Extraction and Isolation of Active Fraction from *Senna multijuga* Bark as Antifungal Agents against *Ganoderma Boninense*

Subeki[1,*], Diah Pangastuti Rahayu[1], Erdi Suroso[1], Samsul Rizal[1], Ruli Wandri[2], Samsu Alam[2], Shervinia Dwi Ayundra[2], Azharudin Apriansa[2], Dwi Asmono[2], Yuyun Fitriana[3], Rosma Hasibuan[3] and Radix Suharjo[3]

[1]Department of Agricultural Product, Faculty of Agriculture, University of Lampung, Lampung-Indonesia 35145

[2]Division of Sustainability, Research and Development, PT Sampoerna Agro Tbk, Jakarta-Indonesia 12930

[3]Department of Plant Protection, Faculty of Agriculture, University of Lampung, Lampung-Indonesia 35145

Abstract. *Ganoderma boninense* is a fungus that causes basal stem rot disease in oil palm plants. This fungus disrupts the transport of water and nutrients from the soil, leaf chlorosis, stem decay, fruitlessness, and ultimately death. This study is one of the first to specifically explore the bark of *Senna multijuga* as a source of antifungal compounds against *Ganoderma boninense*. Previous studies have focused more on leaf extracts from other *Senna* species, so the use of *S. multijuga* bark provides a new contribution to the search for biological agents to control *G. boninense*. This study aims to extract and isolate the active fraction from *S. multijuga* bark as antifungal agents against *G. boninense*. The study was conducted in three stages. The extraction and isolation of the active fraction from *S. multijuga* bark as antifungal agents against *G. boninense*, the determination of the chemical composition of the *S. multijuga* bark, and the antifungal activity of the active fraction against *G. boninense in vitro*. Based on the GC-MS results for fraction-1 from *S. multijuga* bark showed the main compounds of 1-hexadecene (5.51%), 1-octadecene (8.29%), hexadecanoid acid (10.46%), 2-ethoxycyclohexanol (5.53%), 9,12-octadecadienoic acid (8.91%), 9,12,15-octadecatrienoic acid (7.70%), 2-hexadecen-1-ol (5.48%), 1-docosene (3.33%), 2-pyridol (6.28%), and calotropenol acetate (6.52%). A concentration of fraction-1 at 1.50 ppm can inhibit the growth of *G. boninense* (0.32 cm/day), inhibition of *G. boninense* (67,46%), and produce thin white mycelium that collects in the middle of the medium.

1. Introduction

Oil palm is one of the fastest growing plantation sectors in Indonesia. Currently, oil palm plantations are developing in 22 provinces in Indonesia, spread across the islands of Sumatra and Kalimantan. Approximately 90% of oil palm plantations in Indonesia are located in these two regions [1]. Oil palm is one of the country's foreign exchange earners with vast plantation areas, making Indonesia the world's largest exporter of oil palm. Oil palm plays an important role in the economy as a contributor to foreign exchange earnings. According to 2021 data from the Central Statistics Agency (BPS), palm oil contributed USD 26.75 billion to the

* Corresponding author: subeki.1968@fp.unila.ac.id

country's foreign exchange earnings, increasing to USD 27.76 billion in 2022. Problems often arise in the management of palm oil, causing a decline in palm oil production. This is due to attacks by plant pests.

One of the diseases that causes stem rot can be caused by the fungus *Ganoderma boninense*. The fungus *G. boninense* is a disease pathogen that causes the most damage to oil palm plants. This fungus is highly contagious through root contact between oil palm plants, allowing it to spread rapidly in oil palm plantations. The disease caused by the fungus *G. boninense* can attack plants from seedlings to mature trees and result in a decline in fruit production. This disease can cause damage 50-80% of oil palm plants [2]. Therefore, the problem of stem rot caused by the *G. boninense* fungus must be addressed immediately to maintain high oil palm production.

The fungus of *G. boninense* belongs to the Basidiomycetes class of the Ganodermataceae family, which can degrade wood lignin compound. This lignin degradation process involves peroxidase enzymes, cellulase, and xylanase, which mineralize polymers into carbon dioxide and cause plant tissue to rot [3]. One effort to control the *G. boninense* in oil palms is with antifungal compounds from the *Senna multijuga* bark. This study is one of the first to specifically explore the *S. multijuga* bark as a source of antifungal agents against *G. boninense*. Previous studies have focused more on leaf extracts from other *Senna* species, so the use of *S. multijuga* bark provides a new contribution to the search for biological agents to control plant diseases. Unlike previous studies, which generally only reported the antifungal activity of crude extracts, this study emphasized the extraction and isolation of active fraction from the *S. multijuga* bark. This approach enabled the identification of specific compounds that play a role in antifungal activity to control *G. boninense* fungus.

S. multijuga is a tropical plant that grows naturally on the island of Sumatra, Indonesia. This plant species is generally used as an ornamental plant, roadside barrier, intercrop, and rain barrier due to its ability to grow and adapt in dry areas [4]. However, the potential of *S. multijuga* has not been widely developed. Previous studies have shown that the Senna genus contains phytochemical compounds such as alkaloids, terpenoids, glycosides, tannins, saponins, steroids, flavonoids, anthraquinones, and polyphenols. The diversity of these compounds opens up great opportunities for their use as natural active ingredients and environmentally friendly antifungal agents [5]. The appearance of plant *S. multijuga* can be seen in Figure 1 [6].

The pharmacological potential of *Senna sp*. plants continues to be studied through various extraction methods such as maceration, decoction, infusion, and the use of exudates from various parts of the plant such as roots, bark, leaves, seeds, and fruit [7]. Pharmacological studies confirm that *Senna sp*. extracts exhibit antimalarial, antimicrobial, antioxidant, antiinflammatory, and anticancer activities [8]. These compounds, especially flavonoids, phenolics, and anthraquinones, have also been shown to have antifungal properties by damaging the cell walls of *G. boninense* [9].

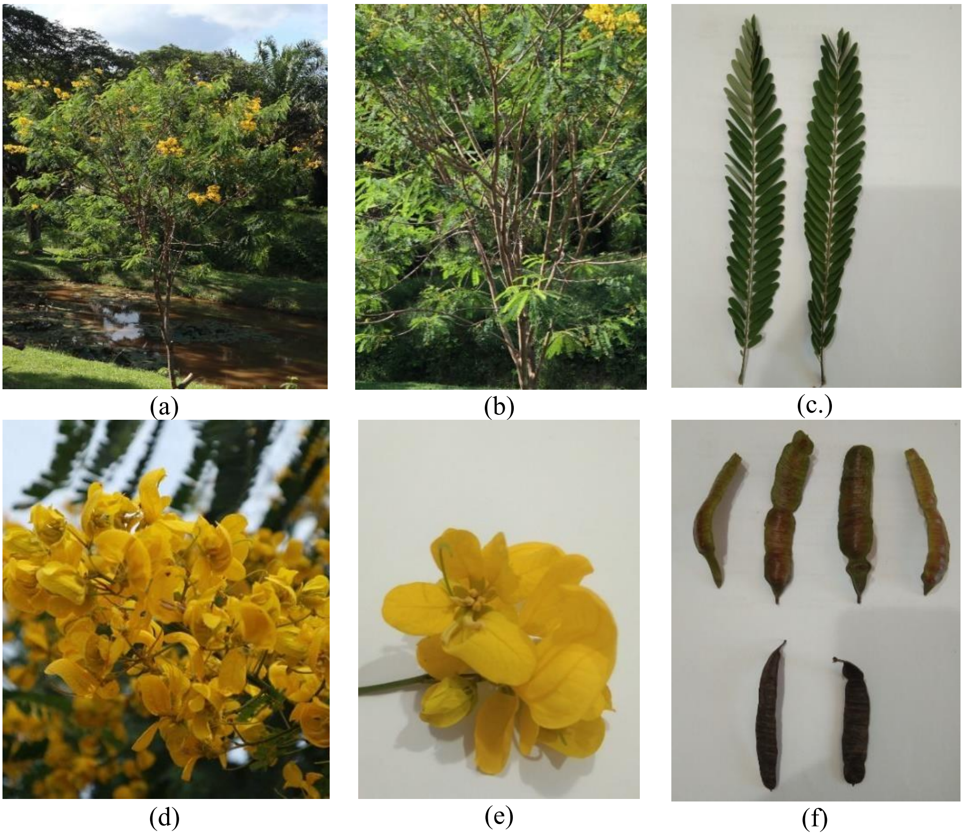

Figure 1. *Senna multijuga* plant. (a) tree, (b) branching, (c) leaves, (d) flowers, (e) petals, and (f) fruit (pod) [6]

Leaf and bark extracts of *S. multijuga* also exhibit inhibitory activity against various microorganisms. The piperidine alkaloids in the leaves of this plant inhibit the growth of *Staphylococcus aureus, S. subtilis, and Candida albicans* fungi [10]. In addition, phenolic compounds from *Senna sp*. show antifungal activity against various species of fungi, including *C. glabrata, C. tropicalis, and C. albicans* [11]. This indicates that *S. multijuga*, which belongs to the Fabaceae family, has strong potential as an antifungal agent against *G. boninense*. This study aims to extract and isolate active fraction from *S. multijuga* bark as antifungal agents against *G. boninense*.

2. Methodology

2.1. Materials and Equipment

The materials used in this study were *S. multijuga* bark, 96% alcohol, methanol, hexane, aluminum foil, ethyl acetate, hexane, chloroform, distilled water, acetone, silica gel 60 (0.2-0.5 mm), PDA medium, and *G. boninense* fungal isolates.

The equipment used in this study included Gas Chromatography-Mass Spectroscopy (GC-MS), hotplate, analytical balance, vacuum rotary evaporator, Petri dishes, laminar air flow, ose needles, bunsen burner, sonicator, incubator, autoclave, glass beakers, erlenmeyer flasks, spatulas, tweezers, measuring cups, and digital calipers.

2.2. Experimental Design and Data Analysis

This study used a single treatment, first column chromatography elution is performed on the ethyl acetate layer from *S. multijuga* bark. The results of column chromatography with eluents of 100% $CHCl_3$, 3% $MeOH/CHCl_3$, 20% $MeOH/CHCl_3$, and 100% MeOH were tested for antifungal activity against *G. boninense in vitro*. The best results were obtained from column chromatography to obtain fractions 1-5. Fraction-1 was then further tested against *G. boninense* at various concentrations, namely 0 ppm, 0.25 ppm, 0.5 ppm, 0.75 ppm, 1 ppm, 1.25 ppm, and 1.5 ppm (w/v). The tests were conducted in three replications. The data obtained were tested for homogeneity using the Bartlett's test and for data addition using the Tukey's test. The data were then analysed using analysis of variance to estimate errors and significance tests to determine the differences between treatments. Furthermore, the data were analysed using the Smallest Significant Difference (SSD) test at a 5% level.

2.3. Research Procedures

Sample Preparation

The *G. boninense* culture was obtained from the Biotechnology Laboratory collection of the Plant Protection Department, Lampung University. Samples of *S. multijuga* bark were obtained from PT. Sampoerna Agro Tbk. The *S. multijuga* bark was cut into small pieces, dried, and then weighed to obtain 5 kg.

Fractionation of *Senna multijuga* Bark

A total of 5 kg of *S. multijuga* bark was soaked for 4 weeks in 15 L of 96% alcohol (ratio 1:3). Next, filtration was carried out to separate the filtrate from the residue. The filtrate obtained was evaporated with a rotary evaporator to obtain a concentrate. The concentrate was added with 1 L of water and then extracted with ethyl acetate to obtain layers of water and ethyl acetate. The ethyl acetate layer was then evaporated with a rotary evaporator to obtain a residue. The residue was subjected in a 100 g silica chromatography column and eluted with 100% $CHCl_3$, 3% $MeOH/CHCl_3$, and 100% MeOH, then tested for compound activity against *G. boninense*.

The $CHCl_3$ fraction was the compound with the strongest inhibitory effect against the *G. boninense*, then evaporated to obtain a residue. The residue was then subjected in a 60 g silica chromatography column and eluted with 25% ethyl acetate/hexane to obtain 5 fractions. Fraction-1 was evaporated until dry and then made into a stock solution of 10 ppm (m/v) by dissolving 1 mg of fraction-1 in 100 mL of distilled water. Fraction-1 was tested against *G. boninense* at concentrations of 1.5, 1.25, 1.0, 0.75, 0.5, 0.25, and 0 ppm (w/v) by taking 3,0, 2.5, 2,0, 1.5, 1.0, 0.5, and 0 mL of the stock solution, respectively, were mixed with agar medium in Petri dishes to a final volume of 20 mL. The extraction and isolation of fraction-1 from *S. multijuga* bark can be seen in Figure 2.

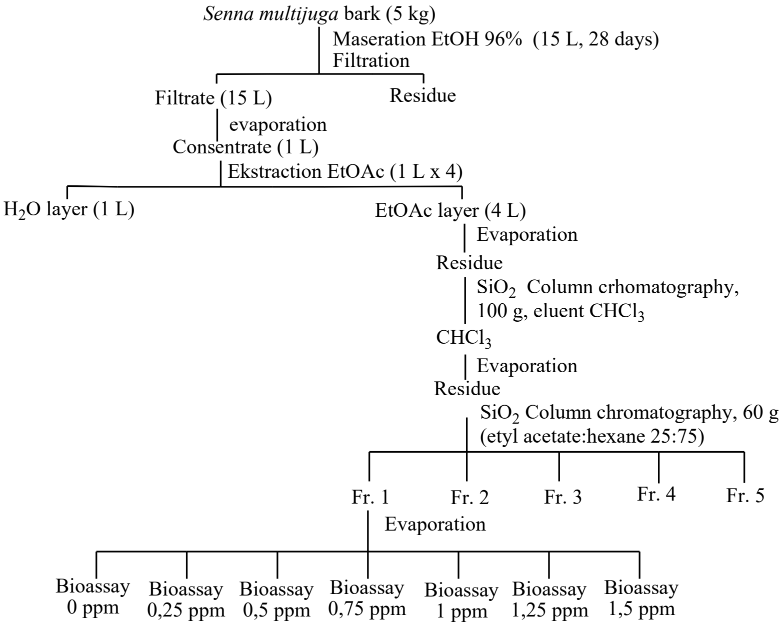

Figure 2. Flow chart of extraction and isolation of fraction-1 from *Senna multijuga* bark

Antifungal Activity *In Vitro*

a. Sterilisation

All instruments used are washed thoroughly and dried. Sterilization is carried out in an autoclave at a temperature of 121ºC for 15 minutes and a pressure of 1 atm. Tubes, pipettes, and containers are closed with cotton plugs, petri dishes are wrapped in HVS paper. Inoculation needles, and borers are sterilized by passing them over a bunsen burner flame.

b. Cultivation of *Ganoderma boninense*

G. boninense cultures were obtained by exploring oil palm plants infested with *G. boninense*. Fresh *G. boninense* isolates were taken from the base of the oil palm bark. The *G. boninense* mushroom bodies were then cut and taken to the laboratory. *G. boninense* was then washed using distilled water and dried. the *G. boninense* mushroom bodies were cut into 1x1 cm pieces using a scalpel. The pieces of *G. boninense* mushroom bodies were then placed in petri dishes containing PDA media. Each petri dish contained 3 pieces of *G. boninense* separately. The Petri dishes were incubated in an incubator at room temperature for 10 days.

The fungal mycelium that grew from the *G. boninense* bodies was re-isolated by aseptically transferring the sterile tissue from the *G. boninense* bodies to Potato Dextrose Agar (PDA) medium. The inoculated petri dishes were then incubated at temperature of 28°C for several days until the mycelium grew and spread across the surface of the medium. The pure isolates obtained from this isolation were then used as inoculum in testing the antifungal activity of the fraction-1.

c. PDA Media Preparation

To prepare the PDA medium, 19.5 g of PDA and 500 mL of distilled water were used. The mixture was then homogenized using a hot plate at a temperature of 85ºC. The homogenized medium was then covered with cotton and aluminium foil. Next, sterilization was carried out in an autoclave at temperature of 121ºC with pressure of 1 atm for 15 minutes.

d. Inhibition Activity of *Ganoderma boninense*

In vitro testing of *G. boninense* inhibition using the fraction-1 from *S. multijuga* bark was conducted based on a modification of the method described by [12]. PDA media was mixed with each treatment concentration in a laminar air flow cabinet. Fraction-1 used in this study was obtained from column chromatography elution after evaporation to remove all ethyl acetate and hexane solvents. The application was carried out by pouring PDA medium and each treatment concentration of fraction-1 into all petri dishes using a micropipette with a final volume of 20 mL and left to solidify. The *G. boninense* fungal mycelium was collected by cutting the PDA inoculated with pure *G. boninense* fungal culture using a sterile borer with a diameter of 0.5 cm. This was done to ensure that the mycelium growth on the PDA medium was the same for each treatment. The *G. boninense* mycelium was placed on PDA media mixed fraction-1 solution right in the middle of the petri dish, then covered with plastic wrap and labelled. The petri dishes were then incubated by placing them in an incubator at room temperature.

2.4. Observation

Growth Rate of *Ganoderma boninense*

The growth rate of the colony was observed daily until the petri dish was filled with *G. boninense* fungus. Measurements were taken using digital calipers with a formula referring to [13] as follows:

$$\mu = \sum X_n - X_{n-1}$$

Description:
μ : Growth rate (mm/day)
X_n : Colony diameter on day n

Growth Inhibition of *Ganoderma boninense*

Growth inhibition values were calculated using the formula developed by [14] as follows:

$$X = \frac{(Y-Z)}{Y} \times 100\%$$

Description:
X : Percentage inhibition value
Y : Control diameter colony
Z : Colony diameter in the treatment

The colony diameter obtained is the average of two measurements of the vertical (d1) and horizontal (d2) diameters. The method for measuring colony diameter is shown in Figure 3.

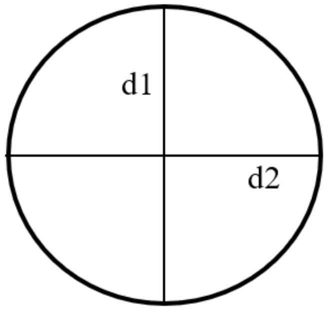

Figure 3. How to measure colony diameter on a petri dish [14]

Macroscopic Characteristics

Macroscopic morphological characteristics were observed by comparing the colour, size, and growth characteristics of *G. boninense* between the control and treatment groups. According to [3], the colour of the colony is white or yellowish-white, and it grows diffusely.

3. Results and Discussion

3.1. Analysis GC-MS

Fraction-1 resulting from the elution of the $CHCl_3$ fraction chromatography column of *S. multijuga* bark was analysed using GC-MS (Gas Chromatography-Mass Spectrometer). This method is a combination of gas chromatography and mass spectrometry techniques to identify organic compounds. Gas chromatography is used to identify compounds that are volatile under vacuum conditions at low pressure. Meanwhile, mass spectrometry is performed to determine molecular weight and molecular formula [15]. The results of GC-MS analysis of fraction-1 from *S. multijuga* bark can be seen in Figure 4.

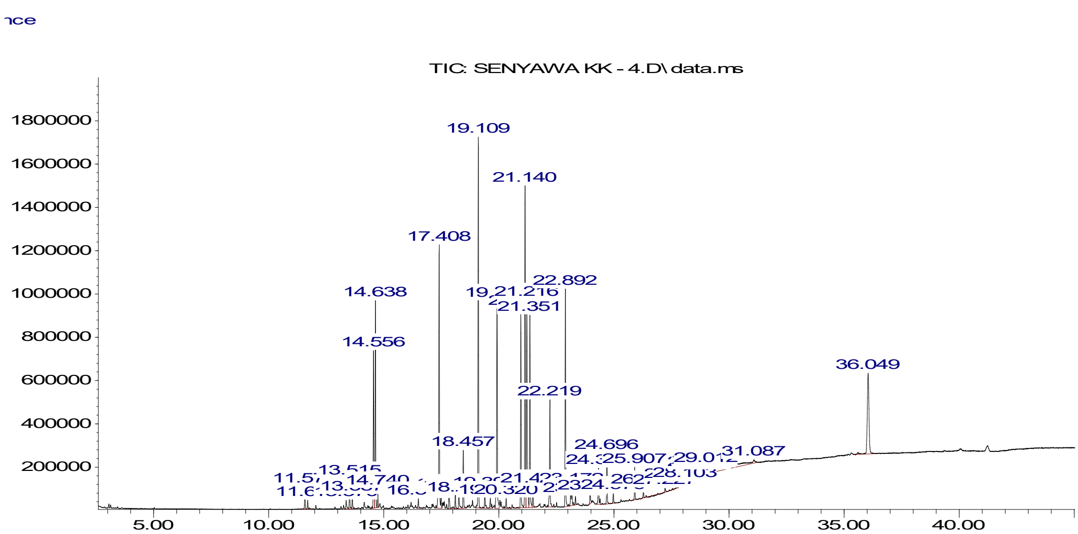

Figure 4. GC-MS analysis of fraction-1 of *Senna multijuga* bark

Figure 4 shows the peaks that appear at retention time. Each peak represents a different compound at a different retention time. GC–MS chromatograms are the result of the separation and identification of compounds based on retention time and mass fragmentation patterns. In the GC–MS analysis, peaks are often encountered that do not originate from the target compound or sample matrix, but rather from the solvent of ethanol, ethyl acetate, chloroform, and hexane used for extraction and isolation, which contain residual impurities that appear as small peaks. The various compounds detected in fraction-1 from the *S. multijuga* bark can be seen in Table 1.

Table 1. Results of GC-MS analysis of fraction-1 from *Senna multijuga* bark

No.	***Read Time*** **(minute)**	**Compound**	**Level (%)**	**SI**
1.	11.574	1-Tetradecene	0.59	99
2.	11.697	Tetradecene	0.28	97
3.	13.515	Phenol	0,84	95
4.	13.637	Dodecanoic acid	0,44	98
5.	14.638	1-Hexadecene	5.51	96
6.	14.740	Hexadecane	0.51	98
7.	16.504	Tetradecanoid acid	0.35	98
8.	17.408	1-Octadecene	8.29	99
9.	17.838	Methyl 13-methyltetradecanoate	0.60	99
10.	18.111	Cyclopentadecanone	0.48	99
11.	18.265	Caffeine	0.58	97
12	19.109	Hexadecanoid acid	10.46	99
13.	19.392	Benzenepropanoic acid	0.63	99
14.	20.320	Heptadecanoic acid	0.44	98
15.	21.140	9,12-Octadecadienoic acid	8.91	99
16.	21.216	9,12,15-Octadecatrienoic acid	7.70	99
17.	21.351	2-Hexadecen-1-ol	5.48	91
18.	21.482	Octadecanoid acid	0.63	99
19.	22.219	1-Docosene	3.33	99
20.	22.892	2-Pyridol	6.28	92
21.	23.331	Methyl 9,10-epoxyoctadecanoate	0.23	90
22.	23.969	9,10-Anthracenedione	0,37	98
23	36,049	Calotropenol acetate	6,52	90

Table 2 shows that there are 23 compounds found in fraction-1 from *S. multijuga* bark. The main components of fraction-1 are 1-hexadecene (5.51%), 1-octadecene (8.29%), hexadecanoid acid (10.46%), 2-ethoxycyclohexanol (5.53%), 9,12-octadecadienoic acid (8.91%), 9,12,15-octadecatrienoic acid (7.70%), 2-hexadecen-1-ol (5.48%), 1-docosene (3.33%), 2-pyridol (6.28%), and calotropenol acetate (6.52%). The chemical structure of the main components in fraction-1 from *S. multijuga* bark can be seen in Figure 5.

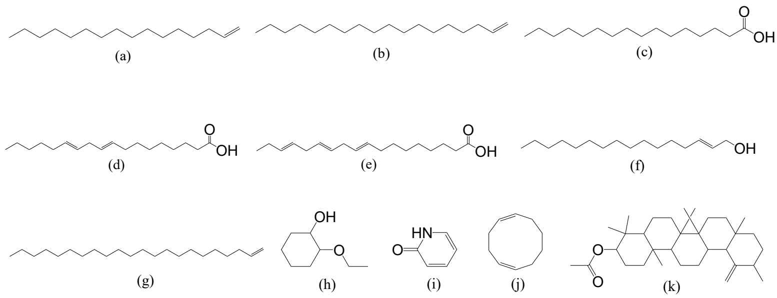

Figure 5. Chemical structures of main compounds of fraction-1 from *Senna multijuga* bark. (a) 1-hexadecene, (b) 1-octadecene, (c) hexadecanoid acid, (d) 9,12-octadecadienoic acid, (e) 9,12,15-octadecatrienoic acid, (f) 2-hexadecen-1-ol, (g) 1-docosene, (h) 2-ethoxycyclohexanol, (i) 2-pyridol, (j) 1,5-cyclodecadiene, and (k) calotropenol acetate

Growth Rate of *Ganoderma boninense*

The results of the variance analysis show that fraction-1 from *S. multijuga* bark has no significant effect on the growth rate of *G. boninense*. The results of the 5% BNT test on the growth rate of *G. boninense* for 10 days can be seen in Table 2.

Table 2. Results of the BNT test on the growth rate of *Ganoderma boninense*

Treatment	Growth Rate (cm/day)
K (-) (distilled water)	0,81 ± 0,04^{a}
P3 (0,75 ppm)	0,38 ± 0,02^{b}
P2 (0,50 ppm)	0,38 ± 0,04^{b}
P5 (1,25 ppm)	0,36 ± 0,06^{b}
P1 (0,25 ppm)	0,33 ± 0,04^{b}
P4 (1 ppm)	0,33 ± 0,02^{b}
P6 (1,50 ppm)	0,32 ± 0,01^{b}
K (+) (*ketoconazole* 0,25 ppm)	0,30 ± 0,07^{b}

BNT (0,05) = 0,08
Note: The average growth rate values in columns with the same letter are not significantly different in the 5% BNT test.

Based on the research conducted on fraction-1 from *S. multijuga* bark, it inhibits the growth of *G. boninense* fungus. The average diameter of the inhibition zone obtained at concentrations of 0.25 ppm, 0.50 ppm, 0.75 ppm, 1 ppm, 1.25 ppm, 1.50 ppm, and positive control were 0.33 cm, 0.38 cm, 0.38 cm, 0.33 cm, 0.36 cm, 0.32 cm, and 0.30 cm, respectively. The mycelium growth rate of the fungus in the negative control had the highest growth rate with a value of 0.81 cm/day. Meanwhile, the growth rate of ketoconazole 0.25 ppm as a positive control produced the lowest growth rate, which was not significantly different with all fraction-1 treatments.

Ketoconazole is a compound that acts as antifungal agent by inhibiting sterol synthesis in cell membranes. This compound can increase cell wall permeability, making it susceptible to osmotic pressure, and can inhibit the performance of oxidase and peroxidase enzymes in cell. Ketoconazole has the ability to inhibit the formation of blastopores into mycelia [16].

Meanwhile, the lowest growth rate in the treatment given 1.50 ppm *S. multijuga* bark fraction was 0.32 cm/day. This shows that concentration of 1.50 ppm gives results close to the positive control. The higher concentration of fraction-1 from the *S. multijuga* bark showed the greater inhibitory effect produced. This is because the amounts of active fraction in it also increases. An increase in concentration results in a greater inhibitory effect.

Antifungal activity tests have shown that fraction-1 from *S. multijuga* bark can inhibit the growth of *G. boninense* mycelium. GC-MS analysis results showed the presence of various compounds of 1-hexadecene, 1-octadecene, hexadecanoid acid, 9,12-octadecadienoic acid, 9,12,15-octadecatrienoic acid, 2-hexadecen-1-ol, 1-docosene, 2-ethoxycyclohexanol, 2-pyridol, 1,5-cyclodecadiene, and calotropenol acetate. These compounds are known to have antifungal activity through various mechanisms, mainly cell membrane damage [17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27], inhibit cell respiration, cell wall synthesis, and cell division [28, 29, 30].

Inhibition Activity *Ganoderma boninense*

The results of the variance analysis show that the treatment of fraction-1 of *S. multijuga* bark has no significant effect on the inhibitory effect on *G. boninense* fungus. The results of the 5% BNT test on the inhibitory effect on *G. boninense* can be seen in Table 3.

Table 3. Results of the BNT test on the inhibitory effect on *Ganoderma boninense*

Treatment	**Inhibition (%)**
K (+) (*ketoconazole* 0,25 ppm)	74,33 ± 5,92[a]
P6 (1,50 ppm)	67,46 ± 4,37[a]
P1 (0,25 ppm)	66,20 ± 8,00[a]
P4 (1 ppm)	64,87 ± 6,98[b]
P2 (0,50 ppm)	63,00 ± 4,44[b]
P3 (0,75 ppm)	62,89 ± 4,44[b]
P5 (1,25 ppm)	62,60 ± 10,00[b]
K (-) (distilled water)	9,81 ± 0,00[c]

BNT (0,05) = 9,81
Note: The average growth rate values in columns with the same letter are not significantly different in the 5% BNT test.

Table 3 shows that the highest inhibitory effect of fraction-1 against *G. boninense* was observed in the positive control ketoconazole at a concentration of 0.25 ppm, with an inhibition of 74.33%. This was not significantly different from treatments P1 and P6 but was significantly different from K (-). The lowest inhibition was obtained in treatment K (-), which was 9.81%. K (-) was a negative control consisting distilled water, so there were no compounds that could inhibit the growth of *G. boninense*. Meanwhile, the highest inhibition was observed in the treatment with the addition of fraction-1 at a concentration of 1.50 ppm, with an inhibition of 67.46%. The 1.5 ppm treatment showed results closest to K (+). The 1.50 ppm concentration can effectively suppress the growth of *G. boninense* fungus because it contains more active fraction such as fatty acids, aliphatic hydrocarbons, alcohols, esters, and aromatic compounds. The higher the concentration of fraction-1 from *S. multijuga* bark given in the treatment, the higher the effectiveness of the extract in inhibiting fungal growth.

The growth inhibition values obtained were classified into a table of fungal growth inhibition percentage categories based on the average growth rate results. The results of the classification of the growth inhibition of *G. boninense* from all treatments can be seen in Table 4.

Table 4. Growth inhibition of *Ganoderma boninense*

Treatment	**Growth Inhibition (%)**
P1 (0,25 ppm)	59,26
P2 (0.50 ppm)	53,09
P3 (0,75 ppm)	53,09
P4 (1 ppm)	59,26
P5 (1,25 ppm)	55,56
P6 (1,50 ppm)	60,49
K (-) (distilled water)	0,00
K (+) (*ketoconazole* 0,25 ppm)	62,96

Based on the growth rate of *G. boninense*, treatment of fraction-1 showed inhibition of *G. boninense* growth by 53.09-60.49% (Table 4). The application of fraction-1 at a concentration of 1.50 ppm resulted in the highest inhibition of *G. boninense* growth at 60.49%, which was relatively similar to the positive control ketoconazole at 62.96%. This inhibition of *G. boninense* growth occurred due to indications of the activity of alkenes, fatty acids, fatty alcohols, esters, aromatic dicarboxylic acids, aromatic heterocyclic compounds, terpenoids, flavonoids, and phenolics contained in fraction-1 of *S. multijuga*. This is in line with previous research conducted by [32], which stated that *Senna* plants contain flavonoids and phenolics that can be used as therapeutic treatments for diseases and disorders caused by oxidative stress, allergic reactions, and pathogen invasion. The inhibitory effect of fraction-1 of *S. multijuga* bark against *G. boninense* fungus is presented in Figure 6.

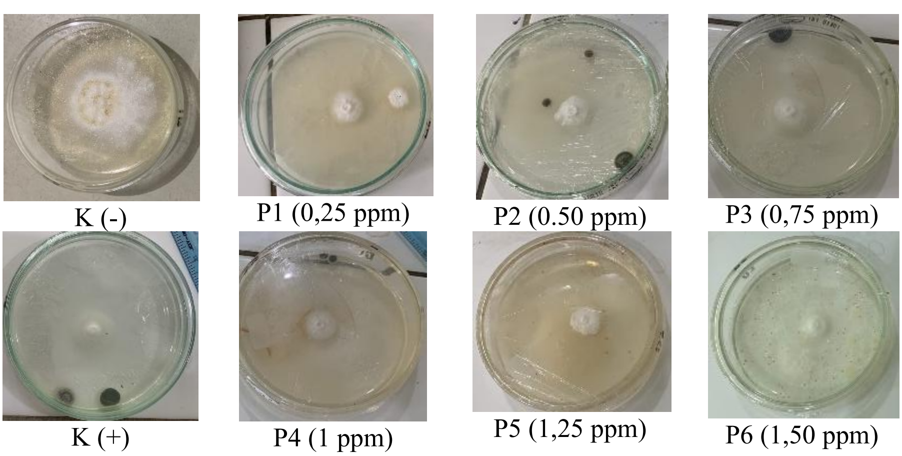

Figure 6. Effectiveness of fraction-1 of *Senna multijuga* bark against *Ganoderma boninense* 10 days after inoculation

The inhibition of *G. boninense* growth occurs due to the presence of active fraction. The compounds in fraction-1 from *S. multijuga*, including 1-hexadecene, 1-octadecene, hexadecanoid acid, 9,12-octadecadienoic acid, 9,12,15-octadecatrienoic acid, 2-hexadecen-1-ol, 1-docosene, 2-ethoxycyclohexanol, 2-pyridol, 1,5-cyclodecadiene, and calotropenol acetate, also have an inhibitory mechanism that works by disrupting cell membranes [17, 18, 20, 21], inhibiting protein synthesis and cell wall synthesis [26, 31].

Morfologi Makroskopis G. boninense

Macroscopic morphological data can be seen in Figure 6. The results showed that there were macroscopic differences between G. boninense fungi in the negative control, positive control, and fraction-1 treatment groups in terms of G. boninense growth after 10 days of incubation. *G. boninense* fungi in the negative control treatment had thick, fine white thread-like mycelium with a wider mycelium distribution pattern that almost filled the petri dish. Meanwhile, *G. boninense* fungi treated with fraction-1 and negative control had thin, fine white thread-like mycelium with a smaller distribution pattern and slow growth. This is in line with research conducted by [32] that the *Senna* plants contain flavonoids and phenols that can inhibit antimicrobial and antifungal activity. The macroscopic morphological of *G. boninense* can be seen in Table 5.

Table 5. Macroscopic morphology of *G. boninense* fungus (Day 10 after inoculation)

Treatment	Color	Appearance	Spread pattern
P1 (0,25 ppm)	Thin white mycelium	flat colony edge	gather in the middle
P2 (0.50 ppm)	Thin white mycelium	flat colony edge	gather in the middle
P3 (0,75 ppm)	Thin white mycelium	flat colony edge	gather in the middle
P4 (1 ppm)	Thin white mycelium	flat colony edge	gather in the middle
P5 (1,25 ppm)	Thin white mycelium	flat colony edge	gather in the middle
P6 (1,50 ppm)	Thin white mycelium	flat colony edge	gather in the middle
K (-) (distilled water)	Thick white mycelium	flat colony edge	Spreading in all directions
K (+) (*ketoconazole* 0,25 ppm)	Thin white mycelium	flat colony edge	gather in the middle

4. Conclusion

Based on the results of GC-MS analysis of fraction-1 from *Senna multijuga* bark, the main components identified were 1-hexadecene (5.51%), 1-octadecene (8.29%), hexadecanoid acid (10.46%), 2-ethoxycyclohexanol (5.53%), 9,12-octadecadienoic acid (8.91%), 9,12,15-octadecatrienoic acid (7.70%), 2-hexadecen-1-ol (5.48%), 1-docosene (3.33%), 2-pyridol (6.28%), and calotropenol acetate (6.52%). A concentration of fraction-1 at 1.50 ppm can inhibit the growth of *G. boninense* (0.32 cm/day), inhibition of *G. boninense* (67,46%), and produce thin white mycelium that collects in the middle of the medium.

Acknowledgments: We express our gratitude to the University of Lampung, for providing Hibah BLU Unila 2025. We also extend our thanks to PT Sampoerna Agro Tbk, Jakarta, Indonesia, for providing materials of *S. multijuga*, we thank Silva Abraham, at the Directorate of Scientific Collection Management, National Research and Innovation Agency (BRIN), Cibinong, Bogor, West Java, Indonesia, for conducting the morphological identification of the *S. multijuga* plants.

References

1. J.H.V. Purba, T. Sipayung, Perkebunan kelapa sawit Indonesia dalam perspektif pembangunan berkelanjutan. Jurnal Masyarakat Indonesia. **43**, 81-94 (2017) [Google Scholar]
2. D.P. Rahayu, E. Suroso, Subeki, R. Suhardjo, S. Rizal, Uji in vitro daya hambat ekstrak kulit *Senna multijuga* terhadap jamur *Ganoderma boninense*. Jurnal Agroindustri Halal. **11**, (2025) [Google Scholar]
3. Y.W. Khoo, K.P. Chong, *Ganoderma boninense*: general characteristics of pathogenicity and methods of control. Frontiers in Plant Science. **14**, (2023) [Google Scholar]
4. R. Wandri, S. Alam, S.D. Ayundra, A. Apriansa, A. Asmono, Subeki, Y. Fitriana, R. Hasibuan, R. Suharjo, First report: *Senna multijuga* Subsp. *multijuga* (Fabales: *Fabaceae*) as an attractant and bioinsecticide for *Oryctes rhinoceros* (Coleoptera: *Scarabaeidae*). Agriculture. 14,1477 (2024) [Google Scholar]
5. K. Bene, K.I. Sinan, G. Zengin, A. Diuzheva, J. Jeko, Z. Cziaky, M.Z. Aumeeruddy, J. Xiao, M.F. Mahomoodally, A multidirectional investigation of stem bark extracts of four African plants: HPLC-MS/MS profiling and biological potentials. Journal of Pharmaceutical and Biomedical Analysis. **168**, 217–224 (2019) [Google Scholar]
6. O.S. Oladeji, F.E. Adelowo, A.P. Oluyori, The genus *Senna* (*Fabaceae*): A review on its traditional uses, botany, phytochemistry, pharmacology and toxicology. South African Journal of Botany. **138**, 1-32 (2021) [Google Scholar]
7. S. Oladeji, F.E. Adelowo, A.P. Oluyori, The genus *Senna* (Fabaceae): A review on its traditional uses, botany, phytochemistry, pharmacology and toxicology. South African Journal of Botany. **138**, 1-32 (2021) [Google Scholar]
8. M.A. Ibrahim, M.S. Islam, Anti-diabetic effects of the acetone fraction of *Senna singueana* stem bark in a type 2 diabetes rat model. Journal of Ethnopharmacology. **153**, 392-399 (2014) [Google Scholar]
9. P, Aditya, W. Lestari, K.D. Sitanggang, I.A.P, Septiyani. Isolasi dan karakteristik jamur dari *Ganoderma* di Desa Pernantian Perkebunan PT. Umada. Jurnal Pengabdian Kepada Masyarakat. **14,** 167-172 (2024) [Google Scholar]
10. P. Sansores-Perazaa, M. Rosado-Valladoa, W. Brito-Loezaa, G.J. Mena-Rejon, L. Quijano, Cassine, an antimicrobial alkaloid from *Senna racemosa*. Fitoterapia. 71, 690-692 (2000) [Google Scholar]
11. M.N. Nascimento, M.M. Martins, L.C. Cunhac, P. Santos, L.P. Goulart, T. Silva, C.H.G. Martins, S.A. de Morais, M. Pivatto, Antimicrobial and cytotoxic activities of *Senna* and *Cassia species (Fabaceae)* extracts. Industrial Crops and Products. **148**, 112081 (2020) [Google Scholar]
12. I. Fletcher, A. Freer, A. Ahmed, P. Fitzgerald, Effect of temperature and growth media on mycelium growth of *Pleurotus ostreatus* and *Ganoderma lucidum* strains. *Cohesive* Journal of Microbiology and Infectious Disease. **2**, 1–5 (2019) [Google Scholar]
13. E. Sulyanti, Yaherwandi, R.M. Ulindari, Aktivitas air rebusan beberapa kulit jeruk (*Citrus spp*) untuk menekan pertumbuhan *Colletotrichum gloeosporioides* pada tanaman buah naga secara *in vitro*. Jurnal Proteksi Tanaman. **3**, 56-64 (2019) [Google Scholar]

14. S.R. Susanti, S. Kusmiadi, S.N. Aini, Uji efikasi ekstrak daun mengkudu, kemangi dan jambu biji dalam menghambat pertumbuhan cendawan *Colletotrichum gloeosporioides* pada buah pepaya. Jurnal Agrosaintek. **1**, 16-22 (2017) [Google Scholar]
15. S.R.Maji, C. Roy, S.K. Sinha, Gas chromatography–mass spectrometry (GC-MS): a comprehensive review of synergistic combinations and their applications in the past two decades. J. Anal. Sci. Appl. Biotechnol. **5**, 72-85 (20230 [Google Scholar]
16. H.V. Bossche, G. Willemsens, P. Marichal, Antifungal activity of ketoconazole. Reviews of Infectious Diseases. **2**, 570–588 (1980) [Google Scholar]
17. S.E. Anderson, R. Meier, Potential mechanisms of acrylate toxicity and allergic contact dermatitis. Chem. Res. Toxicol. **29**, 2079–2090 (2016) [Google Scholar]
18. R. Khan, B. Islam, M. Akram, S. Shakil, A.A. Ahmad, S.M. Ali, M. Siddiqui, A.U. Khan, Antimicrobial Activity of Five Herbal Extracts Against Multi-Drug Resistant (MDR) Strains of Bacteria and Fungus of Clinical Origin. Molecules. **14**, 586–597 (2009) [Google Scholar]
19. Y. Ghasemi, A. Mohagheghzadeh, M.A. Ebrahimzadeh, Antifungal activity of some plant essential oils against *Candida albicans*. Pak. J. Pharm. Sci. **33**, 247-252 (2020) [Google Scholar]
20. A.P. Desbois, V.J. Smith, Antibacterial free fatty acids: activities, mechanisms of action and biotechnological potential. Appl. Microbiol. Biotechnol. **85**, 1629–1642 (2010) [Google Scholar]
21. D. Walters, L. Raynor, A. Mitchell, R. Walker, K. Walker, Antifungal activities of four fatty acids against plant pathogenic fungi. Mycopathologia. **157**, 87–90 (2004) [Google Scholar]
22. D. Walters, R.L. Walker, K.C. Walker, Lauric acid exhibits antifungal activity against plant pathogenic fungi. J. Phytopathol. **151**, 228–230 (2003) [Google Scholar]
23. N.C.C. Silva, Fernandes Júnior A, Biological properties of medicinal plants: a review of their antimicrobial activity. J. Venom. Anim. Toxin.s Incl. Trop. Dis.**16**, 402–413 (2010) [Google Scholar]
24. Y. Zhou, S. Wang, H. Lou, P. Fan, Chemical constituents and antimicrobial activity of volatile oil from *Cassia tora* leaves. Molecules. 24, 1–12 (2019) [Google Scholar]
25. Y. Zhang, Z. Li, S. Wei, C. Xu, M. Chen, Y. Sang, Y. Han, Y. Yan, Z. Li, Z. Cui, X. Ye, Antifungal Activity and Mechanisms of 2-Ethylhexanol, a Volatile Organic Compound Produced by *Stenotrophomonas* sp. NAU1697, against *Fusarium oxysporum* f. sp. *Cucumerinum*. J. Agric. Food. Chem. **72**, 15213-15227 (2024) [Google Scholar]
26. J.S. Raut, S.M. Karuppayil, A status review on the medicinal properties of essential oils. Ind Crops Prod. **62**, 250–264 (2014) [Google Scholar]
27. F. Silva, S. Ferreira, J.A. Queiroz, F.C, Domingues. Coriander (*Coriandrum sativum* L.) essential oil: its antibacterial activity and mode of action evaluated by flow cytometry. J Med Microbiol. **60**, 1479–1486 (2019) [Google Scholar]
28. J. Zhang, L. Li, S.H. Kim, A.E. Hagerman, J. Lü, Anti-cancer, anti-diabetic and other pharmacologic and biological activities of penta-O-galloyl-β-D-glucose. Pharm. Res. **26**, 2066–2080 (2009) [Google Scholar]
29. R.N. Roy, S. Laskar, S.K. Sen, Dibutyl phthalate, the bioactive compound produced by Streptomyces albidoflavus 321.2. Microbiol. Res. **161**, 121–126 (2006) [Google Scholar]
30. L. Das, T. Sengupta, An overview of synthesis and biological activities of 2-pyridone derivatives over the last decade. Discover Applied Sciences. **7**, 1069 (2025) [Google Scholar]

31. S. Kumar, N. Kaushik, R, Edrada-Ebel, P. Proksch, Isolation, characterization, and bioactivity of phthalic acid derivatives from *Phomopsis* sp. Nat Prod Commun. 6, 1231–1234 (2011) [Google Scholar]
32. M.M. Alshehri, C. Quispe, J. Herrera-Bravo, J. Sharifi-Rad, S. Tutuncu, E.F. Aydar, C. Topkaya, Z. Mertdinc, B. Ozcelik, M. Aital, N.V.A. Kumar, N. Lapava, J. Rajkovic, A. Ertani, P. Nicola, P. Semwal, S. Painuli, C. González-Contreras, M. Martorell, M. Butnariu, I.C. Bagiu, R.V. Bagiu, M.D. Barbhai, M. Kumar, S.D. Daştan, D. Calina, W.C. Cho, A review of recent studies on the antioxidant and anti-infectious properties of *Senna* plants. Oxidative Medicine and Cellular Longevity. 6025900 (2022) [Google Scholar]