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Antifungal Potential of Six Herbal Plants against Selected Plant Fungal Pathogens

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ABSTRACT

An investigation was conducted to determine the antifungal potential of *Andrographis paniculata*, *Backhousia citriodora*, *Clinacanthus nutans*, *Ficus deltoidea*, *Phaleria macrocarpa*, and *Piper betle* against selected plant fungal pathogens. Dilutions of crude leaf extracts (5, 10, 15, and 20%) of these plants were screened *in vitro* against *Ganoderma boninense*, *Fusarium oxysporum* f. sp. *cubense* R4 (*Foc*R4), and *Rhizoctonia solani*. The percentage inhibition of diameter growth (PIDG) of test pathogens was measured using poisoned agar method. Aqueous extract of *A. paniculata* was ineffective in inhibiting the mycelial growth of test pathogens at all test concentrations while that of *B. citriodora* markedly inhibited *Foc*R4 growth at 15% (PIDG 70%) and 20% (PIDG 72.9%) concentrations. Methanol extract of *C. nutans* at 20% concentration significantly inhibited *R. solani* growth (PIDG 64.4%) meanwhile that of *P. betle* at 20% considerably inhibited the growth of *Foc*R4 (PIDG 94%), *G. boninense* (PIDG 89.4%), and *R. solani* (PIDG 82.8%). Complete inhibition (PIDG 100%) of *G. boninense* and *R. solani* was obtained at 10% concentration of *F. deltoidea* and *P. macrocarpa* methanol extracts. Leaf extracts of five herbal plants have the potential to be used as bio-fungicides as a safe alternative to synthetic fungicides.

Keywords: Biopesticide, botanical extract, green technology, poisoned agar method

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INTRODUCTION

Immoderate and improper use of synthetic fungicides, which are broadly used in the management of fungal diseases in plants, adversely affects the human health and the environment. Use of plant extracts with antimicrobial properties is therefore regarded as a safer approach in controlling plant fungal diseases. Plants synthesize an assortment of antimicrobial compounds including alkaloids, flavonoids, terpenes and phenolic compounds (Compean & Ynalvez, 2014). Recently, numerous studies have assessed the efficacy of plant extracts in inhibiting the growth of some economically-important plant pathogens. For instance, Satish et al. (2009) had reported the substantial antifungal activities of 12 plants against 8 *Fusarium* species. Besides, the leaf extract of *Artemisia absinthium* L. has been reported to possess antifungal activities against rot-causing pathogens namely, *Alternaria alternata*, *Mucor piriformis*, and *Penicillium expansum* (Parveen et al., 2014). On the other hand, 3 out of 39 plant extracts had been proven effective against *Alternaria solani* (Ravikumar & Garampalli, 2013). Up to now, little attention has been paid to the antifungal activity of plants from Acanthaceae, Myrtaceae, Moraceae, Piperaceae, and Thymelaeaceae. The antifungal potential of plant extracts from these families against a broad spectrum of plant fungal pathogens including *G. boninense*, *F. oxysporum* f. sp. *cubense* R4 (*Foc*R4), and *R. solani* is lacking. The negative impacts of these soilborne fungal pathogens on production of some economically-important crops such as oil palm, banana and rice have been vastly reported. For instance, *G. boninense* is the major pathogen causing the deadly basal stem rot in oil palm where the annual loss caused by this pathogen has been estimated as USD500 million (Arif et al., 2011). Meanwhile, *Foc*R4 is the causal agent of Fusarium wilt in an assortment of banana cultivars including Cavendish with the estimated annual losses ranging from USD14.1 to 253.3 million (Malik et al., 2013; Peng et al., 2013). On the other hand, rice sheath blight caused by *R. solani* is one of the major threats to global rice production where the estimated annual losses of 20% and 10% have been reported in Thailand and India, respectively (Boukaew & Prasertsan, 2014). Therefore, urgent consideration is needed to develop effective and sustainable approaches to control these diseases. Identification of plant extracts with antimicrobial activity could be useful for development of novel biopesticides against these pathogens. The present study explores, for the first time, the effects of leaf extracts of *A. paniculata*, *B. citriodora*, *C. nutans*, *F. deltoidea*, *P. macrocarpa*, and *P. betle* against *G. boninense*, *Foc*R4, and *R. solani*.

MATERIALS AND METHODS

Collection of Plant Leaves

The leaves of *A. paniculata*, *C. nutans*, *F. deltoidea*, and *P. betle* were collected from Herb Garden, University Agriculture Park, Universiti Putra Malaysia (UPM), Serdang, Selangor. Meanwhile, the leaves of *B. citriodora* and *P. macrocarpa* were collected

from Department of Agriculture, Serdang, Selangor. The experiments were carried out in Mycology Laboratory, Department of Plant Protection, Faculty of Agriculture, UPM, Serdang, Selangor. The leaves of *C. nutans*, *F. deltoidea*, *P. macrocarpa*, and *P. betle* were thoroughly washed and air-dried at room temperature (26±2°C). The leaves of *A. paniculata* and *B. citriodora* were treated in the same manner but drying was done at 60°C. All the dried leaf samples were ground separately to fine uniform texture using grinder (Retsch Model SK 100) and stored at room temperature (26±2°C) until use.

Fungal Cultures

The test fungal cultures namely, *G. boninense* (UPMGB002), *F. oxysporum* f. sp. *cubense* R4 (*Foc*R4) (UPMFTR4-01), and *R. solani* (UPMRS01) were obtained from the Department of Plant Protection, Faculty of Agriculture, UPM, Serdang, Selangor. *Foc*R4 and *R. solani* were sub-cultured and maintained on potato dextrose agar (PDA). Meanwhile, *G. boninense* was cultured on malt extract agar (MEA). These cultures were maintained in the culture chamber under laboratory condition.

Preparation of Leaf Crude Extracts

Fifty grams of ground *A. paniculata* and *B. citriodora* leaves were separately soaked in 300 ml distilled water and stirred at 120 rpm for 24 h using an orbital shaker. The leaf crude extract of each plant was collected through filtration using Whatman No-1 filter paper. Then, the solvent was evaporated using Buchi Rotavapor Model R215 (BÜCHI Labortechnik AG, Flawil, Switzerland). The dried extract was collected in an air-tight container and stored at 4°C. The same method was used in preparation of leaf crude extracts of *C. nutans*, *F. deltoidea*, *P. betle*, and *P. macrocarpa*, however 300 ml methanol was used as described by Venkateswarlu et al. (2013).

Screening of Antifungal Activity

The concentrations of leaf crude extract tested in the antifungal test were 5, 10, 15, and 20% (v/v). The stock solutions of the leaf crude extract of *A. paniculata* and *B. citriodora* were prepared separately by diluting the crude extract of each plant with distilled water at the ratio of 1:1 as described by Dadang and Ohsawa (2001). The stock solution of *P. macrocarpa* crude extract was prepared by diluting the leaf crude extract with acetone at the ratio of 1:10 as described by Dadang and Ohsawa (2001). On the other hand, the stock solutions of *C. nutans*, *F. deltoidea*, and *P. betle* extracts were prepared separately by diluting the leaf crude extract of each plant with methanol at the ratio of 1:10. Further serial dilution was done to achieve test concentration. Petri dishes containing 15 ml of poisoned medium were used. Then, a respective fungal plug (0.4 cm diameter) was placed at the centre of a PDA plate containing leaf extract of each plant at the defined concentrations except for *G. boninense* where MEA plates containing leaf extract of each plant at the defined concentrations were used. The antifungal activity of *A. paniculata*, *B. citriodora*, *C.*

nutans, *F. deltoidea*, *P. macrocarpa*, and *P. betle* extracts were separately tested against *G. boninense*, *Foc*R4, and *R. solani*. The plates were incubated at room temperature (26±2°C) until the mycelial growth in control plates for certain fungal species had reached the edge of the plates. The colonial diameter was measured daily and percentage inhibition of diameter growth (PIDG) values was calculated using Equation [1] as described by Lee et al. (2018).

$$PIDG = \frac{D1 - D2}{D1} \times 100\% \qquad [1]$$

where;
D1 = Diameter growth of mycelia in control plates
D2 = Diameter growth of mycelia in treatment plates

Experimental Design and Statistical Analysis

The *in vitro* screenings of antifungal potential of all test plants were conducted in complete randomized design (CRD) with 5 treatments (0, 5, 10, 15, and 20%). There were at least 3 replicates for each treatment. Statistical analysis was conducted using SAS® Software (SAS Institute, North Carolina, USA, Version 9.4, 2012) and comparison of means using Least Significant Difference (LSD) at 5% probability level.

RESULTS AND DISCUSSION

The antifungal activity of *A. paniculata*, *B. citriodora*, *C. nutans*, *F. deltoidea*, *P. macrocarpa*, and *P. betle* extracts differed when tested against *Foc*R4, *G. boninense*, and *R. solani*. According to Kurucheve et al. (1997), the variation in the inhibitory effect of plant extracts is the results of the qualitative and quantitative differences in antifungal properties of the extracts.

As presented in Figure 1, the aqueous extract of *A. paniculata* was ineffective in inhibiting the mycelial growth of *G. boninense*, *Foc*R4, and *R. solani* at any test concentrations. The leaf extract of *A. paniculata* was previously reported to inhibit the radial mycelial growth of *F. oxysporum* (Neela et al., 2014). Furthermore, the antifungal potential of *A. paniculata* leaf extract also has been reported against *Fusarium verticillioides* (Yasmin et al., 2008) and *A. solani* (Nidiry et al., 2015). However, it was not the case in our study. This discrepancy could be attributed to the variations in the components of the *A. paniculata* extracts resulting from the different location and sample collection timing, storage and extraction conditions as suggested by Akbar (2011). For instance, Adegboyega and Oyewole (2013) had reported that the ethanol and methanol extracts of *A. paniculata* contained more phytochemicals as compared to the aqueous extract. Thus, a comparative study on the antifungal activities of *A. paniculata* leaf extracts obtained using different solvents against *G. boninense*, *Foc*R4, and *R. solani* is necessary to elucidate the *in vitro* antimicrobial effect of this plant against the test pathogens.

The aqueous extract of *B. citriodora* was significantly effective against *Foc*R4 at the concentration of 15% and above as

compared to other test pathogens (Figure 2). The concentration of 10% (PIDG 57%) was sufficient to inhibit the mycelial growth of this fungus. Hayes and Markovic (2002) had reported the notable antimicrobial activity of the essential oil extracted from *B. citriodora* and its key constituent namely citral, against an assortment of bacteria, yeast and fungi. In addition, the antimicrobial activity of 4 essential oils with varying citral content of this plant against 8 fungi and 13 bacteria have also been reported (Wilkinson et al.,

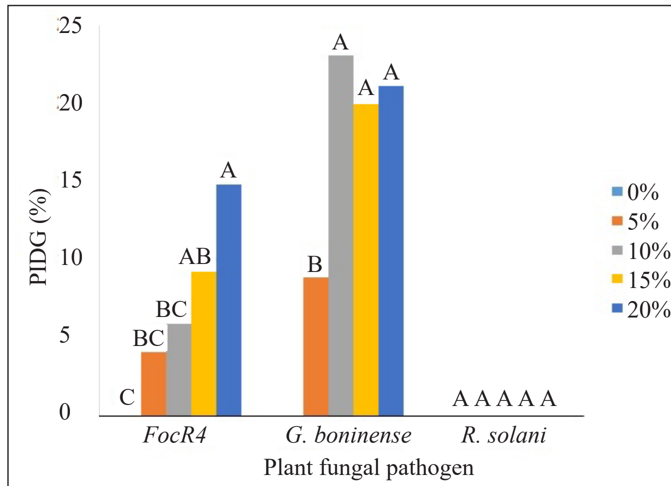

Figure 1. Percentage of inhibition of diameter growth of *Andrographis paniculata* against selected plant fungal pathogens. Measurement made at 11 days after inoculation (DAI) for *Fusarium oxysporum* f. sp. *cubense* R4 (*Foc*R4), 11 DAI for *Ganoderma boninense* and 5 DAI for *Rhizoctonia solani*. Values are the means of 6 replicates. Means with the same letter are not significantly different at P = 0.05

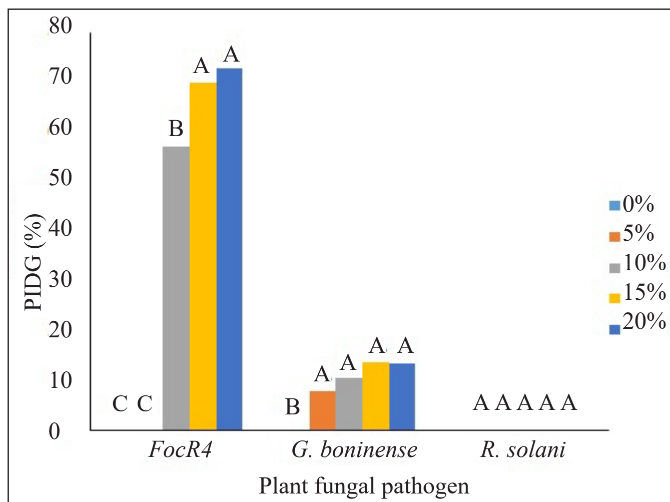

Figure 2. Percentage of inhibition of diameter growth of *Backhousia citriodora* against selected plant fungal pathogens. Measurement made at 11 days after inoculation (DAI) for *Fusarium oxysporum* f. sp. *cubense* R4 (*Foc*R4), 11 DAI for *Ganoderma boninense* and 5 DAI for *Rhizoctonia solani*. Values are the means of 6 replicates. Means with the same letter are not significantly different at P = 0.05

2003). However, the bioactive compounds accountable for antifungal effect of this plant extract against *Foc*R4 were not studied in the present study. To date, no reports of antifungal activities of *B. citriodora* against plant pathogens particularly *G. boninense*, *Foc*R4, and *R. solani* has been found in the literature. This study suggests that *B. citriodora* may serve as a good natural resource of new bioactive compounds for controlling *Foc*R4.

The methanol extract of *C. nutans* at 20% concentration significantly inhibited the mycelial growth of *R. solani* (PIDG 64.4%) as compared to *G. boninense* (PIDG 27.5%) and *Foc*R4 (PIDG 10.4%). Nevertheless, the concentration of 15% (PIDG 56.7%) was sufficient to inhibit the mycelial growth of *R. solani* (Figure 3). The presence of a broad range of bioactive compounds in this plant has been reviewed (Alam et al., 2016). So far, study of *C. nutans* has been only done to determine its antibacterial effect in aquaculture sector, pharmaceutical and medical sector (Arullappan et al., 2014). Use of *C. nutans* as a new source to control *R. solani* is highly recommended since *C. nutans* is widely available in Malaysia.

Strikingly, the methanol extracts of *P. betle* at 20% concentration significantly inhibited the mycelial growth of *Foc*R4 (PIDG 94%), *G. boninense* (PIDG 89.4%), and *R. solani* (PIDG 82.8%) as compared to all test concentrations (Figure 4). Nonetheless, the concentration of 15% was sufficient to inhibit the mycelial growth of *G. boninense* (54.1%) and *R. solani* (PIDG 71.9%). Likewise, effective antifungal activities of *P. betle* leaf extracts against *Aspergillus niger*, wild *Aspergillus* sp., and *Rhizopus* sp. (Pawar et al., 2017) as well as against *F. oxysporum* (Neela et al., 2014) have been reported. Previous studies had suggested that presence of the essential

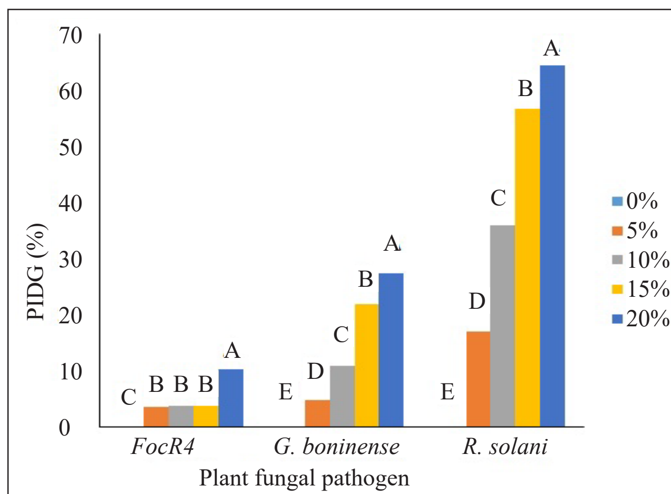

Figure 3. Percentage of inhibition of diameter growth of *Clinacanthus nutans* against selected plant fungal pathogens. Measurement made at 12 days after inoculation (DAI) for *Fusarium oxysporum* f. sp. *cubense* R4 (*Foc*R4), 12 DAI for *Ganoderma boninense* and 4 DAI for *Rhizoctonia solani*. Values are the means of 5 replicates. Means with the same letter are not significantly different at P = 0.05

oils which contained phenolic compounds in *P. betle* might contribute to inhibition of several phytopathogenic fungi (Ali et al., 2010; Begum et al., 2007). The results obtained in this experiment shed light on the potential use of *P. betle* in the management of several economically important plant diseases. Nevertheless, further analysis of the active compounds of this plant is highly recommended.

As shown in Figure 5, the methanol extracts of *F. deltoidea* significantly inhibited the mycelial growth of *G. boninense* and *R. solani* at all concentrations above 10%. However, the concentration of 5% was sufficient to inhibit the mycelial growth of *G. boninense* (PIDG 56%) and *R. solani* (PIDG 53.7%). To date, the antimicrobial activity of *F. deltoidea* leaf extract has been studied only on clinical pathogens (Abdsamah et al., 2012) and fish pathogen (Tkachenko et al., 2016). To our best knowledge, this experiment is the first study done on the plant pathogens. Antifungal activity of *F. deltoidea* methanol extract against test pathogens *in vitro* suggests presence of one or more antifungal secondary metabolites in the leaves of this plant. Nevertheless, further investigation is needed to identify the bioactive compounds of *F. deltoidea* leaves to be used as biocontrol fungicide against *G. boninense* and *R. solani*.

The methanol extracts of *P. macrocarpa* were completely effective against *Foc*R4, *G. boninense*, and *R. solani* at all concentrations above 5% (Figure 6). Nonetheless, the concentration of 5% was sufficient to inhibit the mycelial growth of *G. boninense* (58.3%) as compared to *Foc*R4 (PIDG 0%) and *R. solani* (PIDG 32.2%). According to Altaf et al. (2013), variations in the chemical components of *P. macrocarpa* extract greatly affect the antifungal activities of this plant. For example, the growth

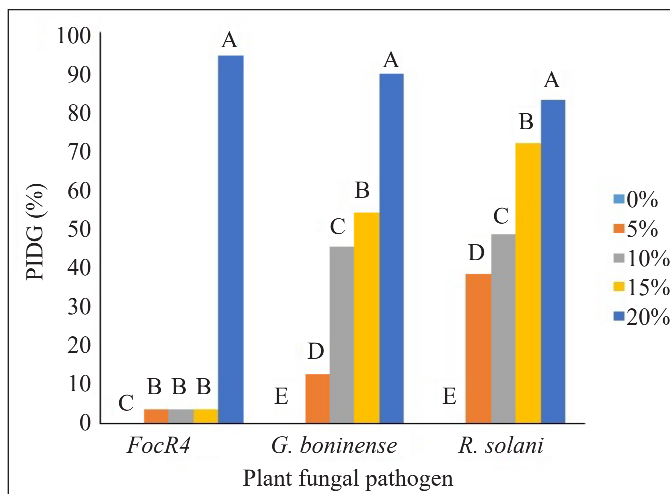

Figure 4. Percentage of inhibition of diameter growth of *Piper betle* against selected plant fungal pathogens. Measurement made at 12 days after inoculation (DAI) *Fusarium oxysporum* f. sp. *cubense* R4 (*Foc*R4), 12 DAI for *Ganoderma boninense* and 4 DAI for *Rhizoctonia solani* (R). Values are the means of 5 replicates. Means with the same letter are not significantly different at P = 0.05

inhibition of *Aspergillus niger*, *Fusarium oxysporum*, *Ganoderma lucidum*, and *Mucor indicus* by phorbolesters in *P. macrocarpa* seeds has been reported (Altaf et al., 2013). Furthermore, flavanoids have been identified as the compound accountable for the antifungal activities in higher plants (Cordell et al., 2001). Thus, identification of the bioactive constituents of *P. macrocarpa* leaves is necessary to develop a bio-fungicide.

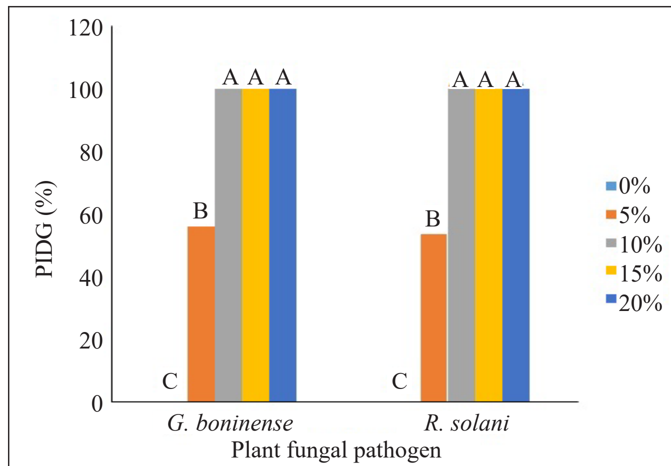

Figure 5. Percentage of inhibition of diameter growth of *Ficus deltoidea* against selected plant fungal pathogens. Measurement made at 7 days after inoculation (DAI) for *Ganoderma boninense* and 5 DAI for *Rhizoctonia solani*. Values are the means of 3 replicates. Means with the same letter are not significantly different at P = 0.05

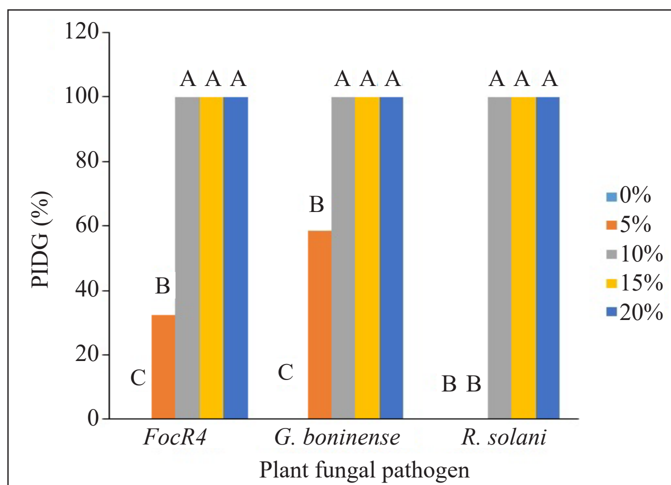

Figure 6. Percentage of inhibition of diameter growth of *Phaleria macrocarpa* against selected plant fungal pathogens. Measurement made at 9 days after inoculation (DAI) for *Fusarium oxysporum* f. sp. *cubense* R4 (*Foc*R4), 11 DAI for *Ganoderma boninense*, and 5 DAI for *Rhizoctonia solani* (R). Values are the means of 6 replicates. Means with the same letter are not significantly different at P = 0.05

CONCLUSION

In this study, *Backhousia citriodora*, *Clinacanthus nutans*, *Ficus deltoidea*, *Phaleria macrocarpa*, and *Piper betle* extracts exhibited different antifungal potential against three economically-important fungal pathogens when tested *in vitro*. Among them, *P. macrocarpa* has been identified as the most effective candidate for development of biofungicide primarily for *Foc*R4, *G. boninense*, and *R. solani* followed by *P. betle*. On the other hand, leaf extracts of *B. citriodora* and *C. nutans* can be used in the management of *Fusarium* wilt disease and soil-borne diseases caused by *R. solani*, respectively. Further experimental investigation into bioactive compounds of these herbal plants as well as trials in both glasshouse and field are strongly recommended for development of novel biofungicides.

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