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Submission date: 10-Apr-2023 05:51AM (UTC+0700)
Submission ID: 2059848994
File name: yustina_sinta_1_2022.pdf (1.67M)
Word count: 5769
Character count: 31714

Science and Technology Indonesia
e-ISSN:2580-4391 p-ISSN:2580-4405
Vol. 7, No. 2, April 2022

Research Paper

Tritirachium oryzae and Other Endophytic Mediated Jambu Bol (*Syzygium malaccense*) are Potential as an Antioxidant

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Abstract

Natural bioactive substances have been discovered produced of intracellular fungi. Intracellular fungi, as well as endophytic fungi, it can be found in organs are leaves, stems, roots, fruits, flowers, and seeds. This study aimed to specify for antioxidant activity of intracellular fungi Jambu Bol (*Syzygium malaccense*) mediated and identify secondary metabolites compounds. The liquid culture was partitioned with ethyl acetate solvent. Using chromatographic techniques, extracts were separated from their secondary metabolites with antioxidant activity apply the DPPH procedure. Its chemical structure was determined using NMR spectroscopic research, and endophytic fungi were recognized using phenotypic characteristics and molecular classification. The endophytic fungus isolation yielded four isolates: YF11, YF12, YF13, and YF14. YF12, with an IC_{50} of 53.03 g/mL, was the fungus that exhibited good antioxidant activity. Pure chemical secondary metabolites compounds were identified as 2-(4-hydroxyphenyl)-4-methoxytetrahydrofuran-3-ol. *Tritirachium oryzae* was identified as the endophytic fungus YF12 based on morphological studies and a phylogenetic tree. To boost its antioxidant activity, more study is needed to perform a semi-synthetic reaction on this pure molecule.

Keywords

Antioxidant, *Tritirachium oryzae*, *Syzygium malaccense*, Endophytic Fungi

Received: 8 December 2021, Accepted: 29 March 2022
https://doi.org/10.26554/sti.2022.7.2.220-227

1. INTRODUCTION

The production of reactive oxygen species known as free radicals often occurs in all cells as part of normal metabolic processes (Goffart et al., 2021; Phaniendra et al., 2015). Free radicals have been implicated in the pathogenesis of many chronic diseases such as cancer, hypertension, and diabetes (Liguori et al., 2018). An antioxidant is needed to inhibit cell damage (Forrester et al., 2018). The use of synthetic antioxidants in inhibiting the activity of free radicals in damaging body cells can cause toxic side effects. Therefore, the search for natural antioxidants is very necessary (Ibrahim et al., 2021).

Endophytic fungi live in the internal tissues of all plant species without causing disease symptoms in their hosts (Nair and Padmavathy, 2014). These endophytic fungi can synthesize bioactive compounds that have proven useful for the process of new drug discovery. The production of bioactive secondary metabolites from endophytic fungi has become a focus of research in recent decades because endophytic fungi represent interesting microorganisms to explore due to their diverse biotechnological potential (Girón et al., 2021).

Plants with ethnographic backgrounds for the treatment of various diseases are promising candidates for obtaining bioactive compounds from their endogenous fungi. Jambu Bol (*S. malaccense*) is found in Southern Sumatra, Indonesia as herbal medicine in treating diseases such as hypertension, diabetes, and diarrh. The results of a literary study, Jambu Bol plants have been used as traditional medicine by humans in various parts of the world. In Brazil, it is used to treat diabetes (Freitas et al., 2015). In India and Nigeria, the fruit, seeds, and bark are used in treating dysentery, diabetes, gastric ulcers, anti-inflammatory, and antimicrobial (Bairy et al., 2005; Oyinlade, 2014). Endophytic fungus isolated from medicinal plants as an antioxidant, *Syzygium aqueum* has been reported to create secondary metabolites (Habisukan et al., 2022). Root bark, stem bark, and leaves of *S. malaccense* have been identified as endophytic fungus with antibacterial activity (Hapida et al., 2021). In this paper, we report the endophytic fungi that live in the fruit tissue of *S. malaccense*, their antioxidant activity, and the secondary metabolites contained in the active extract of the

endophytic fungus.

2. EXPERIMENTAL SECTION

2.1 Materials

This study used PDA and PDB fungal growth media (Merck), alcohol 70%, aqua DM, sodium hypochlorite (NaOCl), DPPH (Sigma Alderich), organic solvents like ethyl acetate, n-hexane, chloroform, TLC silica gel, and chloramphenicol antibiotic.

2.2 Plant Material

The organs used in this study were fresh and healthy fruit from Jambu Bol (*S. malaccense*) obtained in Palembang City, South Sumatra. The taxonomic examination of the Jambu Bol (*S. malaccense*) plant was carried out in the laboratory of the LIPI Botanical Garden Purwodadi. With number: B-302/III/KS. March 1, 2021.

2.3 Isolation of Endophytic Fungi

Endophytic fungi to be isolated from fresh fruit washed under running water, and surface sterilized by soaking in 70% alcohol for ±180 seconds. After that rinsed with aseptic aqua DM in 1 minute, then soaked with 3% sodium hypochlorite (NaOCl) for 60 seconds, sodium hypochlorite (NaOCl) for 60 seconds. The flesh of the fruit used is cut aseptically. Then the samples were plated in Petri dishes containing PDA media, incubated at room temperature for 3-14 days. Morphological observations on different colonies, then purification was carried out (Habisukan et al., 2021a).

2.4 Cultivation and Extraction

Selected endophytic fungi were cultured on PDB (Potato Dextrose Broth) media. A total of (±106 spores/mL) of pure culture was inoculated as much as 10% (v/v) in 50 mL, in a 1-liter bottle containing 250 mL of PDB medium. The fungi were then incubated for 3-4 weeks at 27°C (room temperature). The culture was filtered to lose from the mycelia. The liquid broth is composed and extracted with ethyl acetate, then shaken for 1 hour. The fungal extract was evaporated using an evaporator (Rumidatul et al., 2021; Syarifah et al., 2021).

2.5 Antioxidant Activity Test

In methanol, a 0.05 mM DPPH solution was prepared. The standard liquid was made by dissolving the sample in 1000 g/mL dimethyl sulfoxide (DMSO). Diluting the reference liquid resulted in variations in sample concentration. 3.8 mL of 0.05 mM DPPH solution was added to 0.2 mL of various amounts of sample. The solution combination agreed and stored in an invisible place for 180 seconds to avoid oxidation and protect it from sunlight. A UV-Vis spectrophotometer was used to detect absorption at a λmax of 517 nm. Ascorbic acid was used as a positive control and the same procedure was administered as a sample. The DPPH Radical Absorption Barrier Strength, namely percentage of inhibition and IC_{50} value was used to calculate antioxidant activity (Elfita et al., 2021).

$$\%\text{Inhibition} = \frac{\text{control absorbance} - \text{sample absorbace}}{\text{control absorbance}} \text{x} 100\%$$

2.6 Morphological and Molecular Identification of Endophytic Fungi

Endophytic fungi that have been purified, identified each colony based on the character formed, isolate fungi based on three main characteristics of the fungus, color and reverse colony characteristics, microscopic characteristics and macroscopic characteristics. Then the group was defined (Huang et al., 2012; Nguyen et al., 2021). Endophytic isolates were identified using the method proposed by Metasari et al. (2020), which involves comparing the similarity of sample sequences to a web-based database known as BLAST (Basic Local Alignment Search Tool).

2.7 Measurement of Product Acidity

YF12 endophytic fungi ethyl acetate extract sample (2 g) was preabsorbed with silica gel (70-230 mesh) in a ratio of 1:1. The samples were separated by column chromatography with the eluent system gradient n-hexane–ethyl acetate (10:0–0:10) and ethyl acetate–methanol (9:1–5:5). The eluent was collected every 10 mL in the vials and TLC analysis was performed on each vial to classify column fractions. The column fraction which shows the presence of a purple major stain is then continued with column rechromatography until a pure compound is obtained. The chemical structure of the pure compound was analyzed based on NMR 1 and 2D spectroscopic data (Habisukan et al., 2021b).

3. RESULTS AND DISCUSSION

3.1 Endophytic Fungi of The Fruit of *S. malaccense*

The results of the isolation of the endophytic fungus *S. malaccense* obtained four isolates with codes YF11, YF12, YF13, and YF14 (Figure 1). Fungal identification was carried out based on the main characteristics of the fungus, namely macroscopic, microscopic, and another specific character. The success of identification and observation is influenced by the ability to observe individual morphology, or the technical ability to induce sporulation in agar cultures. The results of this identification were compared with the literature and the key fungal determination (Ueda et al., 2010).

3.2 Morphological Identification of Endophytic Fungi

The four endophytic fungi isolated from *S. malaccense* fruit were identified macroscopically with the following criteria: colony color, colony texture, topography, colony pattern, exudate drops, radial line, and concentric circle. Microscopic identification based on criteria: spore type, spore form, hyphae, and other specific criteria. After observing the endophytic fungal isolate colonies, then microscopic observations were made on each colony. The characteristics of each isolate are based on microscopic observations shown in Tables 1 and 2.

From the results of the identification of macroscopic and microscopic morphology, it was found that isolates from this

Table 1. Macroscopic Characteristics of Endophytic Fungal Isolates from *S. malaccense* Fruit

Code	Colony complexion	Inverse colony	Type colony	Model	Elevation	Exudate drop	Radial line	Concentric circle
YF11	White	Yellowish-white	Velvety	Zonate	Flat	-	-	√
YF12	White	White and pink central	Cottony	Zonate	Umbonate	-	√	√
YF13	White	White, spreading	Cottony	Zonate	Umbonate	-	√	√
YF14	Grayish white	Cream	Cottony	Zonate	Umbonate	-	√	√

Table 2. Microscopic Characteristics of Endophytic Fungi Isolates from *S. malaccense* Fruit

Code	Name spore	Spore form	Hyphae	In character	Genus / species
YF11	Conidia	Ellipsoidal	Septate, aerial	Conidiophores hyalin, simple, bearing spore masses on phialides at the apex: phialide verticillate	*Gliocladium sp*
YF12	Conidia ovoid	Conidiofor	Non septate	Conidiophores upright, long, slender, simple, conidiogenous branches tapering to a rachislike, zigzag, fertile portion; conidia (sympodulo spores) apical on new growing points, hyaline, l-celled, globose or ovoid, saprophytic, conidiophores hyaline; conidia ovoid	*Tritirachium sp*
YF13	Zygospore	Spora	Septate, aerial	Sporangiophores erect, branching sympodial, bearing terminal sporangia, rhizoids. Terminal sporangia, black, ovate	*Mucor sp*
YF14	Sporangiofor	Spora	Septate	Sporangiophores, unbranched. Rhizoids the sporangiophores are terminate with a dark, round sporangium (40–275 mm in diameter) containing a columella and many oval, colorless or brown	*Rizopus sp*

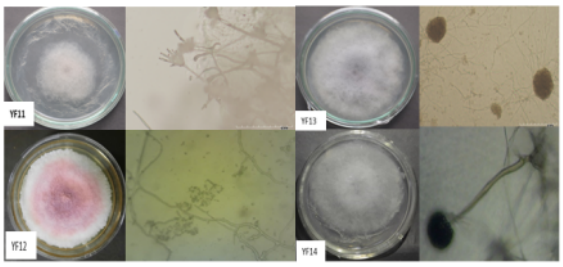

Figure 1. Four Isolates of Endophytic Fungi from *S. malaccense* Fruit YF11 (*Gliocladium sp*), YF12 (*Tritirachium sp*), YF13 (*Mucor sp*), YF14 (*Rhizopus sp*) and Microscopic Observations

fruit belonged to the Ascomycota group, namely YF11 and YF12. YF13 and YF14 isolates belong to the basidiomycota group. Isolation of endophytic fungi that have been carried out, it was found that the number of fungi that could be isolated on the fruit of Jambu Bol (*S. malaccense*) had little and tended not to variety, this is presumably because this endophytic fungus is unique, presence is influenced by place, tissue, environment, and plant ecology. The tissue in the fruit has a smaller amount of tissue compared to the leaves, in the fruit, there is also a phenolic content dissolved in it. Tissues in plant organs provide different micro-habitats for fungal colonies and tissue specificity provides different substrates for the survival of endophytic fungal colonies to give rise to dominant taxa (Huang et al., 2012; Nguyen et al., 2021).

3.3 Antioxidant Activity of Endophytic Fungi

The ethyl acetate extract of endophytic fungi from *S. malaccense* has been tested for antioxidant activity using the DPPH method as shown in Table 3. Based on its IC_{50} value, an extract's antioxidant activity can be classified as high (IC_{50} <100

g/mL), moderate (IC_{50} 100-500 g/mL), or weak (IC_{50} 500-1000 g/mL) and inactive (IC_{50} >1000 g/mL) (Mbekou et al., 2021; Metasari et al., 2020). Only two extracts, the endophytic fungi extract YF12 (IC_{50} 381.04±24.54 g/mL) and the endophytic fungi extract YF14 (IC_{50} 482.83±64.85 g/mL), showed moderate antioxidant activity. Two endophytic fungus extracts, YF11 (IC_{50} 2822.77±442.16 g/mL) and YF13 (IC_{50} 1235.71±144.23 g/mL), were found to be in active as antioxidants. *Rhizopus sp*, isolates from the stems of the *Toona sinensis* plant, Fungus YF14 a member of the *Rhizopus sp*. species, was shown to exhibit substantial (moderate) antioxidant activity such as tannins (Rahmawati et al., 2016). Endophytic fungi extracts that have no antioxidant activity, such as isolates YF11 *(Gliocladium sp*) and YF13 (*Mucor sp*). The fungus *Glocladium sp* has been isolated from *Canna indica* showing high phenolic content but weak antioxidant activity (Eskandarighadikolaii et al., 2015). From the fungus *Gliocladium sp*, compounds ergosterol-5,8-peroxide and allitol were found which can inhibit the activity of *Mycobacterium tuberculosis* (Uc-Cachón et al., 2019).

Table 3. Antioxidant Activity of *S. malaccense* Endophytic Fungi was using The DPPH Method, with Ascorbic Acid as The Antioxidant Standard

Sample	Genus/species of identification	Antioxidant activity IC_{50} (μg/mL)
YF11	*Gliocladium sp*	2822.77±442.16
YF12	*Tritirachium sp*	381.04±24.54
YF13	*Mucor sp*	1235.71±144.23
YF14	*Rhizopus sp*	482.83±64.85
Compound 1 (isolated from YF12)		53.03±0.23
Ascorbic acid		16.03±0.96

Species in the genus *Mucor sp* that have been reported are *Mucor racemosus*, and *Mucor circinelloides*. Ethyl acetate extract of the endophytic fungus *Mucor racemosus* isolated from *Hibiscus sabdariffa* was reported to contain no flavonoids in its secondary metabolites (Khalil et al., 2020). However, the ethanolic extract of *Mucor circinelloides* fungi can bind phenolic compounds, tannins and flavonoids under nutritional stress conditions in the late exponential phase so it can be developed as nutraceuticals and natural antioxidants (Hameed et al., 2017).

3.4 Molecular Identification of Selected Endophytic Fungi

The YF12 endophytic fungus has been chosen to proceed to the molecular identification stage and secondary metabolite isolation because it has a good IC_{50} value among the four other endophytic fungal isolates. The results of molecular data analysis of YF12 isolate using MEGA 11, with the Neighbor-joining method, bootstrap consensus tree with 1000 replications, the evolutionary distance was calculated using the P-distance method to obtain a phylogenetic tree (Figure 2b). The results of a phylogenetic search using the Gene Bank, it was found that the YF12 isolate was a species of *Tritirachium oryzae*. The results of the nitrogen base examination and the phylogenetic tree YF12 isolate can be seen in Figure 2. The its rDNA sequence of the YF12 isolate is shown as follows:

TCACTAATGATCCTTCCGTAGGTGAACCTGCGGA AGGATCATTAGTGAATTTAAAAAATACAGAATTGGAT TGAAAAAAATCCAAATTCTTATTTCTTTATTCTTCTC TTCCACTGTGAAATTTTAAACTATTCGGGCGGTCTT TTGGCCGGTCGAGGTTTAGAGATGGGACTGAGTGA AAAAAAATTGTTGGGGAGTGCCTCCACTTTCAAGTG GAGCGGACGATCTGCAGTTTTAGTTCTTCTGTTCTC TGATCTAGCCGAATTACCCAATTTTTAGAGACAATGT TAAATTTGAATGTGTTTTTTTATTAAACAAATTAAAA CTTTCAGTGACGGATCTCTTGGCTCTCGCATCGATG AAGAACGCAGTAAATCGCGATACGTAATGTGAATTG CAGAAATATGTGAATCATCGAATCTTTGAACGCATCT TGCGCTCTGGGGTACTCCTCAGAGCATGCCTGTTTG AGTGTCTTTTAATTCTCATCTCATAATTTTTTATTAA TTTAAAATAATTATAGGTGGATCGTGGCTGTTTTGA CGACTTAACCTCGTCTCAGCTGAAATATAGAAAGCG ACGTCTAAAATTCAGAGTAATAAGATGTTAACGTCG GCGTGATAAGTAAATTACGCAGTCAGTTTTCTGTCT ATCTCTCTGGTGTTCGCTACGAATTACTATAGTTTT GTTTTCAACATTTGACCTCAAATCAGGTAGGACTAC CCGCTGAACTTAAGCATATCAATAAGCCGG.

Tiraitirachium oryzae is a fungus that comes from the air, its interaction with humans was first known to cause onychomycosis (Naseri et al., 2013). This fungus has the biosynthetic ability of AgNPs because it contains a single carbon and nitrogen in culture media which can be synergized into nanoparticles to inhibit bacterial growth (Khlaifat et al., 2019). Onychomycosis is also reported as a producer of extracellular lipase enzymes that can hydrolyze waste oil (Al-limoun, 2020). This fungus belongs to the basidiomycota group and is often mistaken for Ascomycota, this species produces spores in the air and is often found as endophytes in certain plant species, its presence in mesophyll tissue (Agut, 2001).

3.5 Purification and Identification Bioactive Compound

A total of 2 g of ethyl acetate extract of the endophytic fungus *Tritirachium oryzae* (YF12) was obtained using a gradient (10:0-0:10) in ethyl acetate-methanol (9:1-5:5) to obtain five fractions, namely F1-F5 made. Fraction F4 was re-chromatographed with an n-hexane-ethyl acetate gradient eluent (5:5-0:10) to produce a three-column fraction, F4.1-F4.3. Column fraction F4.2 is a chromatographic column with n-hexane-ethyl acetate (4:6) as eluent and the resulting solid is eluted with 8:2 n-hexane-ethyl acetate to obtain a pure yellowish color compound (compound 1) white solid 36 mg.

The H-NMR spectrum of compound 1 (Figure 3) showed the presence of nine proton signals. There are two doublet signals with the integration of two protons in the aromatic region, namely at δ_H 7.56 and 8.20 ppm. This indicates that

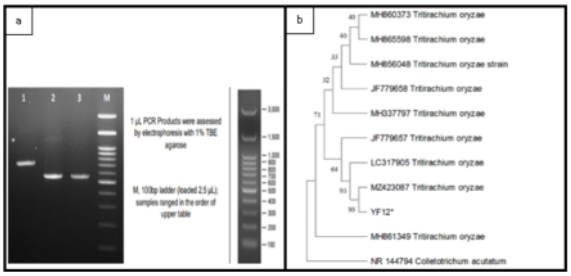

Figure 2. Molecular Examination Results on Selected Fungal YF12 Isolated from *S. malaccense* Fruit (a) The Composition of The Number of Nitrogen Bases Possessed by The YF12 Fungi Isolate was No. 1,753 bp, (b) The Phylogenetic Tree Showing The Species of YF12 Isolate Showed *Tritirachium oryzae* Species, The Scale used was 0.050 m

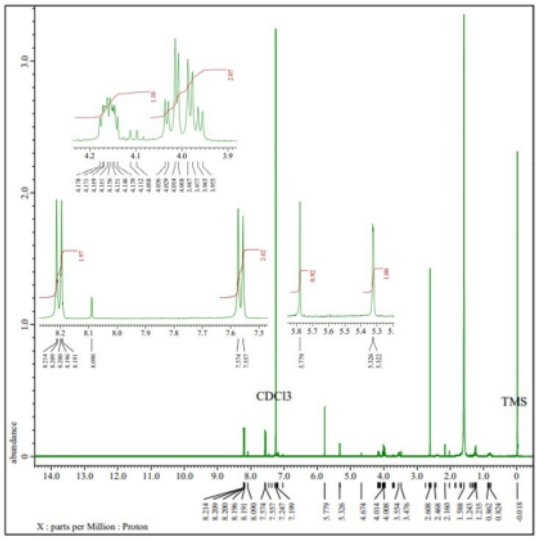

Figure 3. The ^{1}H-NMR Spectrum of Compound 1 (^{1}H-500 MHz in $CDCl_3$)

compound 1 has two pairs of equivalence protons side by side. Thus it is known that compound 1 is an aromatic compound that has a para-substituted benzene ring. Then there are three oxymethine proton signals, namely at δ_H 4.16 (^{1}H, m), 5.32 (^{1}H, d, J= 2), and 5.78 ppm (^{1}H, s), an oxymethylene proton signal at δ_H 3.99 ppm (^{2}H, m), and a methoxy proton signal at δ_H 2.61 (^{3}H, s). In addition, there are also two broad signals that are typical for hydroxyl protons, namely at H 3.55 and 7.19 ppm.

The identification of protons in the ^{1}H-NMR spectrum is supported by data on the ^{13}C-NMR spectrum (Table 4) and HMQC (Figure 3). The ^{13}C-NMR spectrum of compound 1 showed the presence of nine signals. 4 carbon signals appear in the aromatic region, namely two equivalent aromatic methine carbons (δ_H 123.8 and 126.8 ppm), one aromatic quaternary carbon at δC 147.6, and one aromatic oxyaryl carbon at low field δC 164.5 ppm. In addition, five oxygenated carbon signals appear in the δC region of 40.0–75.0 ppm. The analysis of the proton and carbon NMR spectra were confirmed by the data on the HMQC spectrum shown in Figure 4a and Table 4, namely the ^{1}H-^{13}C correlation through one bond. The HMQC spectrum showed seven correlations consisting of two correlations on the aromatic ring and five correlations on oxygenated ^{1}H-^{13}C on aromatic substituents. The other two proton signals at δ_H 3.55 and 7.19 ppm do not correlate with the HSQC spectrum. This indicates that the two protons are hydroxyl protons.

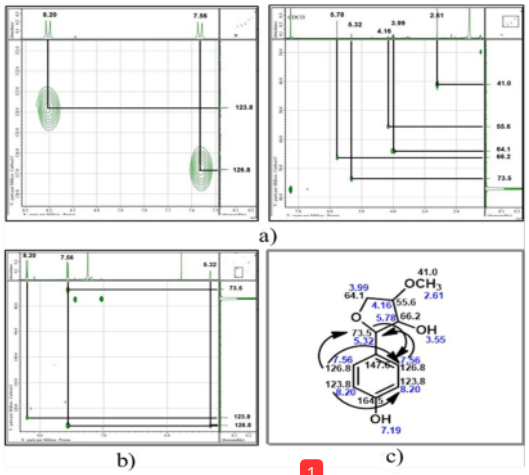

Figure 4. The NMR 2D Spectrum of Compound 1 (^{1}H-500 MHz; ^{13}C-125 MHz in $CDCl_3$ (a) HMQC Spectrum, (b) HMBC Spectrum, (c) The HMBC Correlation and δ-Assignment of Compound 1

The HMBC spectrum (Figure 4) shows the ^{1}H-^{13}C correlation through two or three bonds. The aromatic proton signal at δ_H 8.20 ppm is correlated through three bonds with its equivalent aromatic carbon (δ_C 123.8 ppm). The aromatic proton at δ_H 7.56 ppm is correlated through three bonds with equivalent aromatic carbon (δ_C 126.8 ppm) and oxygenated carbon (δ_C 73.5 ppm). Furthermore, the oxygenated methine proton at δ_H 5.32 ppm was correlated via three bonds with the equivalent aromatic carbon (δ_C 126.6 ppm) indicating that the oxygenated methine group is directly attached to the aromatic ring and is para-substituted with the hydroxyl group. The HMBC spectrum in Figure 4b does not provide all the information for the ^{1}H-^{13}C correlation through two or three bonds. However, the substituent para hydroxyl group is a furan ring-substituted for hydroxyl and methoxyl groups which are indicated by the presence of a hydroxyl proton signal at δ_H 3.55 (^{1}H, broad) and a methoxyl proton at δ_H 2.61 (^{3}H, s).

The 1D and 2D NMR spectral data for compound 1 are shown in Table 4.

Table 4. NMR Data for Compound 1

No. C	δ_C ppm	δ_H ppm (ΣH. multiplicity. J (Hz))	HMBC
2	73.5	5.32 (^{1}H, d, J = 2)	126.8
3	66.2	5.78 (^{1}H, s)	
4	55.6	4.16 (^{1}H, m)	
5	64.1	3.99 (^{2}H, m)	
6	41.0	2.61 (^{3}H, s)	
1'	147.6		
2'	126.8	7.56 (^{1}H, d, J = 8.5)	126.8; 73.5
3'	123.8	8.20 (^{1}H, d, J = 8.5)	123.8
4'	164.5		
5'	123.8	8.20 (^{1}H, d, J = 8.5)	123.8
6'	126.8	7.56 (^{1}H, d, J = 8.5)	126.8; 73.5
3- OH		3.55 (^{1}H, broad)	
4'- OH		7.19 (^{1}H, broad)	

Based on spectral analysis of ^{1}H-NMR, ^{13}C-NMR, HMQC, and HMBC, it can be explained that compound 1 has a para-substituted benzene ring between the hydroxyl group and the furan ring. The oxygenated methine carbon of this furan ring bonds directly to the aromatic carbon. The hydroxyl and methoxyl substituents are bonded to the furan ring. Thus, the proposed chemical structure of compound 1 is 2-(4-hydroxyphenyl)-4-methoxytetrahydrofuran-3-ol as shown in Figure 5.

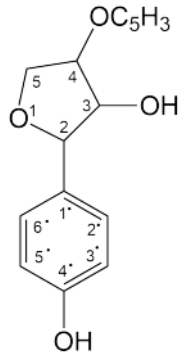

Figure 5. Chemical Structure of Compound 1: 2-(4-Hydroxyphenyl)-4-Methoxytetrahydrofuran-3-ol

The results of the antioxidant test using the DPPH method, the IC_{50} value was 53.03±0.23 with moderate criteria. Chemical structure 2-(4-hydroxyphenyl)-4-methoxytetrahydrofuran-3-ol, contains simple phenolic compounds, phenolic compounds are known as antioxidants and antibacterials (Zeb, 2020). The group of antioxidant compounds present in the host, especially in *S. malaccense* fruit contains more complex compounds such as anthocyanins such as cyanidin 3-glucoside, followed by cyaniding 3,5-glucoside and peonidin 3-glucoside (Batista et al., 2017; Nunes et al., 2016), because anthocyanins are phenol derivatives other than flavonoids (Zeb, 2020). In the essential oil of guava fruit *S. malaccense* 2-phenyl ethanol and its esters (2-phenylethyl acetate, 2-phenylethyl isopentanoate, 2-phenylethyl benzoate and 2-phenylethyl phenylacetate) and herbs (1-octen-3-ol) contributes to the complexity of the aroma (Pino et al., 2004). Similar compounds, such as 2-(4-Hydroxyphenyl)-5-(3-hydroxypropenyl)-7-methoxybenzofuran, which is a derivative of ailanthoidol, have been reported to have been isolated from a neolignan from *Zanthoxylum ailanthoides* and *Salvia miltiorrhiza Bunge*. This compound has a low IC_{50} value, by activating protein kinases with the help of mitogens which can make this compound an effective functional chemical candidate for the prevention of inflammatory diseases (Kim et al., 2013).

Chemical constituents that have been reported are tetrahydroxanthone and oxyanthrone from *Tritirachium sp* associated with *Pseudoceratina purpurea* isolated from marine sponge collected from offshore sites in Sakuraguchi, Ishigaki Island, Okinawa Prefecture, Japan (Ueda et al., 2010). There are three anthraquinone compounds from *Tritirachium sp* by water-soluble Tetrazolium-8 (WST-8) colorimetric assay, were shown to be antiproliferative against Hela cells (IC_{50}: 1, 17, and 17 M respectively) (Ueda et al., 2010). There are 4,5-dihydroxy-10-oxo-9,10-dihydroanthracen-9-yl acetate, and 2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium compounds (Zhang et al., 2017).

The *Pinus wallichiana* plant from the Western Himalayas, has been isolated from the endophytic fungus *Tritirichium oryzae* and has high antifungal activity on *Candida albicans* and a broad spectrum as an antimicrobial (Qadri et al., 2014). In addition, this species is known to cause several infections in humans such as corneal ulcers, otomycosis, onychomycosis, and dermatomycosis, but the genus *Tritirachium* also has potential in biotechnology to produce several enzymes such as proteases, amylase, glucanase, xylanase, pectinases, lipase, and proteinase K, Phylogenetically, it is known that the fungus *Tritirachium oryzae* is closely related to *T. dependents* (Bezerra et al., 2020).

Another host plant known to host *Tritirachium oryzae* is the bark of the Hancornia Speciosa Gomes plant, which has biological activity as an antibacterial against *B. subtilis*, *E. coli*, and *P. aeruginosa* bacteria (Chagas et al., 2017). Isolation of fungi of the *Tritirachium* genus from the soil of various gardens in Shiraz, Iran was found as much as 0.24%, at a pH between 7-8, which are referred to as keratinophilic fungi (Pakshir et al., 2013). There is a diversity of species of *Tritirachium oryzae* isolated from transgenic and non-transgenic "Bt" cotton plants (the commonly farmed cotton plant that has been genetically engineered) on leaves and stems in Brazil (Vieira et al., 2011).

Mangrove plants on the southwest coast of India, the genus *Tritirachium* has antimicrobial activity as well (Maria et al., 2005).

4. CONCLUSIONS

Code YF12 was *Tritirachium oryzae* from *S. malaccense* produced secondary metabolites compound. Compound 1 as 2-(4-hydroxyphenyl)-4-methoxytetrahydrofuran-3-ol with moderate of antioxidant activity. For increasing the antioxidant activity of compound 1, further research is needed, namely a semisynthetic reaction to add a hydroxyl group at the C-3 and/or C-5 positions. So this compound 1 can be used as a basic ingredient of antioxidant compounds.

5. ACKNOWLEDGMENT

The authors thank to the DRPM Kemenristek Republik Indonesia, which provided research funding through Hibah Disertasi Doktor 2021, with contract No. 0166.04/UN9/SB3.LP2M.PT/2021.

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