Total Phenolic Content of Oil Palm Roots (Elaeis Guineensis Jacq.) As Preliminary Health Indicators in Oil Palm Plantation

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Abstract. Phenol and its derivatives are known as major phytochemical compound incorporated in plant defense or resistance. Oil palm (Elaeis guineensis Jacq.) is an important oil-producing species which prone to infection caused by Ganoderma boninense Pat., causing basal stem rot to the plant. Oil palm may accumulate or secrete related phenolic compounds into rhizospheric region as early mechanism in plant defense against G. boninense. This study investigated the role of total phenolic content in oil palm roots as an indicator to health status of plant in plantation. Oil palm roots were sampled from four representative plantations namely Kuala Bekala (KB), Medan Johor (MJ) from Medan city and Bilah Barat (BB), Kualuh Hulu (KH) from Labuhan Batu district. Phenol concentration was measured using Folin-Ciocalteau colorimetry method, expressed as µg GAE/mL from four different root macerates, using 100% MeOH, 100% MeOH with sonication, 80% MeOH and sterile distilled water (H2O). The results showed that phenolic compounds were higher in healthy than diseased plant. Solvent 80% MeOH was considered as the best solvent in extracting phenolic compounds both from diseased and healthy plants (P < 0.001) both quantitatively and qualitatively. The differences of phenol content in oil palm roots may then reflect the fitness status of plant in the presence or absence of G. boninense.

1. Introduction
Oil palm (Elaeis guineensis Pat.) is a superior oil-producing plant species in the world possessing high production capacity in the fruit bunches than any other known species. Productivity of oil palm is influenced by the health status of the plant. Infection caused by notable phytopathogenic fungi (Ganoderma boninense Pat.) causing basal stem rot disease in Indonesian and Southeast oil palm plantation, may lower the oil productivity yet impose a serious threat to the sustainability of oil palm industry [1,2]. Initial attack by G. boninense may not be detected in early phase of plant growth and development. Disease signs and symptoms may appear latter, becoming visible in a badly infected-plant. In addition, control G. boninense becomes more difficult since the pathogen may lurk into plant tissue in latent phase or period. Early detection of infected oil palm plant early to prevent the spread of diseases or pathogens in disease management [3].
Physiologically, the health status of a plant is related to the plant resistance to incoming pathogens either in active or passive ways [4]. Plant secondary metabolites or phytochemicals play a major role in suppression of infection by pathogens. Plant constitutively produced a vast number of phytochemicals as initial protection or signalling pathway to perceive pathogens presence, one of which is phytoanticipin [5]. Phytoanticipin is a low-molecular weight phytocompound with antimicrobial activities [6]. Phenolic compounds are the backbone of phytoanticipins. Other phenolic compounds in oil palm roots, e.g. syringic acid, caffeic acid and 4-hydroxybenzoic acid were reported as phytochemicals responsible to oil palm resistance to *G. boninense* infection [7,8].

Due to phenol attribute in disease resistance, it is assumed that total phenol content in oil palm tissue may reflect a bidirectional relationship between host and pathogen, *G. boninense* in oil palm. Here, we present a preliminary health indicator by assessing total phenol content in oil palm roots from four representative oil palm plantations in Medan city, North Sumatra.

2. Materials and Methods

2.1. Plant material
Oil palm roots were collected from four oil palm plantations, located in North Sumatra. Two locations were from Medan city, namely Kuala Bekala (KB) and Medan Johor (MJ) while the other two were from Labuhan Batu district, namely Bilah Barat (BB) and Kualuh Hulu (KH) (Figure 1). One hundred grams of oil palm roots were sampled in triplicate from each healthy and diseased plants. The diseased plant was confirmed by the presence of *Ganoderma boninense* fruiting body on the basal stem. Root samples were rinsed in running tap water and dried in ambient room temperature. Dried roots were mashed into root powder with a blender and stored in the freezer.

![Figure 1. Map location of study sites](image-url)
2.2. Isolation and extraction of phenolic compound
Phenolic compounds were extracted from oil palm roots using four solvents: sterile distilled water (H₂O), 80% MeOH, 100% MeOH and 100 MeOH of sonicated roots. Root powder was macerated in water and organic solvents (1:10) under agitation of 120 rpm for 24 hr. Water extraction was performed at 60°C while methanolic extraction at room temperature. Macerates were centrifuged at 10,000 rpm for 10 min. The supernatants or root extracts were pooled and stored at -20°C prior experimentation [9].

2.3. Quantification of phenolic compound
Phenolic compounds were measured using Folin-Ciocalteu (F-C) colorimetric method [10]. The root extracts (100 µL) were added into 2 mL sterile distilled water and 200 µL F-C reagent and incubated for 2 min at room temperature. Reaction mixture was added with 2 mL 15% Na₂CO₃ solution (w/v) and incubated further at room temperature for 1 hr. The blue color reaction was measured using spectrophotometer at 750 nm (A₇50). The measurement was compared to standard curve of gallic acid, expressed as µg GAE/mL extract solution.

2.4. Data analysis
Numerical data are presented as mean and analyzed using ANOVA and unpaired t-test. Multiple comparison of data from ANOVA analysis was performed using post-hoc Tukey test. P < 0.05 was considered as statistically significant. Statistical test and graphical image were produced using GraphPad Prism ver. 8.0.

3. Results and Discussions
Phenolic compounds of diseased and healthy plants were detected using four different solvents, yielding various concentration expressed in µg/mL root extract solution based on F-C colorimetry method (Figure 2). Total phenolic content was lower in diseased plant than healthy plant which reflect in all extracts. However, we observed a large variation of results in healthy plant as shown from high deviations.

![Figure 2. Phenol concentration of diseased and healthy plant. Box plots display mean. Error bars: S.D](image-url)
Extraction of diseased root using 100% MeOH with sonication yielded the highest phenol concentration of $425.0 \pm 109.0 \, \mu g/mL$ and the lowest phenol concentration from water extract with concentration of $130.2 \pm 96.92 \, \mu g/mL$. Meanwhile, extraction of healthy root using 80% MeOH yielded the highest phenol concentration of $682.0 \pm 259.7 \, \mu g/mL$ and the lowest phenol concentration from water extract with concentration of $204.9 \pm 192.8 \, \mu g/mL$. Phenol concentration of diseased and healthy roots was very significant in 80% MeOH extract ($t = 3.04, P < 0.01$) and significant in 100% MeOH extract with sonication ($t = 1.92, P < 0.05$). The result then reflect that 80% MeOH may be used as the best solvent for phenol extraction from oil palm roots.

**Figure 3.** Comparison of phenol concentration in diseased and healthy plant. Bars display mean of triplicate results. (Error bars: S.Ds) Statistical test performed is t-test ($P < 0.05$). ns: not significant, *: significant ($P < 0.05$), **: very significant ($P < 0.01$).

Based on sampling sites, the highest total phenolic concent was observed from Kuala Bekala (KB) for diseased plant and in Medan Johor (MJ) and Bilah Barat (BB) for healthy plant (Figure 4). In diseased plant, BB and KH samples showed no significant differences in phenolic concentration, similar to KH and KB. The sampling sites, BB, KH and MJ plantation were identified as community plantation although in MJ, we did not find any disease-related oil palm in the site. It is assumed that many kinds of phenolic derivatives may also be found from each plantation displaying spatial variations.
Ganoderma boninense has caused major loss in oil palm settlement in Indonesia. Under promising discovery of potential biocontrol agents, still an inconsistency existed in disease management of basal stem rot [11]. However, phenomenon occurring in the level of metabolites or molecular level may be investigated further to obtain better understanding regarding Ganoderma infection to oil palm. Induced systemic resistance of oil palm is one effective way through interaction between rhizospheric microorganisms and root region. Phytochemicals derived from these interaction is manifested into accumulation of phenolic compounds as revealed from this study which also showed an indication of oil palm health status. Besides accumulation of phenolic compounds, innate immunity by secretion of other antifungal compounds or chitinase enzyme may also be used by the plant to control Ganoderma infection [12].

Phenolic compounds are naturally synthesized and accumulated in the oil palm roots with reported antifungal activity against G. boninense. Phenolics, e.g. phenol, 2,6-dimethoxy- and phenol, 2,4-bis (1,1-dimethyl ethyl) along with fatty acids have been reported to be involved in oil palm resistance to G. boninense under certain level or concentrations [13]. Laboratory experimentation also supported the view that phenolic acids may inhibit the growth of G. boninense as revealed from hexaconazole-treated soils supporting no growth of G. boninense colonies on Ganoderma Selective Medium (GSM) [8]. However, specificity and effectiveness of phenolic compounds may be differed due to versatility of hemibiotroph G. boninense. Transition from biotrophic to necrotrophic G. boninense hinder our early detection and management of infection in the early phase [14]. In addition, total phenolic content may either be direct or indirectly related to the health status of oil palm. Deeper investigation is needed to support our finding of any phenol-related compounds as chemical marker to infection of G. boninense and fitness level of oil palm.

4. Conclusions
The phenolic content of oil palm root was able to select the fitness status of oil palm. The total phenol content of healthy oil palm was in higher concentration than diseased oil palm. Extraction using 80% MeOH is considered as the best solvent to isolate phenolic compounds from both diseased and healthy oil palm roots.
Acknowledgment(s)
This research was fully funded through DRPM Kemenristekdikti RI 2019 under scheme of Penelitian Dasar Unggulan Perguruan Tinggi (PDUPT) with Project No.173/UN5.2.3.1/PPM/KP-DRPM/2019.

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