicals used for controlling major mango diseases is the most realistic option for solving the problem (Chowdury and Rahim, 2009). Research work in relation to anthracnose disease management of mango is yet to develop effective alternative/options.

Hence, this study was carried out with the aim of providing broader options by evaluating the antifungal activity of crude leaf extracts from selected medicinal plants against phytopathogenic fungi *Colletotrichum capsici* and *Colletotrichum gloeosporioides.*

MATERIALS AND METHODS

The leaves samples were collected from Sarikei, Sarawak. Samples were dried in the oven at 50ºC until the leaves become crunchy and were weighed. 100 g of leaves samples were then pounded using mortar and pestle into coarse powder. Leaves of the plants were extracted in polar solvent (methanol), semi-polar solvent (chloroform), and non-polar solvent (acetone) by soaking in each solvent for at least 48 hours. The extracts solution was filtered with filter paper (Whatman No. 1), transferred into pre-weighed 250mL round bottom flasks and evaporated using Rotavapor. The extracts and the round bottom flask were weighed again after solvent evaporation.

Pathogen culture: The culture of *Colletotrichum gloeosporioides* from *Mangifera india* L. were obtained from Faculty of Agriculture, UPM. Pure cultures were maintained on Potato Dextrose Agar slants.

Antifungal assay: The antifungal assay was carried out according to Alam (2004) with a slight modification. A volume of 19 mL of molten PDA was poured in sterilized Petri dishes along with 1 mL of plant extract and plated. Mycelial discs (10 mm diameter) made using the cork borer were inoculated at the centre of the medium. The antifungal assay was carried out in PDA in five different sets: negative control, crude extract of leaves in methanol, chloroform, and acetone, and positive control (commercial fungicide). Colony growth was measured on the basis of linear dimensions. Minimum inhibitory concentration (MIC) and sporulation were determined.

Sporulation was determined by adding 10ml sterile distilled water to each seven days old plate that were obtained from agar disk method and gently scraping the mycelia with a sterile glass rod to dislodge the spores. The spore suspensions obtained were filtered through sterile cheesecloth into a sterile 50 mL glass beaker and homogenized by manual shaking. The spores were then counted using a haemocytometer.

The percentage reduction (Sr) or stimulation (Ss) of sporulation by each extract was determined using the following formula, (Nduagu *et al*., 2008):

$$Sr = \frac{(S1 - S2) \times 100}{S1}$$

Where;
Sr = Percentage of reduction in sporulation;
S1 = Sporulation on the untreated medium (control); and
S2 = Sporulation on the treated medium.

$$Ss = \frac{(S2 - S1) \times 100}{S2}$$

Where;
Ss = Percentage of stimulation in sporulation;
S2 = Sporulation on treated medium; and
S1 = Sporulation in untreated medium.

RESULTS

Inhibition of radial growth of *Colletotrichum gloeosporioides*: Among the plants screened, only 5 species showed 50% or more antifungal activity against *C. gloeosporioides* at varying concentrations **(Table 1)**. Methanol crude extracts of *A. galanga, P. betle, M. malabathricum, B. balsamifera,* and *P. minus* were most effective. *A. galanga* exhibited highest antifungal activity of 66.78% at 10.00 µg/mL, 66.27% at 1.00 µg/mL, 61.57% at 0.10 µg/mL, and 60.55% at 0.01 µg/mL against *C. gloeosporioides*. This was followed by *M. malabathricum* and *P. Betle,* whose inhibition activity increased as the concentration of plant extracts increased (Table 1).

Table 1: Inhibiton of radial growth (mm) of *Colletotrichum gloeosporioides* by varying concentrations of leaf extracts in methanol.

Leaf Extracts in Methanol	Mean ± S.E of Inhibiton of Radial Growth (mm)			
	0.01µg/mL	0.10µg/mL	1.00µg/mL	10.00µg/mL
***Alpinia galanga* L.**	51.07 ± 0.54*	51.68 ± 0.49*	55.83 ± 0.86*	56.43 ± 0.27*
***Alstonia spatulata* Blume.**	1.36 ± 0.72	3.98 ± 0.66	8.27 ± 0.75	11.36 ± 0.21
***Annona muricata* L.**	15.19 ± 0.58	15.46 ± 0.86	16.15 ± 0.75	17.59 ± 0.61
***Blechnum orientale* L.**	NI	NI	NI	NI
***Blumea balsamifera* L.**	35.91 ± 0.45	37.02 ± 0.52	39.07 ± 0.89	43.05 ± 0.96*
***Centella asiatica* L.**	24.37 ± 0.67	26.46 ± 0.65	29.28 ± 0.61	29.20 ± 0.49
Dicranopteris linearis	NI	NI	3.69 ± 0.76	10.17 ± 0.75
Dillenia suffruticosa	1.75 ± 0.69	2.22 ± 0.59	3.66 ± 0.78	5.72 ± 0.54
***Litsea garciae* Vidal.**	13.31 ± 0.49	15.17 ± 0.55	15.26 ± 0.58	17.22 ± 0.78
***Melastoma malabathricum* L.**	44.12 ± 0.45*	44.94 ± 0.66*	48.85 ± 0.85*	53.09 ± 0.75*
***Momordica charantia* L.**	36.42 ± 0.48	37.93 ± 0.65	40.04 ± 0.42	41.54 ± 0.94
***Nephrolepis bisserrata* (Sw.)**	NI	NI	1.01 ± 0.57	3.31 ± 0.67
***Pangium edule* Reinw.**	24.93 ± 0.68	26.16 ± 0.73	28.44 ± 0.41	30.26 ± 0.50
***Piper betle* L.**	50.41 ± 0.58*	51.58 ± 0.45*	51.94 ± 0.35*	53.73 ± 0.46*
***Polygonum minus* Huds.**	37.71 ± 0.37	39.53 ± 1.11	40.05 ± 0.63	41.88 ± 0.87*

Each value represents mean ± standard error; NI = No Inhibition
* shows crude extracts that effectively inhibit growth

Extracts of *A. galanga* in chloroform also showed high inhibition against *C. gloeosporioides* of 63.69% at 10.00 µg/mL, 61.43% at 1.00 µg/mL, 60.78% at 0.10 µg/mL, and 58.62% at 0.01 µg/mL. This was followed by chloroform crude extract of *P. betle*, *M. malabathrichum*, and *B. balsamifera* (Table 2). Extracts *of A. galanga* in acetone inhibited growth of C. gloeosporioides 62.60% at 10.00 µg/mL, 60.50% at 1.00 µg/mL, 57.05% at 0.10 µg/mL, and 54.67% at 0.01 µg/mL (Table 3).

Minimum Inhibition Concentration (MIC) of plant crude extracts to *C. Gloeosporioides:* Crude extracts of *Alphinia galanga* in all three solvents; methanol, chloroform and acetone exhibited the lowest MIC value against *C. gloeosporioides* of 15.0 µg/mL in methanol, 17.50 µg/mL in chloroform and acetone. This was followed by crude extracts of both *P. betle* and *M. malabathricum* which exhibited no visible growth of *C. gloeosporioides* at 17.50 µg/mL and 20.00 µg/mL. Crude extracts of *C. asiatica, D. suffruticosa, A. muricata, D. linearis, A. spatulata, L. garciae, P. edule,* and *N. bisserrata* in all three solvents exhibited MIC values that were out of the range of the prepared concentrations. Crude extracts of *B. orientale* did not exhibit any antifungal activities against *C. gloeosporioides*.

Table 2: Inhibiton of radial growth of *C. gloeosporioides* by varying concentrations of leaf extracts in chloroform.

Leaf Extracts in Chloroform	Mean ± S.E of Inhibiton of Radial Growth (mm)			
	0.01µg/mL	0.10µg/mL	1.00µg/mL	10.00µg/mL
***Alpinia galanga* L.**	49.41 ± 0.45*	51.00 ± 0.91*	51.68 ± 0.76*	53.77 ± 0.67*
***Alstonia spatulata* Blume.**	14.12 ± 0.74	15.88 ± 0.54	17.19 ± 0.45	20.47 ± 0.54
***Annona muricata* L.**	19.38 ± 0.47	19.98 ± 0.47	20.30 ± 0.69	23.50 ± 0.65
***Blechnum orientale* L.**	NI	NI	NI	NI
***Blumea balsamifera* L.**	36.33 ± 0.67	37.10 ± 0.71	40.10 ± 0.86	42.37 ± 0.47*
***Centella asiatica* L.**	30.33 ± 0.50	32.61 ± 0.77	33.67 ± 0.59	35.31 ± 0.64

Dicranopteris linearis	NI	NI	2.53 ± 1.43	8.48 ± 0.80
Dillenia suffruticosa	1.72 ± 0.67	2.28 ± 0.76	5.36 ± 0.84	8.22 ± 0.56
***Litsea garciae* Vidal.**	14.24 ± 0.51	15.74 ± 0.67	17.73 ± 0.78	19.38 ± 0.48
***Melastoma malabathricum* L.**	41.33 ± 0.51	42.55 ± 0.71*	46.50 ± 0.84*	49.45 ± 0.32*
***Momordica charantia* L.**	35.06 ± 1.07	36.65 ± 0.99	39.44 ± 0.43	40.69 ± 0.56
***Nephrolepis bisserrata* (Sw.)**	NI	NI	4.79 ± 0.99	9.05 ± 0.87
***Pangium edule* Reinw.**	30.88 ± 0.52	32.31 ± 0.84	32.82 ± 0.38	36.38 ± 0.65
***Piper betle* L.**	46.14 ± 0.42*	49.43 ± 0.82*	51.62 ± 0.33*	53.60 ± 0.90*
***Polygonum minus* Huds.**	35.21 ± 0.57	36.81 ± 0.77	38.80 ± 0.47	40.13 ± 0.43

Each value represented the mean ± standard error; NI = No Inhibition
* represented crude extracts effectively inhibits growth

Sporulation : The highest inhibition was recorded in treatments of crude extracts of *A. galanga* which exhibited the highest antifungal activities in inhibiting the sporulation of *C. gloeosporioides* among the 15 medicinal plants (Table 4-6). The methanol crude extract of *A. galanga* at 10.00 µg/mL exhibited the highest inhibition overall of 68.89%. At the lowest concentration of 0.01 µg/mL of acetone crude extract, *A. galanga* still inhibited sporulation by 62.86%.

Table 3: Inhibiton of sporulation (x10^5) of *C. gloeosporioides* by varying concentration of leaf extracts in acetone.

Leaf Extracts in Acetone	**Mean ± S.E of Inhibiton of Radial Growth (mm)**			
	0.01µg/mL	**0.10µg/mL**	**1.00µg/mL**	**10.00µg/mL**
***Alpinia galanga* L.**	46.02 ± 0.67*	47.81 ± 0.43*	50.87 ± 0.34*	52.34 ± 0.39*
***Alstonia spatulata* Blume.**	1.69 ± 0.81	3.53 ± 0.63	5.82 ± 0.56	12.63 ± 0.65
***Annona muricata* L.**	15.41 ± 0.36	16.72 ± 0.46	17.84 ± 0.92	19.54 ± 0.40
***Blechnum orientale* L.**	NI	NI	NI	NI
***Blumea balsamifera* L.**	38.64 ± 0.69	39.17 ± 0.63	42.60 ± 1.20*	45.83 ± 0.85*
***Centella asiatica* L.**	25.63 ± 0.71	27.69 ± 0.86	29.33 ± 0.85	30.25 ± 0.60
Dicranopteris linearis	NI	NI	3.66 ± 0.82	7.47 ± 1.25
Dillenia suffruticosa	7.27 ± 0.73	9.61 ± 0.64	12.40 ± 0.62	13.33 ± 0.42
***Litsea garciae* Vidal.**	18.88 ± 0.69	21.16 ± 0.50	21.78 ± 0.63	22.68 ± 0.56
***Melastoma malabathricum* L.**	37.75 ± 0.56	39.43 ± 0.96	43.74 ± 1.11*	48.54 ± 0.41*
***Momordica charantia* L.**	36.42 ± 0.48	29.04 ± 0.93	32.65 ± 1.02	35.16 ± 0.97
***Nephrolepis bisserrata* (Sw.)**	NI	NI	3.66 ± 0.71	9.01 ± 0.59
***Pangium edule* Reinw.**	26.18 ± 0.72	27.39 ± 0.92	28.48 ± 0.72	31.31 ± 0.60
***Piper betle* L.**	43.91 ± 1.10*	47.72 ± 0.57*	49.50 ± 0.68*	50.16 ± 0.72*
***Polygonum minus* Huds.**	36.56 ± 0.53	38.82 ± 0.99	39.84 ± 0.49	40.15 ± 0.50

Each value represented the mean ± standard error; NI = No Inhibition
* represented crude extracts effectively inhibits growth

Table 4: Inhibiton of sporulation (x10^5) of *C. gloeosporioides* by varying concentration of leaf extracts in methanol.

Leaf Extracts in Methanol	Mean ± S.E of Inhibiton of Sporulation (x10^5)			
	0.01µg/mL	0.10µg/mL	1.00µg/mL	10.00µg/mL
***Alpinia galanga* L.**	2.44 ± 0.02*	2.55 ± 0.02*	2.70 ± 0.02*	2.89 ± 0.02*
***Alstonia spatulata* Blume.**	0.17 ± 0.03	0.18 ± 0.03	0.33 ± 0.03	0.51 ± 0.02
***Annona muricata* L.**	0.84 ± 0.03	0.83 ± 0.03	0.86 ± 0.04	0.86 ± 0.02
***Blechnum orientale* L.**	NI	NI	NI	NI
***Blumea balsamifera* L.**	1.71 ± 0.03	1.68 ± 0.03	1.89 ± 0.04	2.03 ± 0.02
***Centella asiatica* L.**	1.10 ± 0.03	1.17 ± 0.03	1.13 ± 0.03	1.12 ± 0.03
Dicranopteris linearis	NI	NI	0.23 ± 0.02	0.42 ± 0.02
Dillenia suffruticosa	0.13 ± 0.02	0.19 ± 0.02	0.27 ± 0.02	0.29 ± 0.03
***Litsea garciae* Vidal.**	0.68 ± 0.03	0.70 ± 0.02	0.67 ± 0.04	0.68 ± 0.03
***Melastoma malabathricum* L.**	1.98 ± 0.03	2.20 ± 0.03*	2.29 ± 0.03*	2.33 ± 0.04*
***Momordica charantia* L.**	1.55 ± 0.03	1.56 ± 0.02	1.83 ± 0.02	1.88 ± 0.03
***Nephrolepis bisserrata* (Sw.)**	NI	NI	NI	0.08 ± 0.02
***Pangium edule* Reinw.**	1.09 ± 0.03	1.13 ± 0.03	1.32 ± 0.04	1.36 ± 0.02
***Piper betle* L.**	2.10 ± 0.03*	2.28 ± 0.05*	2.45 ± 0.02*	2.64 ± 0.02*
***Polygonum minus* Huds.**	1.73 ± 0.02	1.74 ± 0.03	1.93 ± 0.02	2.00 ± 0.03

Each value represented the mean ± standard error; NI = No Inhibition
* represented crude extracts effectively inhibits growth

Table 5: Inhibiton of sporulation (x10^5) of *C. gloeosporioides* by varying concentrations of leaf extracts in chloroform.

Leaf Extracts in Chloroform	Mean ± S.E of Inhibiton of Sporulation (x10^5)			
	0.01µg/mL	0.10µg/mL	1.00µg/mL	10.00µg/mL
***Alpinia galanga* L.**	2.04 ± 0.02	2.27 ± 0.02*	2.48 ± 0.02*	2.69 ± 0.01*
***Alstonia spatulata* Blume.**	0.70 ± 0.01	0.79 ± 0.02	0.89 ± 0.02	0.86 ± 0.02
***Annona muricata* L.**	1.06 ± 0.02	1.06 ± 0.03	1.09 ± 0.03	1.04 ± 0.03
***Blechnum orientale* L.**	NI	NI	NI	NI
***Blumea balsamifera* L.**	1.65 ± 0.03	1.72 ± 0.03	1.82 ± 0.04	2.06 ± 0.02
***Centella asiatica* L.**	0.92 ± 0.03	0.93 ± 0.03	1.15 ± 0.03	1.14 ± 0.04
Dicranopteris linearis	NI	NI	0.20 ± 0.02	0.45 ± 0.03
Dillenia suffruticosa	0.15 ± 0.03	0.16 ± 0.01	0.38 ± 0.02	0.43 ± 0.02
***Litsea garciae* Vidal.**	0.69 ± 0.03	0.71 ± 0.03	0.71 ± 0.03	0.69 ± 0.02
***Melastoma malabathricum* L.**	1.77 ± 0.03	1.80 ± 0.03	1.89 ± 0.03	2.27 ± 0.04*
***Momordica charantia* L.**	1.56 ± 0.03	1.70 ± 0.02	1.84 ± 0.03	1.86 ± 0.03
***Nephrolepis bisserrata* (Sw.)**	NI	NI	0.22 ± 0.02	0.44 ± 0.02
***Pangium edule* Reinw.**	1.36 ± 0.02	1.43 ± 0.04	1.48 ± 0.03	1.66 ± 0.01
***Piper betle* L.**	1.68 ± 0.02	2.03 ± 0.05	2.22 ± 0.03*	2.48 ± 0.03*
***Polygonum minus* Huds.**	1.55 ± 0.02	1.59 ± 0.02	1.59 ± 0.03	1.70 ± 0.02

Each value represented the mean ± standard error; NI = No Inhibition
* represented crude extracts effectively inhibits growth

Table 6: Inhibiton of sporulation (x10^5) of *C. gloeosporioides* by varying concentrations of leaf extracts in acetone.

Leaf Extracts in Acetone	Mean ± S.E of Inhibiton of Sporulation (x10^5)			
	0.01µg/mL	0.10µg/mL	1.00µg/mL	10.00µg/mL
***Alpinia galanga* L.**	1.89 ± 0.02	2.32 ± 0.03*	2.53 ± 0.02*	2.64 ± 0.03*
***Alstonia spatulata* Blume.**	0.17 ± 0.03	0.22 ± 0.03	0.31 ± 0.03	0.52 ± 0.03
***Annona muricata* L.**	0.91 ± 0.03	0.91 ± 0.03	0.90 ± 0.02	0.90 ± 0.02
***Blechnum orientale* L.**	NI	NI	NI	NI
***Blumea balsamifera* L.**	1.73 ± 0.03	1.80 ± 0.03	2.07 ± 0.04	2.19 ± 0.03*
***Centella asiatica* L.**	0.79 ± 0.03	0.81 ± 0.02	1.03 ± 0.02	1.08 ± 0.03
Dicranopteris linearis	NI	NI	0.14 ± 0.02	0.34 ± 0.02
Dillenia suffruticosa	0.38 ± 0.02	0.47 ± 0.03	0.65 ± 0.02	0.66 ± 0.02
***Litsea garciae* Vidal.**	0.69 ± 0.04	0.71 ± 0.02	0.67 ± 0.04	0.70 ± 0.03
***Melastoma malabathricum* L.**	1.76 ± 0.03	1.75 ± 0.02	1.76 ± 0.03	2.24 ± 0.04*
***Momordica charantia* L.**	1.56 ± 0.03	1.74 ± 0.02	1.80 ± 0.03	1.84 ± 0.03
***Nephrolepis bisserrata* (Sw.)**	NI	NI	0.15 ± 0.02	0.39 ± 0.02
***Pangium edule* Reinw.**	1.29 ± 0.03	1.32 ± 0.03	1.34 ± 0.04	1.38 ± 0.02
***Piper betle* L.**	1.64 ± 0.04	1.79 ± 0.05	2.23 ± 0.02*	2.35 ± 0.02*
***Polygonum minus* Huds.**	1.69 ± 0.02	1.67 ± 0.03	1.71 ± 0.03	1.74 ± 0.01

Each value represented the mean ± standard error; NI = No Inhibition, * represented crude extracts effectively inhibits growth

DISCUSSION

The results obtained from the different studies showed that the crude extracts of *Alpinia galanga* leaves exhibited the highest antifungal activities against *C. gloeosporioides.* The inhibition was expressed as reduced radial growth, dry mycelial weight, and sporulation germination rate of the pathogen. Inhibitory action of *A. galanga* crude extracts was recorded even at very low dose, which is a clear indication that the crude extracts contain active commencements that have antifungal properties.

Antimicrobial activity of extract from *A. galanga* could be attributed to its chemical constituents as reported by Saritnum and Sruamsiri (2003). Phongpaichit and Liamthong (2001) mentioned that chavicol acetate that has been identified as an antifungal component from *A. galanga* plays an antifungal role as in the *Alpinia conchigera* oil which has antifungal activity against *C. gloeosporioides*. The ethanol extracts from *A. galanga* have been found to have pronounced inhibitory activities against a wide variety of human pathogenic fungi, including strains resistant to the common antifungals amphotericin B and ketoconazole (Elfahmi, 2006). 1'-Acetoxychavicol acetate, the active compound from *A. galanga* has antifungal activity against *Trichophyton mentagrophytes*, *T. rubrum*, *T. concentricum*, *Rhizopus stolonifer* and *Aspergillus niger* at a concentration 14 mg/mL (Janssen and Scheffer, 1985). In agreement with this, Abad *et al.* (2007) mentioned that the chloroform extract of *A. galanga* has pronounced antifungal activity against *Cryptococcus neoformans, Microsporum gypseum* and *Trichophyton longifusus*. Many of the medicinal plants selected for this study have been reported to have novel bioactivities. *Piper betle* exhibited the best antifungal activities against *C. gloeosporioides* after *Alpinia galanga.* Johann *et al.* (2007) stated that the chemistry of *Piper* species has been widely investigated and phytochemical investigations from different parts of the world have led to the isolation of a number of physiologically active compounds such as alkaloids/amides, propenylphenols, lignans, neolignans, terpenes, steroids, kawapyrones, piperolides, chalcones, di-hydrochalcones, flavones and flavanones which exhibited high antimicrobial and antifungal properties. According to Lee *et al.* (2004), most *Piper* chemistry has been conducted to find potential pharmaceuticals or pesticides, and over 90% of the literature focuses on compounds that are cytotoxic, antifungal, antitumor, fragrant, or otherwise useful to humans.The leaves of *Blumea balsamifera* contain icthyothereol acetate and cryptomeridiol, lutein, and β-carotene (Ragasa, 2004). Antimicrobial tests indicated that icthyothereol acetate has moderate activity against *Aspergillus niger, Trichophyton mentagrophytes* and *Candida albicans* (Ragasa, 2004).

In conclusion, the crude extracts of leaves that exhibited good potential as fungicides of *C. gloeosporioides* should be evaluated further in in-depth

to study the phytoextracts for their potentiality under *in vivo* condition.

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