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# DNA extraction from *Ganoderma* isolates of PT Socfindo collections

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**Abstract.** Method of CTAB (cetyltrimethylethylammonium bromide) is prevalent for low-cost DNA extraction with many published variations for other plants and tissues, including fungi *Ganoderma*. The quantity of DNA extracts from the *Ganoderma sp* fungus from a coconut tree and rubber tree with concentration extraction efficiency and the quality of purity wholeness varied with different DNA characterizations. DNA purity was measured using UV spectroscopy, where a ratio of 260 nm, 280 nm 320 nm was an indicator of different contaminants. The quantity of DNA from the isolate is indicated by 31 ng/ul until 133 ng/ul and range of ratio from 1,279 until 3,6654. The DNA extraction quantity ratio value obtained is below, and above the average, for absorbance 260 nm, 280 nm and 320 nm varies between 1.5 until 1.8 which is an indicator of DNA purity. Pure DNA isolate without contaminants is only found in sample isolate *Ganoderma sp* 141.1 from coconut tree with a ratio value of 2.2707. Furthermore on the other samples thought to be still contaminated with phenols, salts, proteins, polysaccharide or RNA still co-extracted with phenolic acid (chloroform phenol).

## 1. Introduction

*Ganoderma boninense* or Basal Stem Rot (BSR) which attacks oil palm plants cause vast economic losses throughout the world [1]. In addition to that oil palm plantations, *Ganoderma sp* of fungus also attack coconut (*Cocos nucifera* L.), rubber (*Hevea brasiliensis*) and sengon (*Falcataria mollucana*) plantations [2-4]. Currently, reputable oil palm plants Moderat Tahan *Ganoderma* (MTG) commercials that have been studied genetic linkages of molecular RAPD have been reported [5].

Diagnostics because *Ganoderma sp* disease is difficult when only observing visual symptoms and takes a long time, but whether the chemical analysis is expensive [6]. The existence of basic identification methods using molecular techniques for identification of *G. boninense* has been reported [7]. High molecular weight DNA fast isolation methods (50,000 base pairs of length) produce total cellular DNA (in nucleus, chloroplasts or mitochondrial DNA) have been reported [8]. This study aims to DNA extraction isolate as confirmation of the *Ganoderma sp* gene in coconut and rubber collections of PT. Socfindo, Bangun Bandar, North Sumatra.



## 2. Materials and method

The highest level of *Ganoderma* infection chose identification of sample locations on the plantation. Samples of *Ganoderma* sp mushrooms were originating from coconut tree three units and rubber tree two units were collected, from the palm of life with symptoms of high infection (scores 3–4). The fruit body is fresh and not too old with symptoms at the top still smooth and the bottom with white, the thickness of the mycelium in the middle is more than 5 mm.

The sample location (estate, block, line number, tree number) and notes in the form of a *Ganoderma* sample are recorded. Drawings of sample trees and samples from fruit bodies taken. The body of the palm stem and cut in the middle of the fruiting body to identify that the size of the mycelium is sufficient for the sample, then cover with aluminum foil. The plastic clip is used to identify the sample (code sample), and white into a plastic box then insert it and bring it to the Pathology Laboratory in Tanah Gambus, Bangun Bandar, North Sumatra.

Total DNA extracted from *Ganoderma* isolates using the *cetyl trimethyl ammonium bromide* (CTAB) method based on our previous study with minor modification [9]. Briefly, isolation of DNA from isolate sampling used taken from the field was washed and dried with liquid nitrogen.  $\pm 0.5$  gr of the isolate is ground with mortar while adding liquid nitrogen PVPP. The sample is inserted into a filled centrifuge 1 ml with buffer extraction CTAB and  $\beta$ -mercaptoethanol 10  $\mu$ l, then stirred using vortex and incubated during water bath for 30 minutes at temperature 65°C.

Every 10 min the sampling turned back slowly. Furthermore, the sample was incubated at room temperature during 4–5 min, added 1 ml chloroform: isoamyl alcohol (24:1). Then, the sample was centrifuged at 13,000 rpm at room temperature for 10 min. The supernatant obtained is transferred to another centrifuge tube, vortex and centrifuged at 13,000 rpm at a temperature 4°C for 10 min. The supernatant was homogenized by flipping through the tube, stored in the refrigerator (4°C) for 30 min, centrifuged at 13,000 rpm at 4°C for 10 min. The supernatant obtained are discarded then the pellets are air-dried. Dry pellets were dissolved with a buffer TE 100  $\mu$ l, added with absolute cold ethanol, then turned back and forth until homogenized. Then incubated in the freezer (-20°C) for 30 min and then centrifuged at 13,000 rpm at 4°C for 10 min. The supernatant was removed while the pellets were washed using ethanol 70% and air-dried. The dried one DNA pellets are dissolved with 100  $\mu$ l buffer TE, and finally, DNA stored in the freezer (-20°C). Total DNA is used for quantitative analysis with a spectrophotometer for 260 nm, 280 nm, and 320 nm.

## 3. Results and Discussions

Table 1 shows the results of the quantity of DNA with a score of ratio 1,279–3,6654. The quantity of DNA from the isolate *Ganoderma* sp is indicated by 31 ng/ $\mu$ l until 133 ng/ $\mu$ l. DNA purity was measured using UV spectroscopy, where a ratio of 260 nm, 280 nm 320 nm was an indicator of different contaminants. They were a reasonably high the value. The dirtiness released by ether has a significant absorbance in the UV region (260 nm, 280 nm) present in the sample, causing incorrect results in DNA quantification, the absorbance ratio ( $R_{260} / R_{280}$ ) at 260 nm for absorbance and 280 nm varies between 1.5 until 1.8, which is an indicator of DNA purity [10].

**Table 1.** Test the quantity of isolate DNA of *Ganoderma* sp from Coconut and Rubber tree

| No. | Sample       | A[260]nm | A[280]nm | Bg[320]nm | Ratio  | The quantity of DNA<br>(ng/ $\mu$ l) |
|-----|--------------|----------|----------|-----------|--------|--------------------------------------|
|     | Blanko       | 0,0008   | 0,0003   | -0,0001   | 2,187  |                                      |
| 1   | Isolet 141.1 | 0,0276   | 0,0182   | 0,0107    | 2,2707 | 67                                   |
| 2   | Isolet 141.2 | 0,0402   | 0,287    | 0,0226    | 2,8688 | 99                                   |
| 3   | Isolet 141.3 | 0,0133   | 0,01     | 0,0009    | 1,3655 | 31                                   |
| 4   | Isolet 145.1 | 0,0541   | 0,049    | 0,0307    | 1,279  | 133                                  |
| 5   | Isolet 145.2 | 0,0376   | 0,0178   | 0,0104    | 3,6654 | 92                                   |

Classical methods are often used to compromise the integrity of fungal cell walls and membranes, followed by the use of phenol or chloroform to isolate and purify DNA from cell debris [11]. The reference method has the highest proportion of extract with an  $A_{260} \text{ nm} / A_{280}$  ratio above 2.0 most likely because RNA still co-extracted with *phenolic acid* (*chloroform phenol*) [12]. For the

A260/A230 nm ratio, a good range is 1.8 to 2.2, values below this range can indicate *phenol*, salt, protein or *polysaccharide* contamination [13].

Several reported studied by researchers, direct comparison of DNA produced by methods using *cetyltrimethylammonium bromide* (CTAB) showed lower enzyme inhibition rates and results were still sufficient for use in molecular biology and DNA conditions [14]. The high sensitivity of the PicoGreen test to detergent known as CTAB is very popular for low-cost DNA extraction with many published variations for other plants and tissues, including fungi [15].

#### 4. Conclusions

The DNA extraction quantity ratio value obtained is below and above the average. Pure DNA isolate without contaminants is only found in sample isolate *Ganoderma* sp 141.1 from coconut tree with a ratio value of 2.2707. Furthermore on the other samples thought to be still contaminated with phenols, salts, proteins or polysaccharide.

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