Terpenoid as Antibacterial Produced by Endophyte *Fusarium oxysporum* LBKURCC41 from *Dahlia variabilis* Tuber

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Abstract. *Fusarium oxysporum* LBKURCC41 is an endophytic isolated from *Dahlia variabilis* tubers from Padang Luar, West Sumatra. The strain was fermented using modified Huang’s media, *i.e.* using corn as a carbon source for 15 days. The purpose of this study is to isolate secondary metabolites from LBKURCC41 strains. The filtrate from the liquid fermentation media was extracted using EtOAc:MeOH at a ratio of 95:5 to 50:50. Purification using fast chromatography was performed with a mixture of BAW (5:1:4). The structure was elucidated by FTIR and ¹H NMR spectroscopy. The isolate was identified as a sesquiterpenoid, with weak antimicrobial activity against *E. coli* and *S. aureus* with 2.1 mm clear zone and concentration of 10 ppm.

Keywords: antibacterial, endophyte, fermentation, *Fusarium*, terpenoid

1. Introduction

*Fusarium oxysporum* LBKURCC41 is an endophytic isolated from *Dahlia variabilis* tubers from Padang Luar, West Sumatra, isolated by [1], [2] revealed that *Fusarium* genus are endophytic fungi and in recent decades are known to produce active metabolites. Crude extract of secondary metabolites LBKURCC41 strain from Huang medium by [3] has been tested its ability to inhibit the growth of *Staphylococcus aureus* with the largest clear zone of 9.5 mm and 17 mm respectively in 15 days of fermentation. Antimicrobial tests of crude extracts of LBKURCC41 strain secondary metabolites and positively inhibited the growth of *Staphylococcus aureus* and *Eschericia coli* [4]. Quinine and synconidine compounds [5] and the compound chitotanes and its derivatives [6] from *Fusarium* genus by fermentation which has antimalaria and anticancer activities.

On the other hand, [7] has optimized the production of secondary metabolites of LBKURCC41 strain. They have varied the carbon source in Huang's media by using corn. Secondary metabolites produced from the fermentation media are thought to be antibacterial compounds of terpenoid groups which have been tested for their ability to inhibit the growth of *Staphylococcus aureus* and *Eschericia coli*. Based on research results from [8] beauverisin is a secondary metabolite of *Fusarium oxysporum* which has antibacterial activity. Several studies have indicated the possible prospect of endophytes from *Fusarium* genus as a promising resource of antibacterial compounds from the crude extracts [2]. From [2], fungi belonging to *Fusarium* genus represent one of the most important groups of fungi because of their implication as plant pathogens. Fortunately, the nonpathogenic species from this genus, particularly endophytes are of equal importance because of their outstanding biosynthetic ability. The studies can showed that crude extracts from *Fusarium* has ability as antibacterial, so these findings fits well with the general claim that fungi can produce a pure compound with antibacterial activity.
Based on the information above, the secondary metabolite production from LBKURCC41 strain will be carried out. The isolate production media used corn as a carbon source based on Huang’s media which optimizationed from Marlinda et al, and it was fermented for 15 days. Crude extract of secondary metabolites obtained will be purified and elucidated structure.

2. Materials and Methods

2.1. Sample Preparation
Corn, potato and sweet potato bought at traditional market in Pekanbaru, Riau, Indonesia were washed and dried at sunlight for 8 h and 40°C for 48h. All sample were blendered separately and sieved for 80 mesh. The powder was stored separately at room temperature.

2.1.1. Cultivation and preparation of Fusarium oxysporum LBKURCC41
LBKURCC41 strain was cultivated in potato dextrose agar aseptically and incubated for 4 days at room temperature. Fresh isolate rinsed with NaCl 0.8%, scraped and filtered using sterile glasswoll. Suspension of spores inoculated into potato dextrose broth at ~7 x 10^12 spore/mL was determined optical density using spectrophotometer Ultra Violet-Visible (UV-Vis) Genesis 10 S (Thermo Scientific) then incubated for 4 days at room temperature using rotary shaker shaking incubator model LSI 301 6R (Daihan Lab Tech Co. LTD) with 150 rpm.

2.1.2. Preparation of Fermentation Process
Inoculum starter was placed to Huang production medium with modification in corn as carbon source with fermentation time 15 days. After fermentation filtrate was separated by Buchner funnel through filter paper Whatman No.40. The filtrate was transferred aseptically into a conical flask and stored at 4°C for further assay. The culture filtrate was extracted 3 times with EtOAc. The EtOAc phase was separated from aquos using separating funnel. EtOAc layer concentrate was evaporated using vacuum rotary evaporator Heidolph WB 2000, and stored at room temperature for further assay.

2.2. Extraction and isolation
After an incubation period of 15 days is to do filtration. The fermented culture media mixture was filtered using a Buchner funnel filter, dialed with Whatman paper number 40. The filtrate portion was selected to take secondary metabolite compounds, then extracted using maceration method, ie using ethyl acetate solvent in the separating funnel repeatedly until the compound did not emit gas [9]. The extraction results were evaporated using a rotary evaporator to obtain concentrated extracts from LBKURCC41 isolates secondary metabolites.

The separation stage is carried out using flash chromatography, using various solvents, namely ethyl acetate, methanol, and BAW (butanol: acetic acid: water; with a ratio of 5: 1: 4). The preparation is done by preparing the column, silica slurry is first made by mixing silica with ethyl acetate solvent and stirring thoroughly, then pouring it into the column slowly using a funnel and then eluting until silica density in the maximum column.

Next, elute in a gradient with EtOAc (100%) EtOAc:MeOH (97.5:2.5%), and so on until reaching EtOAc:MeOH (50: 50%). Next with 100% BAW solvent. The elution process is assisted by the pressure of nitrogen gas, so the separation process is faster. Each fraction that comes out is accommodated using a vial. The fraction is heated and allowed to dry, for the next step. The fraction with the existing sediment is taken for further testing, which is to see the stain on TLC. The resulting stain is marked using a pencil with the help of a UV lamp. If the stain found on the TLC plate has the same retardation factor (Rf) value then a vial is merged. The next step is to purify using the preparative TLC method to isolate the compounds contained in the TLC by looking under far and near UV beam.
2.3. Elucidation of the compound isolate

Subsequently samples obtained from flash chromatography were tested using UV-Vis. This step aims to see whether or not there are conjugated double bonds in the compound fraction. This is because the use of the UV-Vis instrument is only successful if the fraction or pure compound tested has a conjugated double bond, so that the electrons in the double bond can be absorbed at wavelengths greater than or equal to 400 nm. After testing using UV-Vis, the next step is to re-test the FT-IR compound to find out the functional groups in the compound. Then using H-NMR to determine the number of protons to determine the molecular weight of the compounds obtained.

2.4. Preparation of Antibacterial test

The pure extract was tested for antimicrobial activity using agar well diffussion method against *E. coli* and *S. aureus* [10]. The extract was solved in methanol with a series of concentration of 10 mg/mL. Amoxan 3.8 mg/mL were used as positive control while methanol as negative control.

Observations were made by measuring the clear zone formed around the disc paper on the media [11].

\[ R = \frac{X_1 - X_2}{X_2} \]  

Notes: 

- \( R \) = ratio of clear zone
- \( X_1 \) = clear zone diameter
- \( X_2 \) = disc paper diameter

3. Results and Discussion

In this research, purification and determination of secondary metabolite compounds produced from *Fusarium oxysporum* LBKURCC41 endophyte from *Dahlia variabilis* tuber. The secondary metabolite produced was the result of fermentation of LBKURCC41 strain for 15 days on media with carbon sources in the form of corn. The fermentation is carried out with the effect of 150 rpm agitation aimed at providing oxygen intake during the fermentation process. This is consistent with the optimization carried out [7], and fermentation takes place at room temperature.

After that, we approved the compound is sesquiterpenoid. The secondary metabolite compounds obtained from LBKURCC41 strain fermented for 15 days with carbon sources from corn is sesquiterpenoid. C15 was obtained from the crude metabolite extract test results using HPLC and TLC [7]. Terpenoid can be produced from *F. oxysporum* [12]. The *F. oxysporum* can carry out terpenoid biosynthesis in the presence of DXP (deoxy-d-xylulose 5-phosphate) compounds, which can be obtained from carbohydrate-rich plants such as corn. Terpenoids are produced from several plants, where these compounds can be used as antimicrobial agents by plants [13]. Terpenoid can be used as medicinal ingredients such as antibacterial, antifungal, insecticide [14].

Most terpenoids are able to inhibit two crucial processes which are essential to microbial survival, this includes oxygen uptake and oxidative phosphorylation. Aerobic microbes require oxygen in order to yield energy for their growth. It was proven that low oxygen concentrations caused limitation in bacterial respiration rates. On other hand, oxidative phosphorylation is a crucial biochemical process responsible for cellular respiration that takes place in the cytoplasmic membrane. Thus, terpene interaction leads to alteration in cellular respiration which later causes uncoupling of oxidative phosphorylation in the microbe. The higher antimicrobial activity was related to the presence of hydroxyl groups (phenolic and alcohol compounds) [15]. Meanwhile, the chemical elucidated were determinated used FTIR and H¹ NMR.

The IR spectra of purificated EtOAc extract compounds from LBKURCC41 strain are 3650 (alkyl O-H stretch), 2998 (alkane C-C), 1663 (cis C = C alkene), and 1390 (methyl C-H stretch). Some aliphatic protons are shown from the 1H NMR spectrum, as in the following table 1.
Table 1. $^1$H NMR spectroscopic data (500 MHz)

| Multiplicity | Chemistry shift |
|--------------|-----------------|
| M            | 0.91 (1H)       |
| T            | 1.24 (4H, 7.15 Hz, 7.1 Hz) |
| M            | 1.31 (4H)       |
| s            | 2.01 (3H)       |
| s            | 2.52 (3H)       |
| m            | 3.13 (1H)       |
| s            | 3.34 (3H)       |
| m            | 3.48 (2H)       |
| m            | 3.65 (7H)       |
| m            | 3.78 (2H)       |
| d            | 3.82 (2H, 14.25 Hz) |
| q            | 4.12 (3H, 14.35 Hz) |

The chemical elucidation results obtained from this study are similar to those of sesquiterpene [2]. This is reinforced by the presence of alcohol groups, double bonds, and the amount of carbon in accordance with those stated by Marlinda et al. The structure of these compounds can be seen below on figure 1. The compound was tested with antibacterial activity test for known the ability of it.

![Figure 1](attachment:figure1.png)

**Figure 1.** (1R,2S,3R)-3-((S)-2-hydroxy-6-methylhept-5-en-2-yl)-1,2-dimethylcyclopentanol

Based on the antibacterial activity test of the pure compound obtained against *S. aureus* and *E. coli* obtained a clear zone with a diameter of 2.1 mm. The clear zone is lower than the test results from Marlinda et al. The extract for the test is a crude extract [7], while in this study using a pure extract. This is probably due to the presence of other compounds that affect the test results of the crude extract.

4. **Conclusion**

The tuber of *Dahlia variabilis* strain of LBKURCC41 can produce sesquiterpenoid using modified Huangs’ media. The ability of antibacterial activity of this compound is relatively weak with a concentration of 10 ppm (*in vitro*).
Acknowledgment
This research was financed by the Directorate General Research Empowerment and Development (DRPM) of Research, Technology, and Higher Education of the Republic of Indonesia 2019 with contract number: 815/UN.19.5.1.3/ PT.01.03/2019, scheme Penelitian Dasar Unggulan Pertinggian Tinggi (PDUPT) awarded to Saryono as principal investigator.

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