

Bioantifungal activity of selected medicinal plant extracts against root rot of fungal disease

Rozihawati Zahari¹, Normala Halimoon¹, Ahmad Said Sajap², Mohd Farid Ahmad³,
Mohamad Roslan Mohamed²

¹Department of Environmental Science, Faculty of Environmental Studies, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia

²Department of Forest Management, Faculty of Forestry, University Putra Malaysia, 43400 Serdang, Selangor, Malaysia

³Department of Biodiversity, Forest of Research Institute Malaysia (FRIM), Kepong, Selangor, Malaysia

Email address:

mala_upm@upm.edu.my (N. Halimoon)

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Abstract: Root rot disease of fungal such as *Ganoderma philippii*, *Phellinus noxius* and *Rigidoporus microporus* are well known destructive fungus to plant root. The *in vitro* antifungal screening of selected medicinal plants was studied against the disease. The twelve of Malaysian medicinal plants including the leaves of *Aglaia argentea*, *A. leucophylla*, *A. grandis*, *A. odorata*, *A. odoratissima*, *A. varrisquama*, *Alium sativum* (bulbs) and *Cassia alata*, *Catharanthus roseus* stems and leaves, *Derris elliptica* leaves and *Tinospora baenzigeri* stems were extracted using different types of solvents extraction i.e, dichloromethane (DCM), acetone and methanol at the concentration of 20 mg/mL. The extracts were studied for antifungal activities against three species of fungal disease including *G. Philippii*, *P. noxius* and *R. microporus*. The antifungal activities of the extracts were determined by the presence or absence of fungal inhibition zone growth on Potato dextrose agar (PDA). The extracts shows a significant results but varying in their antifungal activities on the selected fungal. The DCM and acetone extracts of *C. roseus* stems had the highest antifungal activities against *R. microporus* fungus compared to methanolic extract. On the other hand, acetone extracts of *A. argentea* leaves also gave the highest antifungal activities against *G. philippii* compared to other extracts. However, all of the extracts didn't show any inhibition zone on *P. noxius* culture. In general, the DCM extracts of *C. roseus* stems contain the most of bio-antifungal of active compounds against *R. microporus* of fungal disease.

Keywords: Bio-Antifungal Activity, Medicinal Plants, Root Rot, Fungal Disease

1. Introduction

Ganoderma philippii, *Phellinus noxius* and *Rigidoporus microporus* are the fungal that causes root rot disease commonly attacked to the forest plantation [1,2]. These pathogenic fungi have the most economically damaging disease to the high mortality rates of trees observed during second and third years of rotation cycle [3]. The problem caused by the root rot disease that may affect the growth performance of plants and finally, the plant will die if not control properly. *G. philippii* of fungi (causes red root disease) is a major of root rot disease that attack *Acacia mangium* and rubber plantations mostly occur in South-East Asia [4]. *Phellinus noxius* is not only the host pathogen of the tropical forest species but also diseases of several crops plantation. In Taiwan, the fungal causes a brown root rot disease on

tropical fruit plantation such as longan, litchi, carambola, loquat avocado and sugar apple [5]. *R. microporus* of fungi causes white root disease and well known destructive agent to several crops and fruit trees including rubber trees (*Hevea brasiliensis*) [6,7]. The white and brown root rots disease have causes a significant damages to rubber plantations established on clearance of forest areas in West Africa [8]. Reference [9] reported that these fungal were also affected against the four major plantation tree species including *Acacia mangium*, *Azadirachta excelsa*, *Tectona grandis* and *Hevea brasiliensis* in Peninsular Malaysia. Detection of infection at early stages of its attacked is difficult because the trees are rapidly killed by the fungal. Reference [10] stated that the aboveground symptoms of the trees are mostly beyond treatment and recovery, as rapid progress of infection makes the plant death imminent.

Organic fungicide is one of other alternative as biocontrol of plant diseases because of negative public perceptions using chemical pesticide. Organic fungicide products especially from plant extracts act as disease control agents have been studied, since they tend to have low mammalian toxicity, less environmental effects and wide public acceptance [11]. The increased usage of different chemical pesticides in agriculture sector resulted in various problems to environmental ecology including effect of chemicals residual on the quality of agriculture products, increased the resistance of chemicals to target pathogens and causes environmental pollution. Due to this problem, medicinal plants have been chosen as one of the sources of antimicrobial agents as organic pesticide to control the specific of plant diseases. For example, the seed of *Aglaia forbesii* extracts have significantly higher antifungal activities against plant fungal diseases such as *Phytophthora botryose*, *P. palmivora* and *R. microporus* [7].

Medicinal plants extract such as *Aglaia* spp., *Cassia alata* and *Catharanthus roseus* have shown highly antifungal properties that promising bioactivity against fungal disease. Reference [12] stated that benzofuran compounds from *Aglaia* spp. leaves were shown causes high mortalities on tested anti-fungal. Study by [13] that *Catharanthus roseus* extract has 2,3-dihydroxybenzoic which is potentially used to control fungal of *Phytium aphanidermatum*. The methanolic extract of *Cassia alata* leaves exhibited very strong activity against two types of bacteria (*Dermatophilus congolensis* and *Actinomyces bovis*) and five of fungi (*Microsporum canis*, *Blastomyces dermatitidis*, *Trichophyton mentagrophytes*, *Candida albicans* and *Aspergillus flavus*) with maximum activity in the fractions containing alkaloid [14]. Although, those medicinal plants growth well and abundantly found in

Malaysia, concerns regarding to the species are still less or may unnoted as noxious species to control the root rot disease. Thus, the *in-vitro* screenings of selected medicinal plant extracts as antifungal agent were carried out against the common of plant fungal.

2. Materials and Methods

2.1. Preparation of Plant Extracts

The plant samples were collected from Peninsular Malaysia as shown in Table 1. The free disease and insect pest of plant samples were selected for the study. The specimens of samples were cleaned with 1% of sodium hypochlorite and distilled water. The samples were then air dried at room temperature. The dried samples were then ground to powder. The plant materials were then soaked for three days in different solvents of extraction namely dichloromethane (DCM), acetone and methanol. The solvent was removed using a rotary evaporator unit to produce concentrate extract.

2.2. Microorganisms

The three types of root fungal including *G. philippii* (FRIM589), *P. noxius* (FRIM154) and *R. microporus* (FRIM646) were obtained from the Pathology Laboratory, FRIM. The fungus were cultured on Potato dextrose agar (PDA) and incubated in culture room (25±2°C) for six days.

The maximum growth rates of fungal culture were then tested for antifungal activity using the prepared of plant extraction.

Table 1. The collection of plant samples in Peninsular Malaysia

No.	Plant species	Family	Location	Plant part
1	<i>Aglaia argentea</i>	Meliaceae	Gombak Forest Reserve	Leaves
2	<i>Aglaia leucophylla</i>	Meliaceae	Gombak Forest Reserve	Leaves
3	<i>Aglaia grandis</i>	Meliaceae	Pasoh Forest Reserve	Leaves
4	<i>Aglaia odorata</i>	Meliaceae	Serdang, Selangor	Leaves
5	<i>Aglaia odoratissima</i>	Meliaceae	Gombak Forest Reserve	Leaves
6	<i>Aglaia variisquama</i>	Meliaceae	Gombak Forest Reserve	Leaves
7	<i>Alium sativum</i>	Alliaceae	Serdang, Selangor	Bulbs
8	<i>Cassia alata</i>	Leguminosae	Dungun, Terengganu	Leaves
9	<i>Catharanthus roseus</i>	Apocynaceae	Paka, Terengganu	Stems
10	<i>Catharanthus roseus</i>	Apocynaceae	Paka, Terengganu	Leaves
11	<i>Derris elliptica</i>	Fabaceae	Serdang, Selangor	Leaves
12	<i>Tinospora baenzigeri</i>	Menispermaceae	Jerangau, Terengganu	Stems

2.3. Screening of Antifungal Activity

The sterile Whatman No.1 filter papers with 6mm in diameter were soaked in the respective extracts [15]. The filter paper was then placed on the PDA surface containing a

fungal culture in the petri dish. The filter paper without treated with plant extract was used as the control. The PDA plates were then incubated at 25±2°C for six days. The experiment was conducted in five replications and subjected to factorial design. After six days of incubation, the

antifungal activities of the extracts were observed by the presence or absence of inhibition zone on PDA culture. The inhibition diameter (mm) of fungal culture was measured using scanning electron microscope (SEM). The inhibition data was subjected to the three-ways analysis of variance (ANOVA). The treatment means were separated using Tukey HSD at $\alpha = 0.05$.

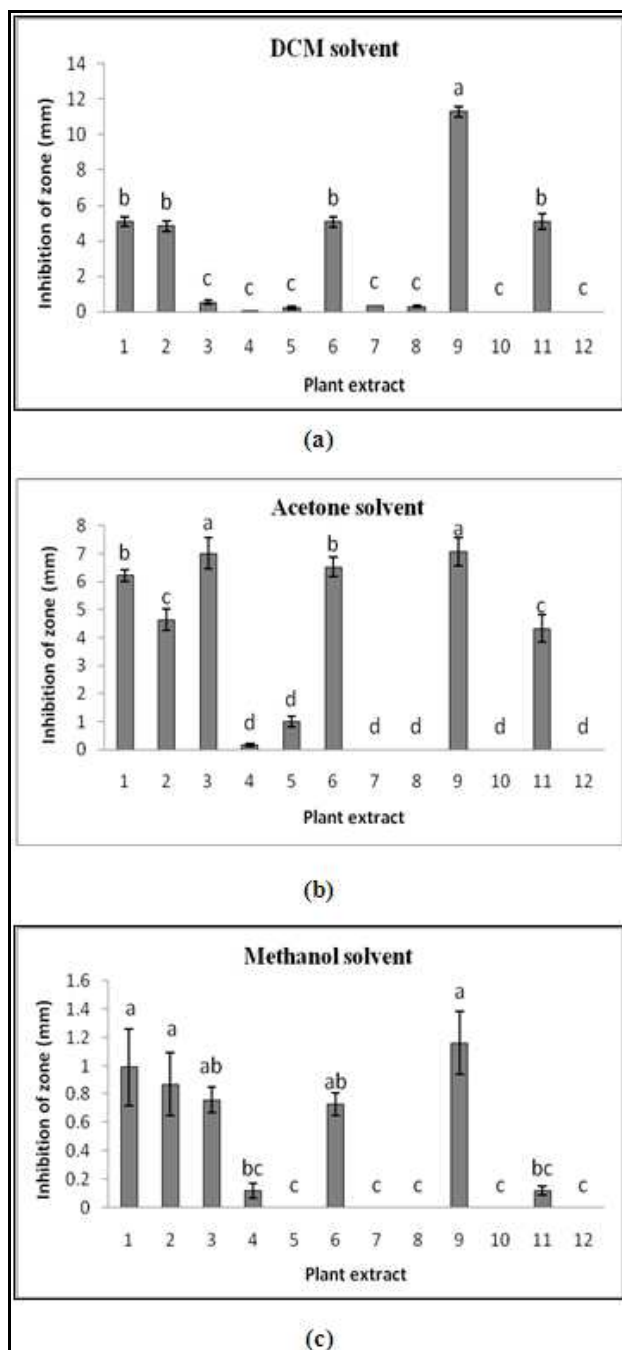


Figure 1. The inhibition zone of selected plant extracts against plant fungus of *R. macroporus* using different solvents of extraction. The means were significantly different at 5% level by Tukey HSD. 1: *A. argentea*; 2: *A. leucophylla*; 3: *A. grandis*; 4: *A. odorata*; 5: *A. odoratissima*; 6: *A. varisquama*; 7: *A. sativum*; 8: *C. alata*; 9: *C. roseus* (stems); 10: *C. roseus* (leaves); 11: *D. elliptica*; 12: *T. baenzigeri*.

3. Results and Discussion

3.1. Antifungal Activity

The DCM extracts of *C. roseus* stems were shown the highest antifungal activities against *R. microporus* (11.29 mm) and *G. philippii* (4.09 mm) compared to other plant extracts (Figures 1 and 2). Prajakta and Jai (2010) have studied that the leaves extract of *C. roseus* have high contained of indole alkaloids and some phenolic compounds which highly shows antimicrobial activity. The alkaloids also found in the aerial parts of *C. roseus* with the most abundant are the monomers of catharanthine and vindoline, bearing the indole and the indoline chromophore, respectively [16].

DCM extracts of *Aglaia* species namely *A. argentea* (5.07 mm), *A. leucophylla* (4.84 mm) and *A. varisquama* (5.07 mm) also exhibited high antifungal activity against *R. microporus* compared to other species. Several *Aglaia* species including *A. argentea* and *A. grandis* also have been isolated, where there are mainly characterized by cycloartane- the type of triterpenes [17]. Moreover, the species, particularly *A. argentea* contain alkaloid compounds that also have antifungal activities [18].

A. sativum of plant species also appears to consist of antifungal activities against *G. philippii* of fungi especially for acetone and methanolic solvents. It contained abundant of sulfur compounds such as allicin (*S*-allylcysteine sulfoxide) [19] that active act as antifungal agents [20].

D. elliptica species was shown the significant result of antifungal activity for all types of solvent extraction. DCM and acetone extracts of *Derris elliptica* leaves showed more significant activity against *R. microporus* with 5.09 mm and 4.02 mm of inhibition zone. Reference [21] stated that the flavonoid and rotenoid compounds were presented in the extract of *D. indica* leaves contain antifungal activities against two types of fungus involved *Aspergillus flavus* and *Penicillium tubesulum*.

Methanolic extract of *T. baenzigeri* was shown the significant inhibitory activity against *G. philippii* of fungus with 1.38 mm of inhibition zone. Based on the previous study, the extract of *Tinospora cordifolia* was also found to be the most effective and high antifungal activity against *Helminthosporium* sp., *Acorus calamus* and *Alternaria solani* [22]. It contained about six different of phenolic acids such as benzoic, cinnamic, caffeic, ferulic, gallic and tannin acids. Several researchers also supported this statement that phenolic acid compounds can act as antifungal activity against plant fungi [23,24,25]. However, all of the plant extracts in different solvents of extraction did not show any inhibition zone on *P. noxius* fungus in the study.

3.2. Solvent Extractions

3.2.1. Dichloromethane Extraction

Based on the polarity of solvents extraction, non-polar solvent of dichloromethane (DCM) extracts exhibited the maximum results of antifungal activity compared to semi

polar (acetone) and polar (methanol) extracts. The previous study noted that the DCM extracts of *Michelia champaca* and *Antidesma madagascariense* have the maximum number of growth inhibiting compounds against *Cladosporium cucumerinum* fungus [15]. The solvent extracts of *Senna didymobotryo* also had good antifungal activities against *Trichophyton mentagrophyte* and *microsporum gypseum* of fungus [26]. Possibility the effect of antifungal compounds on spore germination leading to its inhibition or may be due to effect of these compounds on the cell wall altering its permeability [27].

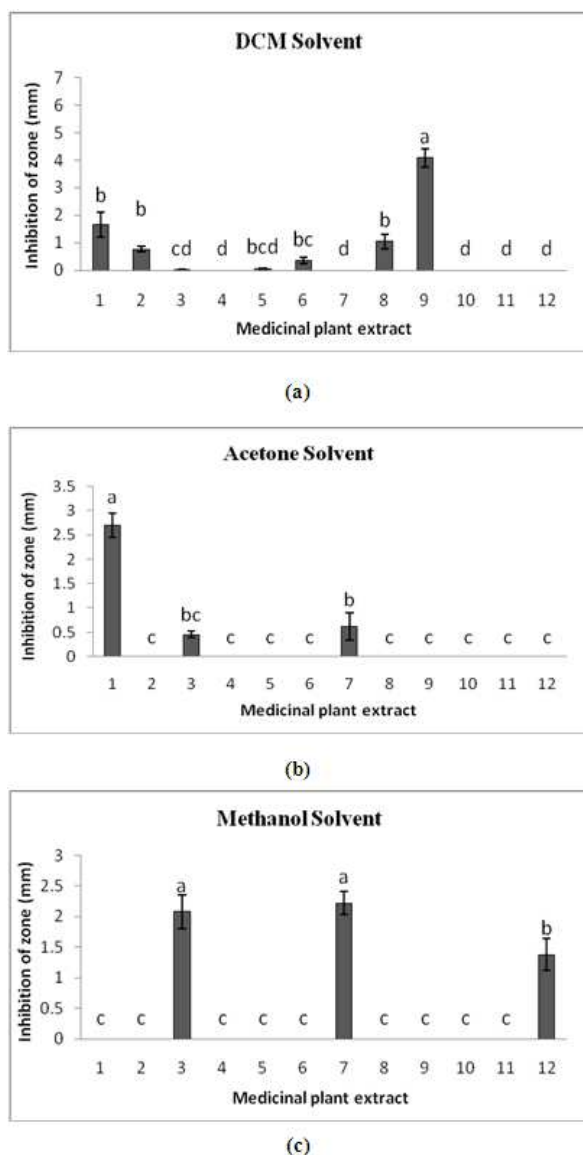


Figure 2. The inhibition zone of selected plant extracts against plant fungus of *G. philippii* using different solvents of extraction. Note: The means were significantly different at 5% level by Tukey HSD. 1: *A. argentea*; 2: *A. leucophylla*; 3: *A. grandis* 4: *A. odorata*; 5: *A. odoratissima*; 6: *A. varrisquama*; 7: *A. sativum*; 8: *C. alata*; 9: *C. roseus* (stems); 10: *C. roseus* (leaves); 11: *D. elliptica*; 12: *T. baenzigeri*.

3.2.2. Acetone Extraction

Acetone is a semi-polar solvent also shows a good solvent of extraction with the most extracts of selected medicinal

plants; especially *C. roseus* and *Aglaia* species extracts with evident the significant results of inhibition zone (mm) against *R. microporus*. However, most of the acetone plant extracts were not effective as antifungal agent against *G. philippii* except *C. roseus* of acetone extract as shown in Figure 2 and Table 3. Research studied by [28] also found that acetone extract of *Combretum molle* stems bark have the highest antifungal activity against selected bacterial diseases. Reported by [29], saponins and tannins have high abundance present in plants could be extracted by acetone solvent. The reason is because alcoholic solvents of acetone give a larger spectrum of polar materials, while non-polar solvents have more lipophilic components in the yields. Acetone is usually preferred to be used as solvent of extraction because it can extract both polar and non-polar compounds in plant [29].

3.2.3. Methanol Extraction

In the study, most polar solvent of methanolic extracts shows less antifungal activities against root rot diseases compared to non-polar and semi-polar solvents. Previous study have published that polar solvent of methanolic extracts possess antifungal activities against various plant fungal diseases; for example, the *Barringtonia racemosa* leaves of methanolic extract can also detected stronger inhibitory activity effect against seven types of fungi species [30]. Similarly reported by [30], the methanolic extracts of 22 medicinal plants in Malaysia produced significant antifungal activity against *Candida albicans*, *Rhodotorula rubra* and *Torulopsis glabrata*. The methanolic extract of *J. curcas* stem bark was also found have more effective antifungal activities than ethanolic extracts against fungal and bacterial pathogens [32].

4. Conclusion

The DCM extract of *C. roseus* stems have a significant inhibitory to the production of toxin form the root rot disease of fungal tested during the investigation study. It's also happened in several *Aglaia* species extracts that shows strong antifungal activity against *R. microporus* and *G. philippii* of fungal. The possibility of antifungal agent of plant extract against fungal diseases might be attributed to the various phytochemical constituents present in the crude plant extracts. The purified compounds in plant extracts have potential inhibition of fungal growth. Further study on identification the types of phytoconstituents and determination structural of the active compounds can revealed the specific active compounds function in inhibiting to several microbes activity. This encourage developing a novel broad spectrum of antifungal bioformulation in the future.

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