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Full Length Research Paper

Antifungal activity of selected plant leaves crude extracts against a pepper anthracnose fungus, *Colletotrichum capsici* (Sydow) butler and bisby (Ascomycota: Phyllachorales)

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The antifungal activities of the leaves extract of 15 selected medicinal plants; *Alpinia galanga* (L.) Willd., *Alstonia spatulata* Blume., *Annona muricata* L., *Blechnum orientale* L., *Blumea balsamifera* L., *Centella asiatica* L., *Dicranopteris linearis* (Burm. f.) Underw., *Dillenia suffruticosa* (Griff ex Hook.f. and Thomson) Martelli, *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, *Colletotrichum capsici* which was isolated from chilli. The antifungal assay was carried out in potato dextrose media in five different treatments, which were distilled water as the negative control, crude extract of leaves in methanol, chloroform, acetone and Kocide 101 as the positive control. They were carried out in three replicates. The two-way analysis of variance (ANOVA) was carried out on all the data to justify the difference between critical difference (CD) of mean (P = 0.05) and coefficient of variation (CV %) in terms of mean percent reduction in colony diameter, sporulation and minimum inhibitory concentration (MICs) of *C. capsici* to take statistical decisions. Crude extract of *P. betle* in all the solvents was found to be the most effective and exhibited the highest antifungal activities. Crude extract of *P. betle* in methanol inhibited 85.25% of radial growth of *C. capsici* followed by 78.53% leaves crude extract in chloroform and 73.58% leaves crude extract in acetone at the concentration of 10 µg/ml ($p < 0.05$). The exact concentrations that had definite potential to fully restrict the growth (100% inhibition) of *C. capsici* (MIC) by *P. betle* was 12.50 µg/ml in methanol, 17.50 µg/ml in chloroform and 15.00 mg/ml in acetone. The sporulation assay also revealed that, *P. betle* leaves crude extracts showed the highest inhibition of spore germination rate of *C. capsici* overall at the concentration of 10 µg/ml; with 80.93% inhibition by leaves crude extracts in methanol, 74.09% by leaves crude extracts in chloroform and 72.91% by leaves crude extracts in acetone. Concentration of plant leaves crude extracts that inhibited 50% or more of the radial growth and sporulation was considered as effective (LC ≥ 50). As a conclusion, the leaf crude extracts that exhibited effectiveness by showing more than 50% inhibition against *C. capsici* should be considered for further evaluation. *P. betle* leaf crude extracts was the most effective in inhibiting the fungus respectively and thus, exhibited the highest potential as a new leading biofungicide in the agriculture field.

Key words: Plant leaves crude extracts, antifungal activities, *Colletotrichum capsici*.

INTRODUCTION

Pepper fruit anthracnose which is caused by *Colletotrichum* species is one of the most serious disease which leads to serious yield loss and quality deterioration in many Asian countries and in tropical areas (Oanh et al., 2004; Sang et al., 2007). The most destructive disease of pepper anthracnose is caused by *Colletotrichum capsici* (Sydow) Butler and Bisby (1931) (Ascomycota:

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Phyllachorales) (Amusa et al., 2004). It is well known for infection on leaves, stems, mummification of unripe green pepper fruits, pre-mature fruit drop and fruit rot (Agrios, 1988; Marvel, 2003). It also has been reported that, pre- and post-harvest fruit losses of up to 50% was caused by this fungi (Boali, 1991). Generally, *Colletotrichum* diseases can be controlled by a wide range of chemicals such as copper compounds, dithiocarbamates, benzimidazole and trizole compounds; other fungicides such as chlorothalonil, imazalil and prochloraz are also effective against *Colletotrichum* (Waller, 1992). Although, the use of systemic fungicides simplifies the management strategy, not many systemic fungicides are practically in use on chilli. This limits the choice of systemic fungicides on chilli, thus, there is a strong need to find alternative systemic fungicides to the existing chemical carbendazim, which is the only systemic fungicide currently used in chilli fields (Gopinath et al., 2006). Furthermore, there is also the raise of concerns for problems of fungicide insensitivity, residues on edible produce and for tree crops; efficiency of spraying has increased in importance (Bailey and Jeger, 1992). One approach might be the testing of plants traditionally used for their antifungal activities as potential sources for drug development.

Hence, this study provided broader options in agriculture by evaluating the antifungal activity of plant's leaves crude extracts from selected medicinal plants as *Alpinia galanga* (L.) Willd., *Alstonia spatulata* Blume*., Annona muricata* L*., Blechnum orientale* L., *Blumea balsamifera* L*., Centella asiatica* L., *Dicranopteris linearis* (Burm. f.) Underw*., Dillenia suffruticosa* (Griff ex Hook.f. and Thomson) Martelli, *Litsea garciae* Vidal., *Melastoma malabathricum* L*., Momordica charantia* L., *Nephrolepis biserrata* (Sw.)., *Pangium edule* Reinw., *Piper betle* L., and *Polygonum minus* Huds., in order to test their antifungal potential against phytopathogenic fungi *C. capsici.* These plants were selected for extraction due to their well-known medicinal properties in traditional uses by local ethnics in Malaysia. The collected plant samples were identified at Herbarium, Department of Biology, Universiti Putra Malaysia. Leave samples were extracted in methanol, chloroform and acetone. Then, *C. capsici* was treated with different concentration of plant crude extract and antifungal activities were determined by measuring the percentage inhibition of radial growth, sporulation rate and minimum inhibition concentration (MIC).

MATERIALS AND METHODS

Plant collection and extraction

The leaves of 15 plants (*A. galanga*, *A. spatulata, A. muricata, B. orientale*, *B. balsamifera., C. asiatica*, *D. linearis, D. suffruticosa*, *L. garciae*, *M. malabathricum, M. charantia*, *N. biserrata*, *P. edule*, *P. betle*, and *P. minus*) were collected locally from the nearby areas of Sarikei, Sarawak (Table 1).

The leaf samples were air dried and weighed. Leaves samples were then, ground 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 following cold percolation method (Valsaraj et al., 1997). Leaves sample were then, soaked in three different solvents; methanol, chloroform and acetone, at room temperature for 72 h. The obtained extract was then filtered through Whatman (no.1) filter paper extracts solution and was transferred into 250 ml round bottom flasks which were previously weighed. Then, the extracts solution was evaporated using Buchi Rotavapor R-210, Switzerland to concentrate the extracts. Concentrated extracts were allowed to dry in fume cupboard, weighed again and were kept in 4°C for bioassays evaluation. Their volume was made up to obtain respective concentrations.

Source of isolate

The culture of *C. capsici* from *Capsicum annuum* L. was obtained from the Faculty of Agriculture, Universiti Putra Malaysia. Pure cultures were maintained on potato dextrose agar (PDA) and slants (Gupta, 2004).

Antifungal assays

Agar-dilution assay

The agar dilution assay was carried out according to Alam (2004) with a slight modification. 39 g of potato dextrose agar (PDA) powder was boiled until the agar completely dissolved in 1 L of distilled water. The solution was then transferred to the blue cap bottle and sterilized using autoclave at 121°C for 15 min. 19 ml of the sterilized PDA and 1 ml of plant extract were mixed and plated on the sterilized Petri dishes (8.5 mm in diameter). 10 mm diameter of mycelia discs were inoculated at the centre of the medium. The antifungal assay was divided into five different treatments as crude extract of leaves in methanol, chloroform and acetone, commercial fungicide Kocide 101 as positive control and a negative control. Colony growth was determined on the basis of linear dimensions.

Assay was carried out in three replicates. The percent reduction (Rr) or stimulation (Rs) of colony diameter by each extract was determined using the following formula (Nduagu et al., 2008):

$$Rr = \frac{(R1 - R2) \times 100}{R1}$$

$$Rr = \frac{(R2 - R1) \times 100}{R2}$$

Where, Rr = percent reduction in colony diameter; Rs = percent stimulation in colony diameter; R1 = colony diameter on the untreated medium (control); and R2 = colony diameter on the treated medium.

Minimum inhibitory concentration (MIC) assay

Minimum inhibitory concentration (MIC) was determined using agar-dilution method (Yazdani et al., 2007). 1 ml of various crude extracts concentrations (0.001, 0.005. 0.050, 0.500, 5.0, 12.5, 15.0, 17.5 and 20.0 µg/ml) were prepared in the laboratory universal bottle containing 9 ml of PDA and were sterilized. The mixture of PDA and extracts were poured into Petri dish under sterile condition. Then, 2 µl of adjusted spore suspension was added to

Table 1. Details about plants used, their family, English and local names.

Plant used	Family	English name	Local name
A. galanga (L.) Willd.	Zingiberaceae	Greater galangal or blue ginger	Lengkuas (Malay), Engkuas/Lankwas/Puar (Iban)
A. spatulata Blume.	Apocynaceae	Hard milkwood or siamese balsa	Pulai puteh/Rejang (Iban), Pulai basong (Malay)
A.muricata L.	Annonaceae	Brazilian pawpaw, soursop, prickly custard apple	Hampun kapal (Kadazan), Durin mekah (Iban), Durian belanda (Malay)
B. orientale L.	Blechnaceae	Oriental blechnum, centipede fern	Paku ikan/Paku Lipan/Paku ular/Paku ulat (Malay)
B.balsamifera L.	Asteraceae	Sambong	Susuoh (Bidayuh), Urok bung (Kayan), Dun supiro (Kiput), Keymabo (Selakau), Sembong (Malay)
C.asiatica L.	Mackinlayaceae	Indian pennywort	Pegaga (Malay)
D. linearis (Burm. f.) Underw.	Gleicheniaceae	Uluhe, staghorn fern, false staghorn, resam	Bengkawang (Iban), Resam (Malay)
D. suffruticosa (Griff ex Hook.f. and Thomson) Martelli	Dilleniaceae	Simpoh ayer	Buan (Iban), Abuan (Semai), Bu'ua (Bidayuh), Simpoh air (Malay)
L. garciae Vidal.	Lauraceae	Bagnolo, wuru lilin	Madang enkala/Pedar (Iban), Pong Labon (Sabah), Ta'ang (Bidayuh)
M.malabathricum L.	Melastomaceae	Malabar melastome, Singapore rhododendron, senduduk	Sekenduduk/kenduduk/seduduk (Iban), Senduduk (Malay)
M. charantia L.	Cucurbitaceae	Bitter melon	Peria (Malay)
N. bisserrata (Sw.)	Polypodiaceae	Giant sword fern	Paku larat (Iban), Paku uban (Malay)
P. edule Reinw.	Salicaceae	football fruit, kepayang, kluwak	Kepayang (Iban, Malay)
P. betle L.	Piperaceae	Betel leaf	Sireh (Malay, Iban)
P.minus Huds.	Polygonaceae	Pygmy smartweed	Kesum (Malay)

each Petri dish plate. The agar without any plant crude extract served as the control. Assay was carried out in three replicates. The minimum inhibitory concentration (MIC) was regarded as the lowest concentration of the extract that did not show any visible growth (100% inhibition) after 14 days of incubation (compared with control). The minimum inhibitory concentration (MIC) was expressed in µg/ml.

Sporulation assay

The sporulation assay was carried out according to Nduagu et al. (2008) with a slight modification. The rate of sporulation was determined by adding 10 ml sterile distilled water to each seven days old plate that were obtained from agar dilution assay and gently scraping with a sterile glass rod to dislodge the spores. The spore suspensions obtained were filtered through sterile cheese cloth into a sterile 50 ml glass beaker and homogenized by manual shaking. The spores were then counted using a haemocytometer.

Assay was carried out in three replicates. The percent sporulation reduction (Sr) or stimulation (Ss) by each extract was determined using the following formula (Nduagu et al., 2008):

$$Sr = \frac{(R1 - R2) \times 100}{R1}$$

$$Ss = \frac{(R2 - R1) \times 100}{R2}$$

Where, Sr = percent reduction in sporulation; Ss = percent stimulation in sporulation; S1 = sporulation on the untreated medium (control); and S2 = sporulation on the treated medium.

Statistical analysis

The two-way analysis of variance (ANOVA) was carried out on all the data to justify the difference between critical difference (CD) of mean ($P = 0.05$) and coefficient of variation (CV %) in terms of mean percent reduction in colony diameter, sporulation and MICs of *C. capsici* to take statistical decisions (Snedecor and Cochran, 1989). Results with $p < 0.05$ were considered to be statistically significant. If the result was significant, CD test was adapted to find which of the concentrations were same or different in their percent reduction in colony diameter, sporulation and MICs. For the calculation of CV, standard deviation was converted into a relative measure of dispersion for the purpose of comparison. If CV was greater, it was said that, the treatment was more variable and less stable in terms of action and vice versa. CD and CV were calculated using the following formula: CD = Standard error difference X table value for error degrees of freedom at 5% level and CV = Standard deviation / mean X 100.

RESULTS

Inhibition of radial growth of *C. capsici* by plant crude extracts

Table 1 shows that only 5 out of the15 plants screened showed more than 50% inhibition of radial growth. These plants were *P. betle, A. galanga, C. asiatica, M. charantia* and *P. minus*. Crude extracts of *P. betle* in all the solvents exhibited significant reduction in colony radial growth against *C. capsici* in all the concentrations (Table 2). Furthermore, these studies revealed that, the percent inhibition of radial growth against *C. Capsici* increased as the concentration of plant crude extract increased. Methanol crude extract of *P. betle* exhibited the highest antifungal activity with 72.30 to 85.18% inhibition against *C. capsici*. This was followed by methanol crude extract of *A. galangal* and *C. asiatica* which exhibited 68.77 to 74.60% and 57.60 to 71.87% inhibition, respectively.

Chloroform crude extracts of *P. betle* also showed the highest inhibition (72.36 to 78.53%) against radial growth of *C. capsici* followed by *A. galangal* (50.72 to 63.57%), *M.* charantia (57.46 to 61.31%) and *C. asiatica* (56.58 to 50.61%) (Table 2). While in *B. balsamifera* and *P. edule* less than 50% antifungal activity was exhibited (46.61to 49.82% inhibition), respectively.

Acetone crude extracts of *P. betle, A. galanga, M. charantia*, *C. asiatica, B. balsamifera* and *P. edule* were found to be effective against *C. capsici* which showed more than 50% inhibition of radial growth with different concentration (Table 2). *P. betle* exhibited 75.02% inhibition in 10.00 µg/ml, 75.38% inhibition in 1.00 µg/ml, 73.73% inhibition in 0.10 µg/ml and 70.36% inhibition in 0.01 µg/ml against *C. capsici*. This was followed by acetone crude extract of *B. balsamifera* which exhibited 74.56% inhibition in 10.00 µg/ml, 74.56% inhibition in 1.00 µg/ml, 70.82% inhibition in 0.10 µg/ml and 65.80% inhibition in 0.01 µg/ml against *C. capsici*. Acetone crude extract of *M. charantia* exhibited 57.24% inhibition in 10.00 µg/ml, 54.94% inhibition in 1.00 µg/ml, 51.44% inhibition in 0.10 µg/ml and 51.53% inhibition in 0.01 µg/ml against *C. capsici*. Acetone crude extract of *D. suffruticosa* exhibited effective antifungal activities only in 10.00 µg/ml with 51.33%.

MIC of *C. capsici* by plant crude extracts

Plant crude solvent extracts with the lowest concentration that did not show any visible growth (100% inhibition) of *C. capsici* after 14 days of incubation (compare to control) were determined as MIC. Among the plants screened, *P. betle* exhibited the lowest MIC value against *C. capsici* in 12.5 µg/ml in methanol crude extract. This was followed by methanol crude extract of *A. galanga* (15.00 µg/ml), *C. asiatica* (17.5 µg/ml) and finally, by *M. charantia* and *B. balsamifera* (20 µg/ml), respectively (Table 3). In chloroform crude extracts, both *P. betle* and *A. galanga* exhibited the lowest MIC value against *C. capsici* in 17.5 µg/ml, followed by *C. asiatica* and *M. charantia* (20.00 µg/ml). The lowest MIC value was observed in *P. betle* (15.00 µg/ml), *B. balsamifera* (17.50 µg/ml) and *M. charantia* and *P. minus* (20.00 µg/ml) in acetone crude extracts (Table 3).

Inhibition/stimulation of sporulation of *C. capsici* by plant crude extracts

Inhibition of sporulation of *C. capsici* by leaf crude extracts in methanol, chloroform and acetone of the 15 medicinal plants species are summarized in Table 4. Plant crude solvent extracts with concentration that inhibited more than 50% of the normal sporulation were considered as effective (Begum et al., 2007). Among the plants screened, five species showed 50% and more of antifungal activity against; *C. capsici A. galanga, C. asiatica, B. balsamifera*, *M. charantia*, *P. betle* and *P. minus. P. betle* exhibited the highest antifungal activity in all the chosen four treatments of solvent extracts. *P. betle* leaf crude extract of 10.00 µg/ml exhibited the highest percent of inhibition against *C. capsici* between 72.91 and 80.93%, respectively (Table 4).

DISCUSSION

The findings from this study revealed that, leaf crude extracts of *P.betle* exhibited the highest antifungal activities overall in inhibiting the mycelial growth of *C. capsici* among the 15 medicinal plants. The methanol crude extract of *P. betle* in 10.00 µg/ml exhibited the highest inhibition overall with 85.25% inhibition ($P < 0.05$). Compared with the positive control, commercial *C. capsici* fungicide (Kocide 101), the percentage of inhibition by methanol crude extract of *P. betle* in 10.00 µg/ml exhibited almost the same percentage of Kocide 101 (87.24%) of the respective concentration. At the lowest concentration of acetone crude extract which was 0.01 µg/ml, *P. betle* still effectively inhibited the mycelial growth with 70.36% inhibition. This showed that the inhibitory action of the *P. betle* crude extracts was recorded even at very low dose, which is a clear indication that the crude extract contained active components that have antifungal properties.

The methanol crude extract of *P. betle* in 10.00 µg/ml exhibited the highest inhibition in sporulation of *C. capsici* overall with 80.93% inhibition ($P < 0.05$). The positive control, commercial *C. capsici* fungicide (Kocide 101), exhibited 91.73% inhibition of the respective concentration. At the lowest concentration of acetone crude extract which was 0.01 µg/ml, *P.betle* still effectively inhibited the sporulation with 68.96% inhibition. This showed that the inhibitory action of the *P. betle* crude

Table 2. Mean ± S.E of inhibiton of radial growth (mm) of *C. capsici* by leaf extracts in methanol, chloroform and acetone with various concentrations.

Leaf extract in methanol	Mean ± S.E of inhibiton of radial growth (mm)			
	0.01 (µg/ml)	0.10 (µg/ml)	1.00 (µg/ml)	10.00 (µg/ml)
A. galanga L.	55.22 ± 1.08*	56.99 ± 0.59*	59.31 ± 1.28*	62.78 ± 1.51*
A. spatulata Blume.	19.13 ± 0.92	19.89 ± 1.04	22.06 ± 0.56	23.64 ± 0.91
A. muricata L.	30.25 ± 1.23	31.86 ± 1.21	35.60 ± 1.20	39.96 ± 1.54
B. orientale L.	NI	NI	2.68 ± 1.07	4.02 ± 1.13
B. balsamifera L.	25.86 ± 0.73	27.24 ± 1.19	30.37 ±1.22	34.33 ±1.46
C. asiatica L.	54.78 ± 1.48*	46.90 ± 1.80*	55.26 ± 1.58*	59.07 ± 1.44*
D. linearis	4.44 ± 1.35	5.84 ± 0.77	7.85 ± 0.90	9.78 ± 0.85
D. suffruticosa	30.57 ± 0.59	31.08 ± 0.77	33.77 ± 0.78	36.71 ± 0.76
L.garciae Vidal.	13.59 ± 0.84	15.83 ± 1.22	29.34 ± 0.66	31.02 ± 1.41
M. malabathricum L.	30.71 ± 2.52	30.27 ± 1.24	33.41 ± 1.08	34.51 ± 2.16
M.charantia L.	42.17 ± 1.32*	42.42 ± 2.81*	46.25 ± 1.59*	48.32 ± 1.57*
N.bisserrata (Sw.)	NI	NI	2.89 ± 0.11	3.67 ± 1.13
P.edule Reinw.	NI	NI	1.68 ± 0.43	4.26 ± 1.17
P. betle L.	61.22 ± 0.53*	65.50 ± 1.10*	66.59 ± 0.89*	71.87 ± 0.78*
P. minus Huds.	39.10 ± 1.72	40.20 ± 1.42	40.37 ± 2.03	46.92 ± 1.11*
Leaf extracts in chloroform				
A. galanga (L.) Willd.	42.64 ± 1.30*	46.40 ± 0.72*	48.97 ± 1.36*	53.38 ± 1.05*
A. spatulata Blume.	24.96 ± 1.13	24.74 ± 1.12	27.44 ± 0.72	27.40 ± 0.73
A. muricata L.	8.39 ± 0.84	10.61 ± 1.16	12.65 ± 1.19	18.01 ± 0.92
B. orientale L.	NI	NI	1.77 ± 1.01	5.33 ± 1.13
B. balsamifera L.	35.50 ± 0.43	39.17 ± 1.35	44.87 ± 1.10*	46.26 ± 1.34*
C. asiatica L.	38.69 ± 1.87	40.82 ± 1.54	42.61 ± 1.01*	47.41 ±1.82*
D. linearis (Burm. f.) Underw.	32.05 ± 1.02	34.23 ± 0.62	36.19 ± 1.13	37.89 ± 0.62
D. suffruticosa	21.41 ± 0.50	22.10 ± 0.93	23.98 ± 1.26	29.90 ± 2.20
L. garciae Vidal.	1.94 ± 1.70	5.84 ± 1.26	8.85 ± 1.99	9.68 ± 1.53
M. malabathricum L.	25.58 ± 1.55	23.56 ± 0.69	27.27 ± 1.13	41.12 ± 1.55
M. charantia L.	48.12 ± 1.53*	50.24 ± 2.12*	51.85 ± 1.94*	50.09 ± 1.56*
N. bisserrata (Sw.)	NI	NI	3.27 ± 0.15	5.60 ± 0.62
P. edule Reinw.	36.93 ± 1.43	37.73 ± 0.70	41.24 ± 1.17	44.01 ± 1.40*
P.betle L.	61.27 ± 0.75*	63.90 ± 1.72*	65.37 ± 0.90*	66.20 ± 1.15*
P. minus Huds.	20.23 ± 2.27	25.62 ± 1.51	29.43 ± 0.52	31.57 ± 1.38
Leaf extracts in acetone				
A. galanga L.	19.70 ± 0.95	21.68 ± 0.66	23.17 ± 1.55	26.94 ± 1.26
A. spatulata Blume.	28.87 ± 0.97	31.06 ± 0.84	34.65 ± 0.43	37.27 ± 1.06
A. muricata L.	16.00 ± 0.85	17.56 ± 1.04	28.75 ± 1.01	33.55 ± 1.25
B. orientale L.	NI	3.77 ± 1.32	2.83 ± 1.15	4.70 ± 1.47
B. balsamifera L.	55.86 ± 0.36*	60.06 ± 1.00*	62.55 ± 1.15*	62.44 ± 1.13*
C. asiatica L.	14.47 ± 0.84	24.16 ± 1.24	28.48 ± 1.23	35.28 ± 1.59
D. linearis	21.74 ± 0.99	23.28 ± 1.26	28.25 ± 1.21	34.80 ± 0.91
D. suffruticosa	39.14 ± 0.49	39.14 ± 1.42	38.78 ± 1.38	43.23 ± 1.50*
L. garciae Vidal.	NI	NI	24.48 ± 1.38	25.48 ± 1.26
M. malabathricum L.	22.74 ± 1.25	31.07 ± 1.63	28.98 ± 1.89	37.72 ± 0.84
M. charantia L.	43.16 ± 0.72*	43.23 ± 1.25*	46.45 ± 0.94*	47.78 ± 1.28*
N. bisserrata (Sw.)	NI	NI	2.05 ± 0.92	3.18 ± 1.00
P. edule Reinw.	8.12 ± 1.28	36.91 ± 2.03	36.92 ± 1.27	35.36 ± 0.75
P. betle L.	59.57 ± 1.39*	62.17 ± 1.18*	63.77 ± 0.55*	63.56 ± 0.77*
P. minus Huds.	25.34 ± 1.88	27.65 ± 1.73	28.08 ± 1.30	42.64 ± 0.78
Positive control (Kocide 101)	61.60 ± 0.64	66.08 ± 0.90	72.55 ± 0.71	73.54 ± 1.06
Negative control (distiiled water)	84.66 ± 0.24	84.34 ± 0.57	85.00 ± 0.00	84.30 ± 0.65

Each value represented the mean (3 replicates) ± standard error; NI = no inhibition; * represent crude extracts that effectively inhibited growth ($P < 0.05$).

Table 3. Mean ± S.E of inhibiton of radial growth (mm) of *C. capsici* by leaf extracts in methanol, chloroform, and acetone with various concentrations.

Leaf extracts in methanol	Minimum inhibition concentration (µg/ml)
A. galanga L.	15.00
A. spatulata Blume.	>20.00
A. muricata L.	>20.00
B. orientale L.	>20.00
B.balsamifera L.	20.00
C. asiatica L.	17.50
D. linearis	>20.00
D.suffruticosa	>20.00
L. garciae Vidal.	>20.00
M. malabathricum L.	>20.00
M. charantia L.	20.00
N.. bisserrata (Sw.)	>20.00
P.edule Reinw.	>20.00
P.betle L.	12.50
P.minus Huds.	>20.00
Leaf extracts in chloroform	
A.galanga L.	17.50
A.spatulata Blume.	>20.00
A. muricata L.	>20.00
B.orientale L.	>20.00
B. balsamifera L.	20.00
C. asiatica L.	20.00
D.linearis	>20.00
D. suffruticosa	>20.00
L.garciae Vidal.	>20.00
M. malabathricum L.	>20.00
M.charantia L.	>20.00
N.bisserrata (Sw.)	>20.00
P. edule Reinw.	>20.00
P.betle L.	17.50
P.minus Huds.	>20.00
Leaf extracts in acetone	
A.galanga L.	20.00
A. spatulata Blume.	>20.00
A.muricata L.	>20.00
B.orientale L.	>20.00
B.balsamifera L.	17.50
C. asiatica L.	20.00
D.linearis	>20.00
D. suffruticosa	>20.00
L.garciae Vidal.	>20.00
M.malabathricum L.	>20.00
M.charantia L.	>20.00
N. bisserrata (Sw.)	>20.00
P. edule Reinw.	>20.00
P.betle L.	15.00

Table 3. continued

P.minus Huds.	>20.00
Positive control (Kocide 101)	12.50
Negative control (distilled water)	NI

NI = No Inhibition

Table 4. Mean ± S.E of inhibiton of sporulation (x10^5) of *C. capsici* by leaf extracts in methanol, chloroform, and acetone with various concentrations.

Leaf extracts in methanol	Mean ± S.E of inhibition/stimulation of sporulation (x10^5)			
	0.01 (µg/ml)	**0.10 (µg/ml)**	**1.00 (µg/ml)**	**10.00 (µg/ml)**
A.galanga (L.) Willd.	2.40 ± 0.07*	2.62 ± 0.06*	2.57 ± 0.12*	2.73 ± 0.10*
A. spatulata Blume.	0.84 ± 0.02	0.86 ± 0.03	1.11 ± 0.02	1.09 ± 0.03
A. muricata L.	0.94 ± 0.04	0.91 ± 0.03	1.35 ± 0.06	1.59 ± 0.06
B.orientale L.	NI	NI	NI	NI
B.balsamifera L.	1.29 ± 0.08	1.28 ± 0.10	1.43 ± 0.04	1.62 ± 0.08
C.asiatica L.	1.49 ± 0.13	1.57 ± 0.14	2.03 ± 0.10*	2.41 ± 0.09*
D.linearis (Burm. F.) Underw.	0.22 ± 0.04	0.17 ± 0.03	0.32 ± 0.05	0.48 ± 0.04
D. suffruticosa	1.08 ± 0.08	1.30 ± 0.03	1.58 ± 0.13	1.61 ± 0.06
L.garciae Vidal.	0.40 ± 0.02	0.54 ± 0.02	0.93 ± 0.03	0.95 ± 0.04
M. malabathricum L.	1.46 ± 0.03	1.39 ± 0.05	1.59 ± 0.11	1.54 ± 0.12
M.charantia L.	1.65 ± 0.11	1.70 ± 0.14	2.00 ± 0.10*	2.01 ± 0.04*
N.bisserrata (Sw.)	NI	NI	NI	NI
P.edule Reinw.	NI	NI	NI	NI
P.betle L.	2.78 ± 0.09*	2.92 ± 0.06*	2.95 ± 0.03*	3.20 ± 0.07*
P. minus Huds.	1.81 ± 0.08	1.73 ± 0.03	1.77 ± 0.07	2.06 ± 0.05*
Leaf extracts in chloroform				
A. galanga (L.) Willd.	1.91 ± 0.05	1.96 ± 0.15*	2.25 ± 0.09*	2.49 ± 0.06*
A. spatulata Blume.	1.23 ± 0.02	1.17 ± 0.03	1.19 ± 0.03	1.29 ± 0.03
A.muricata L.	0.12 ± 0.03	0.11 ± 0.04	0.45 ± 0.04	0.55 ± 0.04
B. orientale L.	NI	NI	NI	NI
B. balsamifera L.	1.53 ± 0.06	1.63 ± 0.13	1.92 ± 0.08	2.13 ± 0.07*
C.asiatica L.	1.50 ± 0.09	1.52 ± 0.15	1.82 ± 0.10	2.00 ± 0.06*
D. linearis (Burm. f.) Underw.	1.16 ± 0.02	1.60 ± 0.03	1.58 ± 0.04	1.57 ± 0.04
D. suffruticosa	1.00 ± 0.04	1.06 ± 0.04	1.07 ± 0.03	1.11 ± 0.03
L. garciae Vidal.	0.06 ± 0.02	0.07 ± 0.02	0.21 ± 0.03	0.30 ± 0.02
M. malabathricum L.	1.09 ± 0.07	1.05 ± 0.11	1.26 ± 0.08	1.76 ± 0.08
M. charantia L.	1.74 ± 0.12	1.88 ± 0.05	2.02 ± 0.05*	2.11 ± 0.04*
N.bisserrata (Sw.)	NI	NI	0.20 ± 0.03	0.20 ± 0.03
P.edule Reinw.	1.39 ± 0.06	1.58 ± 0.05	1.58 ± 0.04	1.83 ± 0.04
P.betle L.	2.71 ± 0.06*	2.84 ± 0.04*	2.86 ± 0.13*	2.93 ± 0.04*
P.minus Huds.	1.03 ± 0.09	1.25 ± 0.04	1.30 ± 0.09	1.43 ± 0.05
Leaf extracts in acetone				
A.galanga (L.) Willd.	0.98 ± 0.08	1.01 ± 0.04	1.01 ± 0.07	1.21 ± 0.10
A. spatulata Blume.	1.31 ± 0.03	1.33 ± 0.03	1.49 ± 0.03	1.47 ± 0.03
A. muricata L.	0.60 ± 0.05	0.53 ± 0.04	1.06 ± 0.04	1.09 ± 0.04
B. orientale L.	NI	NI	NI	0.10 ± 0.03
B. balsamifera L.	1.91 ± 0.07	2.48 ± 0.15*	2.73 ± 0.10*	2.80 ± 0.10*
C. asiatica L.	0.66 ± 0.10	0.89 ± 0.08	1.43 ± 0.06	1.81 ± 0.09
D. linearis (Burm. f.) Underw.	1.05 ± 0.05	1.08 ± 0.03	1.47 ± 0.04	1.56 ± 0.04

Table 4. Continued

D. suffruticosa	1.70 ± 0.04	1.67 ± 0.12	1.64 ± 0.03	1.77 ± 0.09
L. garciae Vidal.	NI	NI	0.83 ± 0.04	0.89 ± 0.04
M. malabathricum L.	1.06 ± 0.04	1.40 ± 0.05	1.41 ± 0.05	1.62 ± 0.10
M.charantia L.	1.66 ± 0.13	1.85 ± 0.06	1.93 ± 0.04*	1.99 ± 0.06*
N. bisserrata (Sw.)	NI	NI	NI	0.07 ± 0.01
P. edule Reinw.	NI	1.17 ± 0.04	1.38 ± 0.05	1.42 ± 0.03
P.betle L.	2.71 ± 0.07*	2.82 ± 0.02*	2.83 ± 0.12*	2.88 ± 0.07*
P. minus Huds.	1.14 ± 0.06	1.26 ± 0.13	1.33 ± 0.03	2.05 ± 0.04*
Positive control (Kocide 101)	0.76 ± 0.09	0.79 ± 0.05	0.55 ± 0.04	0.33 ± 0.02
Negative control (distilled water)	3.93 ± 0.02	3.95 ± 0.01	3.97 ± 0.01	3.95 ± 0.02

Each value represented the mean (3 replicates) ± standard error; NI = No Inhibition; * represent crude extracts that effectively inhibited growth ($P < 0.05$).

extracts was recorded even at very low dose, which is a clear indication that the crude extract contained active components that had antifungal properties. Phangthip et al. (2005) found that *Piper betle* leaves have antimicrobial activity due to the essential oils of the plant which contained phenolic compounds such as cavicol, cavibetol, carvacrol, eugenol and allilpyrocatechol. These compounds are assumed could inhibit bacteria and fungi. Begum et al. (2007) had found the extract of *P.betle* to have a wide spectrum of antifungal activity. It has also been reported that the leaves of *P. betle* possess various medicinal properties such as antioxidant, antibacterial, digestive, stimulant, antifungal and nematocidal properties (Phangthip et al., 2006).

Johann et al. (2007) stated that the chemistry of *Piper* species has been widely investigated and phytochemical investigations from all 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 antimicrobial properties of different species of the genus *Piper* have also been studied (Johann et al., 2007). In a screening for medicinal plants with antimicrobial activity in Colombia, the methanolic extract of the leaf of *Piper lanceafolium* showed activity against *Candida albicans*, *Klebsiella pneumoniae*, *Enterococcus faecalis*, *Mycobacterium phlei*, *Bacillus subtilis* and *Staphylococcus aureus*. *Piper nigrum* (black pepper) is known to have antifungal activity due to lactones, terpenoids, alkaloids and saponins. 4,5-Dimethoxy-2,3-(methylenedioxy)-l-allylbenzene, a natural isolate of *Piper hispidum* and *Piper aduncum,* also has strong antimicrobial activity. This natural product and three other related compounds, [4-(5'-hydroxy-5'-nonanyl)-1,2 (methylenedioxy) benzene, 4-(5'-non-4'-enyl)-1,2-(methylenedioxy) benzene and 6-methoxy-2,3-(methylenedioxy)-4-allylphenol], were synthesized from piperonal and screened for their biological activity. These four compounds showed high levels of antifungal and antibacterial activity against several fungi and bacteria.

In conclusion, the leaves crude extracts that exhibited good potential and showed effectiveness as fungicides of *C. capsici* should be drawn with an in-depth study of testing the phytoextracts for their potentiality under *in vivo* condition.

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