Phytochemicals of Oil Palm Root Extracts and Antifungal Activity against *Ganoderma boninense*

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ABSTRACT This study was conducted to investigate the phytochemicals composition of oil palm seedling roots before and after elicited with copper sulfate ($CuSO_4$). Apart from that, the antifungal activity of methanol, ethanol, acetone, ethyl acetate, chloroform and petroleum ether oil palm root extracts were also evaluated against *G. boninense*, the causal pathogen of basal stem rot (BSR) disease in oil palms. Qualitative chemical tests for both $CuSO_4$-elicited and non-elicited oil palm root extracts revealed the presence of various phytochemicals in methanol extract (ME) and ethanol extract (EE). Both extracts contain alkaloids, saponins, terpenoids, cardiac glycosides and tannins except for the absent of flavonoids. Those phytochemicals constituents were strongly presence in ME from $CuSO_4$-elicited oil palm root as compared to other extracts for both treatments. Chloroform extract (CE) from non-elicited oil palm root and petroleum ether extract (PEE) for both treatments showed negative finding for all tests. Meanwhile, the results of antifungal tests for the non-elicited oil palm roots of ME showed a higher percent of inhibition (8.15 %) against *G. boninense* mycelial growth followed by EE (5.56 %) and acetone extract (AE) (3.7 %). However, there is no significant difference between ME and EE, and similar results obtained for EE and AE. The $CuSO_4$-elicited oil palm root showed that ME gave the highest significant antifungal activity (14.07 %) followed by EE (8.14 %) and AE (6.67 %). In contrast, ethyl acetate extract (EAE), CE and PEE exhibited significantly weak antifungal activities with lower percent of inhibition for both treatments. Prior application of $CuSO_4$ as an elicitor to trigger plant defense responses and stimulate the production of secondary metabolites could determine the outcome of a plant-pathogen interaction.

KEYWORDS: Oil palm; Phytochemicals; Antifungal; Elicitation; *Ganoderma boninense*

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INTRODUCTION

Basal Stem Rot disease caused by *Ganoderma boninense* is one of the most important diseases of oil palm in South East Asia. Wide range of different types of research have been conducted to manage this disease, which include biological control of the pathogen using *Scytalidium parasiticum* (Goh *et al.*, 2016) to study of survival and pathogenicity of the pathogen (Tung *et al.*, 2018). Nonetheless, the disease remains economically importance with losses of multimillion USD recorded yearly.

Plants exhibit a huge array of defense strategies against pathogen attack. The resistance against pathogens is achieved by both pre-existing (constitutive) and induced defense systems. In addition to essential primary metabolites for instance carbohydrates, lipids and amino acids which is required to make and maintain cells, higher plants are also able to synthesize a wide variety of low molecular weight compounds known as the plant secondary metabolites (PSMs). Although PSMs do not have an immediate effect in the survival of plants, but still they give a long-term effect for the survival and fitness of plants via strong interaction with the environment, thus making it as essential as primary metabolites (Costa et al., 2012; Kliebenstein, 2012). The best understood secondary metabolites in plants are implicated in defence against pathogens or sensing and signalling.

Meanwhile, several studies have been conducted to extract and identify bioactive compounds of the infected oil palm roots with *G. boninense* (Chong et al., 2011; Nusaibah et al., 2016, Rozlianah et al., 2015). However so far, work on the screening and selection of the best solvent to obtain the highest yield percentage with ability to extract various phytochemical constituents and greatest antifungal activity is lacking.

The possibility of sensitizing a plant by prior application of elicitors, which are capable of inducing biochemical responses in association with plant disease resistance, has become a favorable for plant disease management. Furthermore, the research into the nature of plant disease resistance has emerged the idea that acceleration of the plant response by the application of resistance inducers could provide early information of the phytochemical constituents produced after the elicitation. In this study, copper sulphate was chosen as the abiotic elicitor to observe the accumulation of phytochemical constituents and antifungal activity that presence in the oil palm roots which was extracted with different organic solvent.

METHODOLOGY

Collection and Preparation of Plant Materials

Oil palm seedlings of the Deli x AVROS progeny initially grown in polybags were supplied by Sawit Kinabalu Sdn. Bhd. They were aged six-month-old. Upon arrival of the oil palm seedlings, they were kept at the greenhouse for one week and maintained with regular watering and fertilizing.

Elicitation of Oil Palm Roots with Copper Sulfate ($CuSO_4$)

The ability of oil palm roots to accumulate different metabolites or defensive compounds was investigated based on the method of (Ong & Chong, 2009) with slight modifications. Treatment was consisted of oil palm roots elicited with $CuSO_4$ and non-elicited oil palm roots treated with sterile distilled water served as control. The healthy six-month-old oil palm seedlings were uprooted and cleaned. Experiment was conducted by immersing the seedling roots into a beaker containing 50 mM (5%) of $CuSO_4$ and as for control, seedlings roots were only immersed in sterile distilled water for 30 min respectively. All seedlings roots were then incubated overnight under high humidity in dark with temperature of 25 ^{o}C $\pm$ 2 ^{o}C.

Extraction of Oil Palm Roots

Fresh weight (50 g) of oil palm seedling root samples elicited with $CuSO_4$ and non-elicited were weighed and ground using Waring laboratory blender. Samples were extracted as described by Hamza et al. (2006) with some modifications. Samples were soaked with 100 % (v/v) methanol solvent in ratio of 1:10; which is 1 g sample to 10 mL of methanol and left for 24 hours at 4 ^{o}C. The methanol extracts were then filtered through a Whatman No. 1 filter paper. The procedure was repeated three times to ensure exhaustive extraction of the plant material. The extracts were pooled together and concentrated using a Stuart RE300DB rotary evaporator under reduce pressure at 38 ^{o}C. The $CuSO_4$-elicited and non-elicited oil palm roots samples were also extracted with ethanol, acetone, ethyl acetate, chloroform and petroleum ether respectively in the same manner as followed for methanol. The dried crude extracts of those samples were kept in -20 ^{o}C until further analysis. Upon test, the crude extracts will be redissolved with their respective solvents in 100 mg mL^{-1} concentration.

Phytochemical Screening of Oil Palm Roots

Qualitative phytochemical analysis was carried out for each of the methanol, ethanol, acetone, ethyl acetate, chloroform and petroleum ether of the $CuSO_4$-elicited and non-elicited crude extracts. Samples were centrifuged at 5000 rpm for 10 minutes and diluted to 10 mg mL^{-1}. All tests were done in triplicate; i) Alkaloids; 2 mL of each extracts was added + six drops of Dragendorff's reagent and Wagner's reagent respectively; a prominent yellow and reddish-brown precipitate was observed indicating the presence of respective alkaloids (Raaman, 2006); ii) Saponins; 1 mL of each extracts + 5 mL distilled water in closed test tube; frothing persistence indicated presence of saponins (Parekh & Chanda, 2007); iii) Flavonoids; 2 mL of each extracts was + 1 mL of concentrated HCl + 3 cm magnesium tape; pink-tomato red colour (Parekh and Chanda, 2007); iv) Terpenoids; Salkowski test, 2 mL of each extracts + 1 mL chloroform + 2 mL concentrated H_2SO_4; reddish-brown colouration of the interface); v) Cardiac glycosides; Keller-Kiliani test, 2.5 mL of each extracts + 1 mL glacial acetic acid + six drops of ferric chloride solution + 1 mL of concentrated H_2SO_4; production of brown ring of the interface, a violet ring may appear below the brown ring, while in the acetic acid layer, a greenish ring may form just gradually throughout thin layer; vi) Tannins; six drops of 0.1 % ferric chloride + 1 mL of each extracts; brownish green or a blue-black colouration. Terpenoids, cardiac glycosides and tannins were conducted as described by Edeoga et al. (2005) with slight modification.

In Vitro Antifungal Activity of Oil Palm Crude Extracts

A dilution agar method was used to determine the *in vitro* antifungal activity among the different oil palm crude extracts as described previously by Yen et al. (2008) with slight modifications. Potato dextrose agar (PDA) of 60 mL was prepared and autoclaved prior to the addition of the extracts. Extracts were dissolved in 1 % dimethylsulfoxide (DMSO) and filtered through 0.45 µm PTFE syringe filters, to remove possible contaminants, before mixing with PDA (at 45 °C) to final concentrations of 1 mg mL^{-1}. Sterile PDA with added test compound was then dispensed in 20 mL volumes into 9 cm petri dishes prepared in triplicate. Each plate was swirled carefully until the agar began to set and left for two hours to allow the solvent to evaporate. After the agar solidified, a 7 mm mycelial disc cut from the edge of a seven-day old *G. boninense* culture was seeded in the centre of each petri dish and incubated at 28 $\pm$ 2 °C for eight days. Blank plates containing 1 % DMSO without the tested compounds served as control. The diameter of the fungal growth was measured and expressed as percentage growth inhibition of three replicates. Growth inhibition (%) = [(A - B)/ A] x 100 was calculated, where A represents the length of mycelia in the negative control plate and B = the length of mycelia in the treated plate (Park et al., 2008).

Data Analysis

Data were analyzed using a one-way analysis of variance (ANOVA) to determine statistical differences and the means compared by the Tukey's Studentized Range (HSD) comparison method at $p \leq 0.05$. Statistical Package for Social Sciences (SPSS) software Version 21 in Mac OS X operating system was used for analysis.

RESULTS AND DISCUSSION

This finding suggests that choices of solvents play an essential role in terms of the total extract yields (Table 1) and the ability of the respective solvent to extract the secondary compounds in the oil palm roots in response to copper sulfate and non-elicited oil palm roots as control. Methanol extract (ME) gave the highest yield percentage for non-elicited and $CuSO_4$-elicited oil palm roots with values of 2.54 % and 1.86 % respectively. However, the results for ME and EE were not significantly different. Generally, EAE, CE and PEE were considered less effective as solvents extraction for oil palm roots. Plant secondary metabolites are currently the subject of much research

interest, but their extraction as part of phytochemical or biological investigations presents specific challenges that must be addressed throughout the solvent extraction process. Due to a fresh plant material was required in this study, the extraction process was conducted as soon as possible using organic solvents that will deactivate enzymes present in the plant.

Furthermore, the composition of phytochemicals for the non-elicited and $CuSO_4$-elicited oil palm roots extracted with different solvents were assessed and compared as shown in Table 2. Qualitative chemical tests for both non-elicited and $CuSO_4$-elicited oil palm root extracts revealed the presence of various phytochemicals in ME and EE. Those phytochemicals constituents were strongly present in ME from $CuSO_4$-elicited oil palm root as compared to other extracts for both treatments.

Table 1. Yield of different solvents for non-elicited and $CuSO_4$-elicited oil palm roots extraction.

Solvents	Non-elicited		$CuSO_4$-elicited	
	Yield (g)	Yield (%)	Yield (g)	Yield (%)
ME	1.27 ± 0.15	2.54^{a}	0.93 ± 0.10	1.86^{a}
EE	1.12 ± 0.09	2.24^{ab}	0.74 ± 0.12	1.48^{ab}
AE	0.90 ± 0.05	1.80^{b}	0.60 ± 0.07	1.20^{b}
EAE	0.55 ± 0.09	1.10^{c}	0.22 ± 0.11	0.45^{c}
CE	0.38 ± 0.04	0.76^{c}	0.06 ± 0.02	0.13^{c}
PEE	0.37 ± 0.03	0.74^{c}	0.06 ± 0.01	0.12^{c}

Means within each column followed by the same letter are not significantly different at $p \leq 0.05$. The values are the means of three replicates, with ± SD indicates the standard deviation from the mean.
Notes: ME = Methanol extract, EE = Ethanol extract, AE = Acetone extract, EAE = Ethyl acetate extract, CE = Chloroform extract and PEE = Petroleum ether extract.

Table 2. Qualitative analysis of the phytochemicals for the non-elicited and $CuSO_4$-elicited oil palm roots extracted with different solvents.

Oil Palm Treatments	Crude Extracts	Alkaloids		Saponins	Terpenoids	Flavo-noids	Cardiac glycosides	Tannins
		Dragendorff's reagent	Wagner's reagent					
Non-elicited	ME	+	+	+	+	-	+	+
	EE	+	+	+	+	-	+	+
	AE	+	+	+	+	-	-	+
	EAE	+	+	-	+	-	+	-
	CE	-	-	-	-	-	-	-
	PEE	-	-	-	-	-	-	-
$CuSO_4$-elicited	ME	+++	+++	+++	+++	-	+++	+++
	EE	+	+	++	++	-	++	+++
	AE	+	+	++	+	-	-	++
	EAE	+	+	-	+	-	+	-
	CE	-	-	+	-	-	+	-
	PEE	-	-	-	-	-	-	-

Scores: (-) = not present, (+) = weakly present, (++) = moderately present, (+++) = strongly present.

Meanwhile, Table 3 shows the *in vitro* antifungal activity of non-elicited and $CuSO_4$-elicited among the different oil palm crude extracts obtained in this study eight days after incubation. The results of antifungal tests for the non-elicited oil palm roots of ME and EE showed no significant difference with a value of 8.15 % and 5.56 % respectively. Meanwhile, $CuSO_4$-elicited oil palm roots showed that ME gave the highest significant antifungal activity (14.07 %) compared to other

treatments. EAE (2.59 %), CE (1.11 %) and PEE (0.74 %) exhibited significantly weak antifungal activities. Type of solvent has been the most studied factor (Singh et al., 2014; Uma et al., 2010) to ensure the efficiency of extraction instead of other investigated variables for instance the pre-treatment of the sample, solvent/sample ration, time and temperature of extraction. Plant materials have diverse chemical profile and it is impossible to develop a universal solvent that is suitable for the all kinds of antioxidant compounds extraction from plants.

Table 3. *In vitro* antifungal activity of non-elicited and $CuSO_4$-elicited among the different oil palm crude extracts eight days after incubation.

Solvents	Mycelial growth inhibition (mean ± SD)			
	Non-elicited		$CuSO_4$-elicited	
	Growth (mm)	% Inhibition	Growth (mm)	% Inhibition
ME	82.67 ± 1.15	8.15^{a}	77.33 ± 1.53	14.07^{a}
EE	85.00 ± 1.00	5.56^{ab}	82.67 ± 1.53	8.14^{b}
AE	86.67 ± 1.53	3.70^{b}	84.00 ± 1.00	6.67^{b}
EAE	89.33 ± 0.58	0.74^{c}	87.67 ± 0.58	2.59^{c}
CE	89.67 ± 0.58	0.37^{c}	89.00 ± 1.00	1.11^{c}
PEE	89.67 ± 0.58	0.37^{c}	89.33 ± 1.15	0.74^{c}

Means within each column followed by the same letter are not significantly different at $p \leq 0.05$. The values are the means of three replicates, with ± SD indicates the standard deviation from the mean.

Therefore, it is worthwhile to perform several trial extractions using different solvents in order to get an optimum recovery of total extract yields, compounds of interest or intensity of biological activity as it indicates which method gives the best results. The presence of various phytochemical constituents revealed that many of the secondary metabolites of organisms, including plants, serve important biological and ecological roles, mainly as chemical messengers and defensive compounds. Therefore, results obtained in this study generally showed that the oil palm roots treated with $CuSo_4$ revealed the presence of various phytochemical constituents and greater antifungal activity as compared to non-elicited oil palm roots extracts. This finding is in agreement with Bota & Deliu, 2011 and Ramakrishna & Ravishankar, 2011 who had reported that an effective accumulation of Cu have been shown to induce higher yields of secondary metabolites. In the presence of elicitors the amounts of phytoalexins can increase very much up to ten times (Bota & Deliu, 2011). Other than salts of heavy metals for instance Al, Cd, Ag, Ni and Cr, copper salts whether exist as sulphate or chloride were also successfully used as abiotic elicitors in several research studies (Aziz et al., 2006; Engelmann et al., 2009; Nur Sabrina et al., 2012). Indeed, the exposure of plants to heavy metals may lead to protection against pathogens as well as becoming an elicitors of plant defence mechanisms (Maksymiec, 2007; Poschenrieder et al., 2006).

CONCLUSION

This article provides an overview of the basic and important approach for the process of screening the best organic solvent to extract oil palm roots which give an optimum recovery of yield extraction and phytochemical constituents as well as good antifungal activity. Obviously, the crude methanolic of oil palm root extracts showed the best results in this study. Prior to conduct the bulk collections of oil palm root extracts for further analysis or fractionation in metabolomics research, this preliminary study is paramount as it gives general overview in selection of the best solvent system.

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REFERENCES

[1] Aziz, A., Trotel-Aziz, P., Dhuicq, L., Jeandet, P., Couderchet, M., & Vernet, G. (2006). Chitosan oligomers and copper sulfate induce grapevine defense reactions and resistance to gray mold and downy mildew. *Phytopathology*, *96*(11), 1188–94.

[2] Bota, C., & Deliu, C. (2011). The effect of copper sulphate on the production of flavonoids in *Digitalis lanata* cell cultures. *Farmacia*, *59*(1), 113–118.

[3] Chong, K. P., Rossall, S., & Atong, M. (2011). HPC fingerprints and in vitro antimicrobial activity of syringic acid, caffeic acid and 4-hydroxybenzoic acid against *Ganoderma boninense*. *Journal of Applied Sciences*, **11**(13), 2284–2291.

[4] Costa, T. D. S. A., Vieira, R. F., Bizzo, H. R., Silveira, D., & Gimenes, M. A. (2012). Secondary metabolites. *In*: Dhanarasu S (Ed.) *Chromatography and its Applications*. InTech Publishers, Croatia, pp. 131–164.

[5] Edeoga, H. O., Okwu, D. E., & Mbaebie, B. O. (2005). Phytochemical constituents of some Nigerian medicinal plants, **4**(7), 685–688.

[6] Engelmann, N. J., Reppert, A., Yousef, G., Rogers, R. B., & Lila, M. A. (2009). *In vitro* production of radiolabeled red clover (*Trifolium pratense*) isoflavones. *Plant Cell, Tissue and Organ Culture*, **98**(2), 147–156.

[7] Goh, Y. K., Marzuki, N. F., Lim, C. K., Goh, Y. K., & Goh, K. J. 2016. Cytotoxicity and acute oral toxicity of ascomycetous mycoparasitic *Scytalidium parasiticum*. *Transactions on Science and Technology*, **3**(3), 483-488.

[8] Hamza, O. J. M., van den Bout-van den Beukel, C. J. P., Matee, M. I. N., Moshi, M. J., Mikx, F. H. M., Selemani, H. O., Mbwambo, Z. H., Van der Ven, A. J. A. M., & Verweij, P. E. (2006). Antifungal activity of some Tanzanian plants used traditionally for the treatment of fungal infections. *Journal of Ethnopharmacology*, **108**(1), 124–32.

[9] Kliebenstein, D. J. (2012). Making new molecules-evolution of structures for novel metabolites in plants. *Current Opinion in Plant Biology*, **16**, 1–6.

[10] Maksymiec, W. (2007). Signaling responses in plant to heavy metal stress. *Acta Physiol. Plant.*, **29**, 177–187.

[11] Nur Sabrina, A. A., Sariah, M., & Zaharah, A. R. (2012). Suppression of basal stem rot disease progress in oil palm (*Elaeis guineensis*) after copper and calcium supplementation. *Pertanika J. Trop. Agric. Sci.*, **35**(S), 13–24.

[12] Nusaibah, S. A., Siti Nor Akmar, A., Idris, A. S., Sariah, M., & Mohamad Pauzi, Z. (2016). Involvement of metabolites in early defense mechanism of oil palm (*Elaeis guineensis* Jacq.) against *Ganoderma disease*. *Plant Physiology and Biochemistry*, **109**, 156–165.

[13] Ong, W., & Chong, K. P. (2009). Aging effect to accumulation of lettucenin a in lettuce after elicitation with various abiotic elicitors. *Modern Applied Science*, **3**(2), 66–70.

[14] Parekh, J., & Chanda, S. V. (2007). In vitro antimicrobial activity and phytochemical analysis of some Indian medicinal plants. *Turk J Biol*, **31**, 53–58.

[15] Park, I. K., Kim, J., Lee, Y. S., Shin, S. C., 2008. In vivo fungicidal activity of medicinal plant extracts against six phytopathogenic fungi. Int. J. PestManage. 54 (1), 63-68.

[16] Poschenrieder, C., Tolrà, R., & Barceló, J. (2006). Can metals defend plants against biotic stress? *Trends in Plant Science*, **11**(6), 288–295.

[17] Raaman, N. (2006). *Phytochemical Techniques*. *New India Publishing Agency*, pp. 19-21.

[18] Ramakrishna, A., & Ravishankar, G. A. (2011). Influence of abiotic stress signals on secondary metabolites in plants. *Plant Signaling & Behavior*, **6**(11), 1720–1731.

[19] Rozlianah, F. S., Jualang, A. G., & Chong, K. P. (2015). Fatty acids and phenols involved in resistance of oil palm to Ganoderma boninense. *Advances in Environmental Biology*, **9**(7), 11–16.

[20] Singh, M., Jha, A., Kumar, A., Hettiarachchy, N., Rai, A. K., & Sharma, D. (2014). Influence of the solvents on the extraction of major phenolic compounds (punicalagin, ellagic acid and gallic acid) and their antioxidant activities in pomegranate aril. *Journal of Food Science and Technology*, **51**(9), 2070–2077.

[21] Tung, H. J., Ong, C. E., Goh, Y. K., Goh, Y. K. & Goh, K. J. 2018. Survival and pathogenicity of monokaryotic and dikaryotic Ganoderma boninense following the different preservation methods. *Transactions on Science and Technology*, **5**(1), 46-52.

[22] Uma, D. B., Ho, C. W., & Wan, A. W. M. (2010). Optimization of extraction parameters of total phenolic compounds for Henna (*Lawsonia inermis*) leaves. *Sains Malaysiana*, **39**, 119–128.

[23] Yen, T., Chang, H., Hsieh, C., & Chang, S. (2008). Antifungal properties of ethanolic extract and its active compounds from Calocedrus macrolepis var. formosana (Florin) heartwood. *Bioresource Technology*, **99**, 4871–4877.

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