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Trichoderma hamatum derived from coffee plant (*Coffea canephora*) rhizosphere inhibit *Candida albicans* Growth

Yuyun Nisaul Khairillah[1], Nampiah Sukarno*[1,2], Irmanida Batubara[2,3]

[1]Biology Department, Faculty of Mathematics and Natural Sciences, IPB University, Indonesia
[2]Tropical Biopharmaca Research Center, IPB University, Indonesia
[3]Chemistry Department, Faculty of Mathematics and Natural Sciences, IPB University, Indonesia
*Corresponding E-mail: nampiah@apps.ipb.ac.id

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Abstract. The *Trichoderma hamatum* produces various secondary metabolites that can be used as anti-*Candida albicans*. This research aimed to isolate *T. hamatum* from the coffee (*Coffea canephora*) rhizosphere and analyze the fungal compounds to control the pathogenic fungus *C. albicans*. *T. hamatum* was isolated using the dilution method, and the fungal identification was used combining morphological and molecular characteristics of ITS rDNA. The potency of *T. hamatum* as anti-*C. albicans* was determined by antagonist test using the double-layer method, while for culture filtrate, ethyl acetate and *n*-hexane filtrate extracts were carried out by the agar diffusion method. The compounds in the most active extract were analyzed by Gas Chromatography-Mass Spectrometry (GC-MS). *T. hamatum* inhibits *C. albicans* growth in antagonistic and filtrate assays with 15.53 mm and 14.40 mm of inhibition zone, respectively. It indicated that both culture and fungal filtrate had similar activities on *C. albicans*. The ethyl acetate extract (minimum inhibitory concentration, MIC of 0.50%) showed more potent against *C. abicans* than *n*-hexane extract (MIC of 15.00%). The potential active compound in the ethyl acetate extract would be 9-Octadecenoic acid (Z) methyl ester. The 9-Octadecenoic acid (Z) methyl ester could be used as an alternative candidate to control *C. albicans*.

Key words: clear zone, filtrate extract, ITS rDNA, *Trichoderma hamatum, 9-Octadecenoic acid (Z) methyl ester*

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INTRODUCTION

T. hamatum is a saprophytic fungus that is commonly found in litter, humus, and plant rhizosphere. The fungus has a wide distribution in nature and lives as cosmopolitan. *T. hamatum* is reported to have various anti-microbial mechanisms, including the production of β-glucanase and chitinase, which act as hydrolytic enzymes during cell wall degradation (Cheng et al., 2015). The fungus is also known to produce gliotoxins (an active compound against *Aspergillus fumigatus*) (Scharf et al., 2016), siderophores (an anti-microbial compounds) (Lehner et al., 2013), 6-pentyl-alpha-pyrone (6PP) (a volatile metabolite compound which could damaging the cell walls of *C. albicans*) (Jelen et al., 2014), and peptaibol (compound which could inhibit *C. albicans* growth) (Sadykova et al., 2015). Therefore this fungus is used as a biocontrol agent (Kandula et al., 2015).

C. albicans is an opportunistic fungal pathogen that causes candidiasis. In general, there are three types of candidiasis, namely superficial candidiasis that attacks the skin, mucocutaneous candidiasis that attacks oral mucosa and vaginal epithelium (Monroy et al., 2016), and systemic candidiasis that infects internal organs (Tsui et al., 2016). Besides being able to infect parts of the human organs, *C. albicans* can also cause disorders of the immune system. About 75% of women worldwide are infected with *Candida*, at least once in their lifetime, but only about 5% cause recurrent infections (Ferrer, 2000). Until to date, candidiasis is treated by a ketoconazole-type drug from the azole group. However, long-term use of this class of drugs can cause some strains of *C. albicans* to become resistant (Monroy et al., 2016). Therefore, it is important to find new alternative bioactive sources for controlling *C. albicans* to overcome their resistance.

The fungus *T.hamatum* has various mechanisms controlling pathogenic fungi, including producing enzymes that damage cell wall permeability and volatile metabolite compounds that have activity against pathogenic fungi by destroying the hyphal cell wall components, such as polysaccharides and chitin. Therefore, this study aimed to characterize the *T. hamatum* isolated from the robusta coffee plant rhizosphere in inhibiting *C. albicans* growth and determine its suggested active compounds as *C. albicans.*

METHOD

Sampling Site

Rhizosphere soil used to isolate *T. hamatum* were collected from robusta coffee plantations in Mekarsari Village, Kepahiang Regency, Bengkulu Province, Indonesia. The sampling site is at S 02º 36'413" longitude and E 960º 24'430" latitude, and 1.786 masl altitude. The plantation has an average rainfall of 233.5 mm/month, an average daily temperature of 23.87 ºC, and 19.65 ºC and 29.87 ºC of minimum and maximum temperatures, respectively.

Isolation of the fungus *T. hamatum* from the robusta coffee plant rhizosphere

The fungal isolation was carried out from the rhizosphere soil of the robusta coffee plant using a serial dilution technique from 10^{-3} to 10^{-5} (Elias et al., 2016). Ten grams of the soil was put into an Erlenmeyer containing 90 ml of sterile distilled water. The sample was shaken at a speed of 120 rpm for 20 minutes and then serially diluted from 10^{-1} to 10^{-5} by sterile distilled water. One ml of the sample of each dilution from 10^{-3} to 10^{-5} was taken and spread on 50% PDA medium containing rose bengal (30 mg.l^{-1}) and chloramphenicol antibiotic (0.5 g.1^{-1}) in the Petri plate. The plates were incubated for 7 to 10 days at 28 ºC. Then, the pure isolates were subjected to identification.

Morphological and molecular identification of the fungus *T. hamatum*

The morphological identification of the fungus was carried out based on the identification key of Barnett and Hunter (1998) and Miles in 1997 using the Riddle method (Riddell, 1950). The morphological characters used in the identification were colony color, colony texture, septate or aseptic hyphae, conidiophores shape, spore characteristics such as color, size, and shape. Molecular identification was carried out using DNA sequence of the ITS1 - 5.8S - ITS2 region of rDNA, with primer pairs of ITS1 (forward) (5'-CTTGGTCATT TAGAGGAAGTAA3') and ITS4 (reverse) (5′TCCTCCGCTTATTGATATGC-3′) (White et al., 1990). Isolation of fungal genome DNA using the CTAB method (Sambrook & Russell, 2013). The fungus was grown on a cellophane membrane placed on a PDA medium for seven days at room temperature. The mycelium was taken with a sterile spatula at harvest time, then put into a 1.5 ml sterile Eppendorf tube containing 500 µl CTAB. The mycelium was ground using a sterile pestle and incubated in a water bath at 65 ºC for 30 minutes. Then the ice shock process was carried out for 5 minutes after adding a 500 µl C: I solution and then inverted. Then the solution was centrifuged at 10.000 rpm for 10 minutes. Then the upper phase was taken, and 500 µl of P:C:I solution was added and centrifuged for 10 minutes at 10.000 rpm. The upper stage was taken and then added 50 µl of NaOAc solution and 100 µl of EtOH with a concentration of 100% and left for 12 hours. Then the solution was centrifuged at 10.000 rpm for 30 minutes.

The upper phase was added 500 µl of EtOH with a concentration of 70%, centrifuged for 10 min at 10.000 rpm, and dried for 30 minutes. Then the solution was added by 0.2x vol RNAse, and 50 µl ddH_2O and then incubated for 10 minutes at 70º C. The isolated DNA quality was checked by 1% agarose gel electrophoresis, which was run at 100 volts for 30 minutes in a 1× TAE buffer solution. The DNA was stored in the freezer until used. The agarose gel was immersed in EtBr solution for 15 minutes, rinsed with distilled water, and observed using a UV transilluminator. Samples showing a single band on 1% agarose gel were used to amplify ITS (*Internal Transcribed Spacers*) region using a PCR machine.

Amplification of the ITS rDNA region was carried out with a total volume of 30 µl, containing 100 ng of template genomic DNA, 15 µl of 2x PCR master mix, and 0.9 pmol of each primer. PCR amplification for each primer was carried out under pre-denaturation conditions at 94 °C (5 minutes), denaturation at 94 °C (30 seconds), annealing with adjustment of optimization results at 55 ℃ (1 minute), elongation at 72 °C (2 minutes), and post-elongation at 72 °C (7 minutes) with 35 cycles. The PCR results were visualized by electrophoresis on 1.2% (w/v) agarose gel in 1x TAE (0.04 M tris-acetate and 0.001 M EDTA), and immersion of the gel in 0.5 g/ml ethidium bromide. Observations were made on DNA bands on 1.2% agarose gel placed on a UV transilluminator and equipped with a digital camera. The standard size used was a Benchtop DNA ladder 100 bp marker (Promega®, U.S.A.) (10ng/µL). The PCR results were sent to Genetika Science for DNA sequencing. The sequencing results were then analyzed and determined for homology with the types of species available in GenBank and Mycobank (http://www.mycobank.org) through the Basic Local Alignment Search Tool (BLAST) program available at http://blast.ncbi.nlm.nih.gov. The fungal phylogenetic tree was created using the Mega five program with bootstrap values calculated from 1000 replicates (Tamura et al., 2007).

The extraction of *T. hamatum* culture filtrate

The *T. hamatum* was grown in a PDB medium for 21 days using a shaking incubator at 125 rpm and

room temperature. The filtrate culture was extracted using two solvents separately, namely *n*-hexane (1:1) v/v and ethyl acetate (1:1) v/v. Each extract was concentrated with a rotary vacuum evaporator. The yield of each extract was determined by dividing the weight of dried extract by the filtrate volume.

Anti-C. albicans* activity test of *T. hamatum

The activity of *T. hamatum* culture on *C. albicans* was determined using antagonist double layer methods. About 1 ml of *C. albicans* culture on PDB (106 CFU/ml*)* was inoculated into 99 ml of PDA medium at 37 °C. The solidified medium containing *C. albicans* was incubated for 24 hours at 37 °C. The clear zone around the colony of *T.hamatum* was measured (Tendencia, 2004).

***Anti-C. albicans* activity test of *T. hamatum* filtrate culture and filtrate extracts**

The agar diffusion method was used in this step. The extracts and nystatin positive control (100.000 U/ml) were dissolved separately in dimethylsulfoxide (DMSO) 10% before the activity test. About 20 µl of each filtrate and extract with a concentration of 0.5% - 80% was separately dropped onto 6 ml sterile paper discs on the surface of PDA medium containing *C. albicans* and incubated at 37°C for 24 hours. The minimum inhibitory concentration (MIC) is determined based on the lowest concentration that shows inhibition (Birch & Ruddat, 2005). The negative control treatment used was 10% DMSO (Horváth et al., 2005).

Fungal secondary metabolite compound determination using gas chromatography-mass spectrometry (GC-MS)

The culture filtrate of *T. hamatum* was extracted by two solvents separately, semi-polar solvent (ethyl acetate) and polar solvent (*n*-hexane). GC-MS was used to identify the prospective compounds in the active extracts. About 5 ml extract was injected into the Shimadzu GCMS QP 2010 Ultra instrument. Conditions used include: mobile phase: Helium; detector; FID; capillary column Rtx-5MS (60 m; 0.25 mm, ID); column temperature: 50 °C; inlet pressure 100 kPa; flow rate 0.85 ml/min; split ratio 112.3; injector temperature 230°C, ion source temperature 200°C; interface temperature 270°C and split 20. Separation was carried out with a programmed temperature of 50℃ for 3 minutes and then increased rate of 5 °C /minute until 150 °C, the final temperature of the column was 270 °C for 2 minutes with a rate of increase of 3 °C /minute. The mass spectrometer was operated in electron ionization mode at 70 eV with a temperature of 200 °C. The mass spectra were then compared with the NIST 11 mass spectrometry database (Narayana et al., 2008).

Data analysis

The activity against *C. albicans* data of the filtrate extract were compared with positive and negative control treatments using the Analysis of Variance (ANOVA) method with the SPSS 24 program. In addition, significant differences between treatments were followed up with the Duncan Multiple Range Test (DMRT) at a level of 5% ($p<0.05$).

RESULTS AND DISCUSSION

Morphological and molecular identification of the fungus isolated from robusta coffee rhizosphere

Based on morphological characteristics, the fungus was identified as *Trichoderma* sp. TN-103-1. The morphological characteristics of the fungus as follows: having a spherical colony with the initial color of the colony surface white at the beginning then gradually becomes light green, at the seventh-day growth becomes a bit dark green with clear borders on the white edges. The reverse colony was yellowish-white. Colony surface texture is granular or rough. It has septate hyphae with a diameter of 2.32-3.49 µm. The conidiophores are branched with phialide round to elliptical bearing conidium that forms in a cluster at the conidiophores' ends. The size of phialides is 11.12-12.31 x 3.12-3.25 µm and conidium size is 1.8-3.1 x 1.7-3.8 µm (Figure 1).

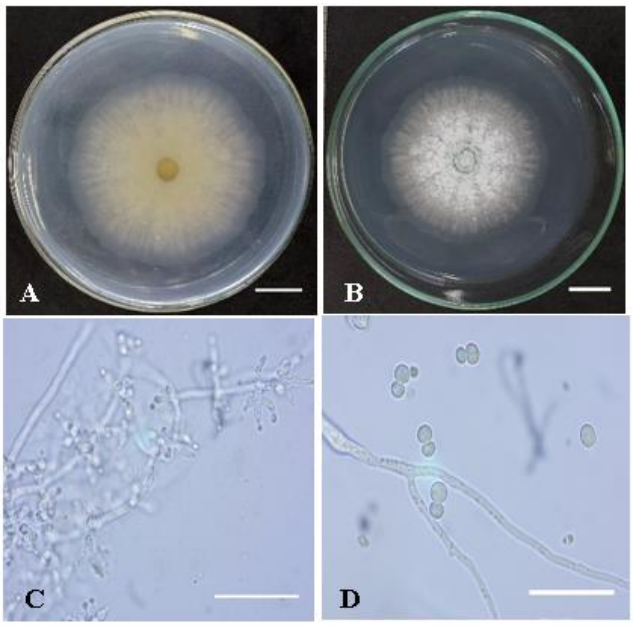

Figure 1. Colony and microscopic characteristics of *T. hamatum* TN-103-1 grown on PDA at 28 ± 4 °C for 7 days incubation. Reverse colony (A), surface colony (B), conidiophores and phialides (C), conidia (D). Scale = 1 cm (A–B), 20 □m (C–D).

Table 1. BLAST analysis of *T. hamatum* TN-103-1 DNA sequences of ITS1-5.8S-ITS2 rDNA.

Isolate	Identification of the fungus	GenBank reference access number	Maximum score	Query cover	Identity	E value
Trichoderma sp. TN-103-1	*T. hamatum*	KF856960.1	1120	100%	100%	0.0

Further identification using DNA sequences of ITS1-5.8S-ITS2 rDNA by BLAST revealed that the fungus has 100% similarity to that of *T. hamatum* with GenBank accession number KF856960.1 (Table 1). Therefore, the fungus obtained in this research is identified as *T. hamatum* TN-103-1. The results of BLAS are supported by that of phylogenetic analysis using *F. acuminatum* strain CBS 131258 as an outgroup. All *Trichoderma* species used for phylogenetic analysis formed a separate clade with the outgroup *F. acuminatum* strain CBS 131258, and the *T. hamatum* TN-103-1 is located in the same clade with all the *Trichoderma* species analyzed. Therefore, it indicates that the phylogenetic tree constructed is suitable. Furthermore, the *T. hamatum* TN-103-1 has the closest relationship with *T. hamatum* DOAM 167057 as a type species reference with a bootstrap value of 95%. Furthermore, *T. hamatum* TN-103-1 is in the same clade with *T. asperellum* with a bootstrap value of 94% (Figure 2).

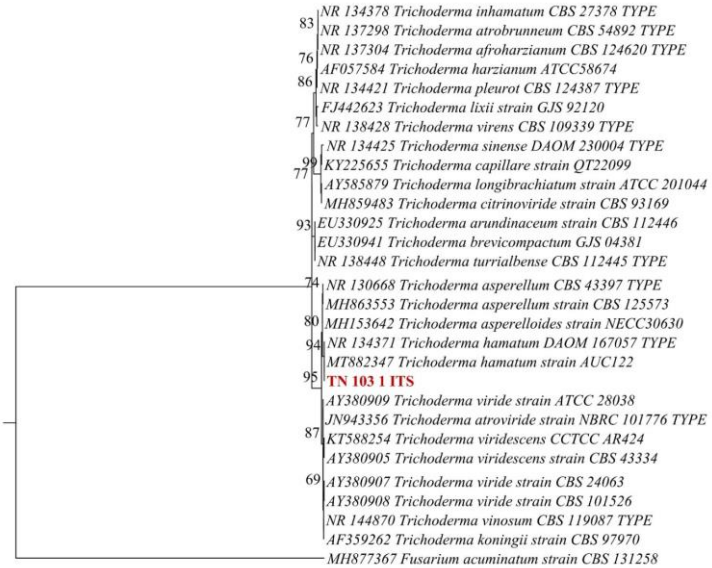

Figure 2. Phylogenetic tree of the fungus *T. hamatum* TN 103-1 based on sequences of ITS rDNA with *F. acuminatum* strain CBS 131258 as outgroup

Antagonistic activity of *T. hamatum* against *C. albicans*

The *T. hamatum* TN-103-1 inhibited the growth of *C. albicans* with an inhibition zone diameter of 15.53 mm (Figure 3). This value is classified as strong inhibition based on the classification reported by Rundengan et al. (2017). Furthermore, the *T. hamatum* TN-103-1 showed better inhibition on *Candida* compared with other species of *Trichoderma* such as *T. viride* isolated from cucumber rhizosphere reported by Awad et al. (2018). The inhibition of *T. hamatum* TN-103-1 was almost twice that of *T. viride.*

The genus *Trichoderma* is known to produce extracellular enzymes that suppress the growth of pathogenic fungi. The enzymes included 1,3-glucanase, cellulase, and chitinase, which can cause lysis and destruct pathogenic fungal hyphal walls. These enzymes play a role in the mycoparasites process, and associated with the cell wall skeleton which composed by chitin, glucan, and protein. *Trichoderma* spp. was reported to synthesize bioactive compounds such as 6-pentyl-2H-pyran-2-one (6-PP), which inhibit pathogenic bacteria (Mutawila et al., 2016). Bioactive compounds such as the 6-pentyl-α-pyrone (6PP) produced by *Trichoderma* spp. are known to have antifungal activity. The 6-pentyl-α-pyrone (6PP) bioactive compound inhibits the production of metabolites released by the pathogenic fungus (Jelen, 2003). In addition, *T. hamatum* was reported to produce antibiotics such as gliotoxin and viridine, which play a role in inhibiting the growth of pathogenic fungi (Mallikharjuna et al., 2016).

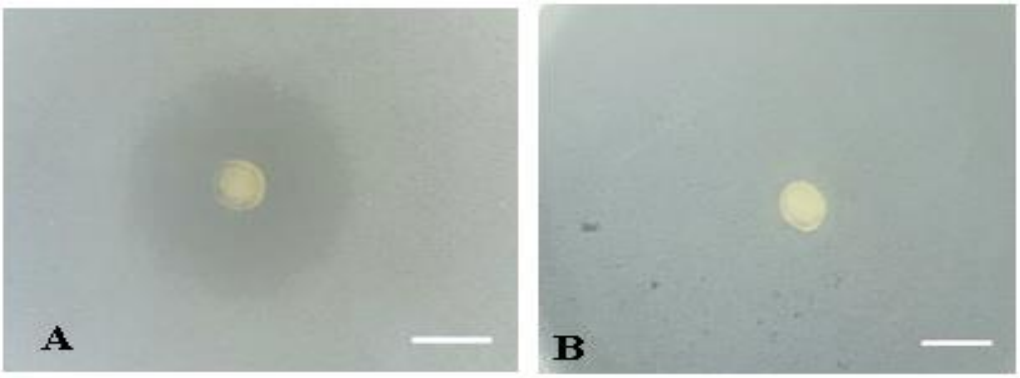

Figure 3. Inhibitory activity of *T. hamatum* TN-103-1 isolated from robusta coffee rhizosphere against *C. albicans* (A) and control treatment (B) showing by a clear zone. Scale = 0.5 cm

Culture filtrate and extracts of *T. hamatum* activities against *C. albicans*

The culture of *T. hamatum* TN-103-1 activity was studied to determine bioactive compounds' nature, whether they are extracellular or intracellular compounds. The culture filtrate of *T. hamatum* TN-103-1 inhibited the growth of *C. albicans* with a clear zone diameter of 14.40 mm (Table 2). It indicated that the active compounds were extracellular metabolites secreted by the fungal cells. The inhibition value of culture filtrate is not significantly different at the 5% confidence level with the inhibition zone of *T. hamatum* in the antagonist test carried out in this study (Figure 4). The results also showed that the *T.hamatum* TN-103-1 has better activity than the positive control, nystatin (100.000 U/mL), a commercial drug to control *C. albicans* infection.

Table 2. Growth inhibition activity of *T. hamatum* TN-103-1 culture filtrate on *C. albicans*

Sample name	Inhibition diameter (mm) ± SE.
Culture filtrate of *T.hamatum* TN-103-1	14.40±1.67b
Positive control (nystatin, 100.000 U/mL)	11.80±0.62bc
Negative control (DMSO 10%)	0.00±0.00a

Note: the number followed by the same letter shows an insignificant result based on DMRT at 5%.

Nystatin inhibits pathogenic fungi growth by binding to sterols, especially ergosterol, on fungal cell membrane, hence disrupting the membrane permeability and transport system. The formation of a polyenergosterol complex resulted in leakage, which causes the release of essential components of the fungal cell and, hence, the growth of pathogenic fungi (Lyu et al., 2016). The negative control, 10% DMSO had no inhibition effect on the growth of *C. albicans*. Our finding agrees with that of Awad et al. (2018) who reported that bioactive compounds obtained from *T. viride*as culture filtrate had *C. albicans* activity. The culture filtrate was extracted using two different solvents, ethyl acetate, and *n*-hexane, separately. The yield of ethyl acetate and *n*-hexane extracts were 0.94% (w/v) and 0.80% (w/v), respectively. The yield of the ethyl acetate extract was higher than that of the *n*-hexane, indicating that *T. hamatum* produced more semi-polar compounds compared to that of non-polar compounds.

Both ethyl acetate and *n*-hexane extracts of *T. hamatum* TN-103-1 inhibited *C. albicans* growth. At the concentration of 15%, the ethyl acetate extract tended to have a higher diameter inhibition than that of *n*-hexane extract, even though the difference was not statistically significant (Figure 4, Table 3). In addition, the minimum inhibitory concentration (MIC) of the ethyl acetate extract was obtained at 0.5%, while *n*-hexane extract was at 15%. Furthermore, the MIC value of the ethyl acetate extract was even better than the positive control (nystatin 100.00 U/ml) with a MIC of 1.0%. (Table 3). The fungal culture ethyl acetate extract had MIC value of 0.5%, while the positive control nystatin had a higher MIC of 1%. It indicates that the ethyl acetate extract of *T. hamatum* TN-103-1 is more potent to inhibit the *C. albicans* pathogenic fungus, and the active compound is a semi-polar. Ethyl acetate is a solvent that is widely used in extracting secondary metabolites because of its semi-polar nature and also had been reported as an efficient solvent for extracting secondary metabolites (Yadav et al., 2014).

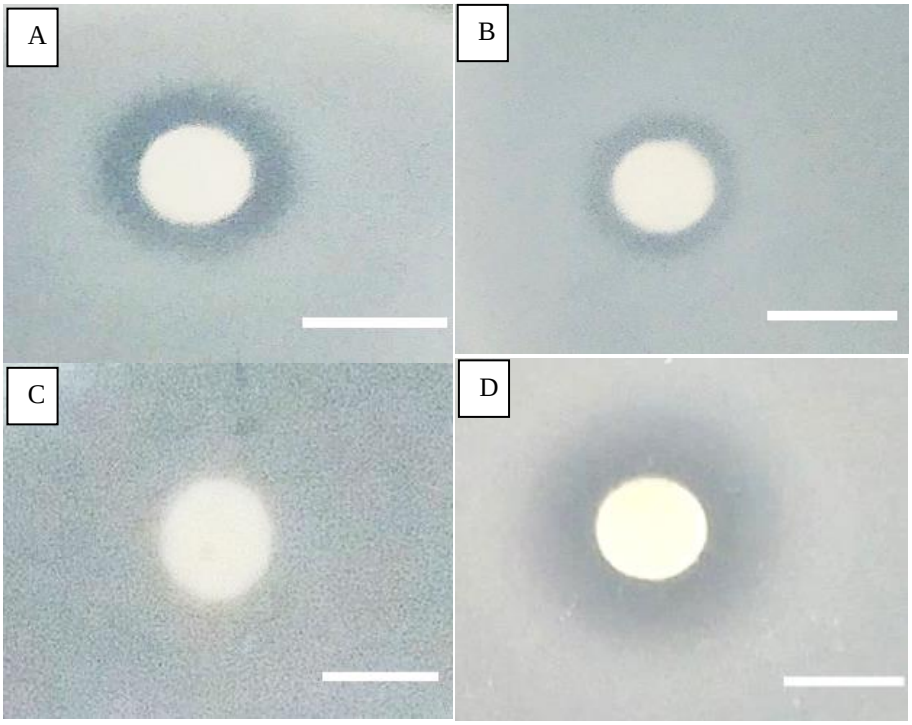

Figure 4. Inhibition zone of 15% concentration of n-hexane and ethyl acetate extracts of *T. hamatum* against *C. albicans. T. hamatum* ethyl acetate extract (A), *T. hamatum* n-hexane extract (B), 10% DMSO negative control (C), nystatin positive control (100.000 U/mL) (D).

Table 3. Growth inhibition activity of *T. hamatum* TN-103-1 culture filtrate extracted by ethyl acetate and *n*-hexane on *C.albicans*

Sample	Sample concentration	Inhibition diameter (mm ± SE)	Minimum Inhibitory Concentration (%)
Ethyl acetate extract of *T. hamatum* TN-103-1	15%	8.83±0.74b	0.5
n-hexane extract of *T. hamatum* TN-103-1	15%	6.50±0.29b	15.0
Positive control (nystatin)	100.000 U/mL	15.50±0.81c	1.0
Negative control (DMSO)	10%	0.00±0.00a	-

Note: the number followed by the same letter shows an insignificant result based on DMRT at 5%.

Predicted active compounds in ethyl acetate extract of *T. hamatum*

T. hamatum TN-103-1 ethyl acetate extract consisted of various compounds with different concentrations (Table 4). Among them, 10 compounds identified in the ethyl acetate extract were reported to have antibacterial or antifungal activities. These compounds come from saturated hydrocarbons, fatty acids, and aliphatic aldehydes. Eight of the ten dominant compounds of which have been reported as anti fungal compounds are 1-Nonadecene, E-15-Heptadecanal, 9-Octadecenoic acid (Z)-methyl ester, 5-Eicosene (E), Octadecane, Heptadecane, Hexadecane, and Tetradecane (Ali et al., 2017; Balamurugan & Selvam, 2013; François et al., 2020; Kamiyama et al., 2013; Kuppuswamy et al., 2013; Rao et al., 2015; Silva & Wansapala, 2016;Vasudevan et al., 2012). The 9-Octadecenoic acid (Z)-methyl ester had the highest peak area among the ten identified compounds.

The 9-Octadecenoic acid (Z)-methyl ester belongs to the fatty acid. The saturated fatty acid compounds and the methyl aster fatty acid were reported to have anti-microbial activity (Lima et al., 2011). The presence of other types of fatty acid groups such as 9-Octadecenoic acid (oleic acid), 9-Octadecenoic acid methyl ester, hexadecanoic acid (palmitic acid), hexadecanoic acid methyl ester (methyl palmitic) is also known as anti microbes (Asghar et al., 2011). For example, the fatty acid methyl ester from the *Excoecaria agallocha* inhibits the growth of four *Candida* species, namely *C. albicans, C. krusei, C. tropicalis*, and *C. parapsilosis*. Similarly, the 6-methylenebicyclo (3.2.0) hep-3-en-2-one also has the same activity (Hussein et al., 2016). The fatty acid compounds will penetrate the microbial cell wall to

disrupt the acid-base balance, disrupt electron transport chain, oxidative phosphorylation, and inhibit enzyme activity in fungal cell membrane, resulting in cell lysis (Desbois & Smith, 2014). The mechanism of action of the 9-Octadecenoic acid (Z)-methyl ester targets the damaging cell structures and membranes of *C. albicans*.

Table 4. Identified compounds in *T.hamatum* ethyl acetate extract and its bioactivity

RT (min)	Compound name	Area (%)	Molecular formula	Similarity (%)	Bioactivity reported
26.88	*1-Nonadecene*	0.99	$C_{19}H_{38}$	97	Antifungal (Kuppuswamy et al., 2013).
22.14	*Heneicosane*	0.60	$C_{21}H_{44}$	97	Antibacteria, anti-inflammatory *(Kamiyama* et al., 2013).
22.11	*E-15-Heptadecanal*	0.99	$C_{17}H_{32}O$	97	Antifungal, antibacteria (François et al., 2020).
21.25	*9-Octadecenoic acid (Z)-, methyl ester*	1.77	$C_{19}H_{36}O_2$	99	Antifungal, antibacteria (Ali et al., 2017).
20.24	*5-Eicosene, (E)*	0.80	$C_{20}H_{40}$	99	Antifungal, antibacteria (Silva & Wansapala, 2016).
19.94	*n-Hexadecanoic acid*	1.36	$C_{16}H_{32}O_2$	97	Anti-inflammatory (Vasudevan et al., 2012).
18.25	*Octadecane*	0.79	$C_{18}H_{38}$	99	Antifungal, antimicrobial (Rao et al., 2015).
17.15	*Heptadecane*	0.60	$C_{17}H_{36}$	90	Antifungal, antibacteria (Francois et al., 2020).
15.99	*Hexadecane*	1.38	$C_{16}H_{34}$	98	Antifungal, antimicrobial (Balamurugan & Selvam, 2013).
13.50	*Tetradecane*	0.64	$C_{14}H_{30}$	97	Antifungal, antibacteria (Francois et al., 2020)

9-Octadecenoic acid (Z)-methyl ester is one of the active compounds commonly found in plants. But so far, it has never been found in the fungus, especially the type of fungus *T.hamatum*, which isolated from the rhizosphere of the robusta coffee plant. So that the compound 9-Octadecenoic acid (Z)-methyl ester is a candidate as an alternative compound that is environmentally friendly with minimum side effects in overcoming several strains of *C. albicans*, which are starting to experience resistance to several drug classes.In addition, the benefits of this research can provide information to the broader community regarding the use of the functional value of rhizosphere fungi, especially in Robusta coffee plants which contain active compounds that are environmentally friendly and can be used as candidates for alternative compounds in overcoming resistance of several strains of *C. albicans*.

This research significantly contributes to Indonesian fungal data base and information about the functional value of rhizosphere fungi (*T. hamatum*), especially those isolated from Robusta coffee plants. *T. hamatum* produces novel active compounds that are environmentally friendly which could be used as an alternative source to control *C. albicans*. The compounds obtained in this study is the first report of the metabolite produced by *T. hamatum.*

CONCLUSION

The fungus isolated from robusta coffee rhizosphere soil is *T.hamatum* TN-103-1. The fungus and the fungal culture filtrate inhibited the growth of *C. albicans*. Both the ethyl acetate and *n*-hexane extracts of the fungal culture filtrate suppressed the growth of *C. albicans* which the effect of the ethyl acetate extract more severe than that of *n*-hexane. The extract also has a better effect than nystatin positive control. The extract's minimum inhibitory concentration (MIC) was 0.50%, whereas the positive control nystatin was 1%. The putative anti-*Candida* compounds present in ethyl acetate extract detected by GCMS obtaied active component 9-Octadecenoic acid (Z), methyl ester, which belongs to fatty acid compounds.

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