Optimization of Bioactive Metabolite Production From 
Fusarium oxysporum LBKURCC41

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Abstract. Fusarium oxysporum is one of endophyte microbes isolated from dahlia tuber (Dahlia variabilis) which has the ability to produce secondary metabolites. In this research, optimization of chemical and physical fermentation conditions were carried out. Corn, potato, and sweet potato with particle size of 80 mesh were used as carbon sources to produce secondary metabolite for 5, 10, 15, 20, and 25 days respectively with ethyl acetate was used to extract secondary metabolites. The assessment of antimicrobial activity was then performed against Candida albican, Escherichia coli and Staphylococcus aureus. A serial of metabolite concentrations 5.7 mg/ml, 3.8 mg/ml, and 1.9 mg/ml were used. The optimum inhibition against microbial pathogen growth was corn for 15 day fermentation (C15). The inhibition zone against Candida albican, E.coli, and S. aureus were 7.62±0.32, 14.15±0.09, and 15.24±0.24 mm respectively at 5.7 mg/ml metabolite concentration. The result of metabolites screening showed overall extract secondary metabolites comprised terpenoid group. The separation of the active ingredients was performed using both Thin Layer Chromatography and High Performance Liquid Chromatography (HPLC) depicted 4 component.

Keywords: Fusarium oxysporum, optimization, antimicrobial, secondary metabolite and terpenoid.

1. Introduction

Endophytic fungi is defined as the fungi which spend the whole or part of their lifecycle colonizing inter and/or intracellularly inside the healthy tissues of the host plant, typically causing no apparent symptoms of disease [1]. In the past decade, many valuable bioactive compounds have been successfully discovered from endophytes. During the long period of co-evolution, a friendly relationship was gradually set up between each endophyte and its host plant. Therefore, endophytes show ability to produce same or similar bioactive substances as those originated from their host plants [2].

Endophytic fungi have also been reported to possess bioactive secondary metabolites and enzymes such as inulinase, chitinase, amylase, and cellulase [3-4]. Endophyte microbial could be classified as bacteria, fungi, and actinomycetes [5]. Fusarium sp LBKURCC73 produced cellulase [6], while Fusarium oxysporum (LBKURCC6) isolated from pink-flowered dahlia tuber have ability to produce inulinase [7].

Fusarium oxysporum (LBKURCC41) is an endophytic fungi isolated from red-flowered dahlia tuber collected from Bukit tinggi, Sumatera barat [8], it was indentified based on the gene sequenced analyse according to a molecular biological protocol by DNA amplification and sequencing of the ITS region [9]. On the other hand, it has been found ability to produce saponin which produced in Huang medium.
production with antimicrobial activity [10-11]. The production of secondary metabolite from the *Fusarium oxysporum* can be influenced by medium and time of fermentation. The aim of this research was to discover the best natural carbon resources and time fermentation in order to produce optimum secondary metabolites through corn, potato, and sweet potato.

2. Materials and Methods

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 blandered separately and sieved for 80 mesh. The powder was stored separately at room temperature.

Cultivation and preparation of *Fusarium oxysporum* LBKURCC41

Isolate LBKURCC41 *Fusarium oxysporum* 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 glasswell. Suspension of spores inoculated into potato dextrose broth at $\sim 7 \times 10^{12}$ spore/ml was determined optical density using spectrophotometer Ultra Vi-let-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.

Fermentation

Inoculum starter was placed to Huang production medium with modification in carbon source (corn, potato, and sweet potato) and fermentation time 5, 10, 15, 20, and 25 days. After fermentation filtrate was separated by Buchner funnel through filter paper Whatman No.1. 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 ethyl acetate. The ethyl acetate phase was separated from aqueos using separating funnel. Ethyl acetate layer concentrate was evaporated using *vacuum rotary evaporator* Heidolph WB 2000, and stored at room temperature for further assay.

Antimicrobial assesment

Crude extract was tested for antimicrobial activity using agar well diffusion method against *Candida albicans*, Gram-negative bacteria (*Escherichia coli*) and Gram-positive bacteria (*Staphylococcus aureus*). Extract was solved in methanol with a series of concentration of 5.7, 3.8, and 1.9 mg/ml. Ketoknazole and amoxan 3.8 mg/ml were used as positive control while methanol as negative control.

Screening of secondary metabolites

Secondary metabolites were screening for their content of terpenoid/steroid, alkaloid, phenolic, flavonoid dan saponin.

Separation Using TLC and HPLC

Extract possessing optimum antimicrobial activity was analyzed further with TLC and HPLC. Aluminum plates pre-coated with silica gel as stationary phase and ethyl acetate 100% as a mobile phase. Chromatograms were observed under UV light, and calculated the retention factor. On the other hand, a number 20 µl extract was analyzed by HPLC Shim-pack VP-ODS (250 x 4,6 mm) using water:methanol sovent with gradient elution system. UV detector was used to determine its maximum wavelength.

3. Result and Discussion

*Fusarium oxysporum* growth and fermentation observation

*Fusarium oxysporum* grew at the second day, produced white mycelia on PDA and purple spore at third day. This inoculum was removed to modified production medium after being incubated for 96 hours. Secondary metabolites produced by *Fusarium oxysporum* were assayed against 3 microbial pathogens. Inhibition zone resulted against *Candida albicans* was showed in Table 1.
### Table 1. Ethyl acetate extract from *Fusarium oxysporum* metabolites and inhibition zone against *Candida albicans*

| Carbon source | Fermentation Day | Concentration       | Inhibition zone diameter (mm) |
|---------------|------------------|---------------------|------------------------------|
| Corn          | 5                | Extract 5.7 mg/ml   | 6.74±0.34<sup>a</sup>       |
|               |                  | Extract 3.8 mg/ml   | 6.81±0.12<sup>b</sup>       |
|               |                  | Extract 1.9 mg/ml   | 6.14±0.04<sup>c</sup>       |
|               |                  | Ketoconazole        | 8.31±0.32<sup>d</sup>       |
|               |                  | Methanol            | 0.00±0.00<sup>e</sup>       |
| Corn          | 10               | Extract 5.7 mg/ml   | 7.22±0.03<sup>b</sup>       |
|               |                  | Extract 3.8 mg/ml   | 6.73±0.00<sup>b</sup>       |
|               |                  | Extract 1.9 mg/ml   | 0.00±0.00<sup>f</sup>       |
|               |                  | Ketoconazole        | 7.37±0.15<sup>g</sup>       |
|               |                  | Methanol            | 0.00±0.00<sup>h</sup>       |
| Corn          | 15               | Extract 5.7 mg/ml   | 7.62±0.00<sup>b</sup>       |
|               |                  | Extract 3.8 mg/ml   | 6.29±0.05<sup>i</sup>       |
|               |                  | Extract 1.9 mg/ml   | 0.00±0.00<sup>j</sup>       |
|               |                  | Ketoconazole        | 7.87±0.13<sup>k</sup>       |
|               |                  | Methanol            | 0.00±0.00<sup>l</sup>       |
| Corn          | 20               | Extract 5.7 mg/ml   | 7.39±0.16<sup>b</sup>       |
|               |                  | Extract 3.8 mg/ml   | 6.44±0.03<sup>c</sup>       |
|               |                  | Extract 1.9 mg/ml   | 0.00±0.00<sup>d</sup>       |
|               |                  | Ketoconazole        | 7.76±0.07<sup>e</sup>       |
|               |                  | Methanol            | 0.00±0.00<sup>f</sup>       |
| Corn          | 25               | Extract 5.7 mg/ml   | 0.00±0.00<sup>b</sup>       |
|               |                  | Extract 3.8 mg/ml   | 6.56±0.18<sup>b</sup>       |
|               |                  | Extract 1.9 mg/ml   | 0.00±0.00<sup>g</sup>       |
|               |                  | Ketoconazole        | 7.43±0.22<sup>h</sup>       |
|               |                  | Methanol            | 0.00±0.00<sup>i</sup>       |
| Potato        | 5                | Extract 5.7 mg/ml   | 6.54±0.05<sup>b</sup>       |
|               |                  | Extract 3.8 mg/ml   | 6.91±0.11<sup>b</sup>       |
|               |                  | Extract 1.9 mg/ml   | 0.00±0.00<sup>c</sup>       |
|               |                  | Ketoconazole        | 7.54±0.24<sup>d</sup>       |
|               |                  | Methanol            | 0.00±0.00<sup>e</sup>       |
| Potato        | 10               | Extract 5.7 mg/ml   | 6.53±0.40<sup>b</sup>       |
|               |                  | Extract 3.8 mg/ml   | 6.26±0.01<sup>c</sup>       |
|               |                  | Extract 1.9 mg/ml   | 0.00±0.00<sup>d</sup>       |
|               |                  | Ketoconazole        | 7.88±0.16<sup>e</sup>       |
|               |                  | Methanol            | 0.00±0.00<sup>f</sup>       |
| Potato        | 15               | Extract 5.7 mg/ml   | 7.18±0.11<sup>b</sup>       |
|               |                  | Extract 3.8 mg/ml   | 0.00±0.00<sup>b</sup>       |
|               |                  | Extract 1.9 mg/ml   | 0.00±0.00<sup>c</sup>       |
|               |                  | Ketoconazole        | 7.43±0.22<sup>d</sup>       |
|               |                  | Methanol            | 0.00±0.00<sup>e</sup>       |
Means were calculated by one-way ANOVA and separated by Duncan least Significant Difference at 5% level. Value with same alphabets does not differ significantly. Ketoconazole as positive control, methanol as negative control.

Based on antifungal activity from secondary metabolites obtained from *Fusarium oxysporum* against *Candida albicans* showed sweet potato for 15 day fermentation possessed highest antifungal activity with inhibition zone diameter 8.01 mm at 5.7 mg/ml.

Inhibition zone diameters of ethyl acetate extract from *Fusarium oxysporum* against *Escherichia coli* is shown in Table 2.

**Table 2.** Ethyl acetate extract from *Fusarium oxysporum* metabolite and inhibition zone diameters against *Escherichia coli*
| Material     | Concentration 1 | Concentration 2 | Concentration 3 | Concentration 4 | Concentration 5 |
|--------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Corn 25      | 3.8 mg/ml       | 1.9 mg/ml       | 5.7 mg/ml       | 3.8 mg/ml       | 1.9 mg/ml       |
|              | 9.97±0.51       | 8.37±0.51       | 11.16±0.39      | 8.66±0.21       | 7.37±0.31       |
|              | c               | d               | b               | c               | d               |
| Amoxan       | 13.83±0.15      | 7.65±0.20       | 7.63±0.27       | 7.67±0.16       | 7.56±0.26       |
| Methanol     | 0.00±0.00       | 0.00±0.00       | 0.00±0.00       | 0.00±0.00       | 0.00±0.00       |
| Potato 5      | 5.7 mg/ml       | 3.8 mg/ml       | 1.9 mg/ml       | 5.7 mg/ml       | 3.8 mg/ml       |
|              | 0.00±0.00       | 0.00±0.00       | 0.00±0.00       | 0.00±0.00       | 0.00±0.00       |
| Amoxan       | 7.65±0.20       | 7.63±0.27       | 7.87±0.12       | 7.56±0.26       | 7.63±0.22       |
| Methanol     | 0.00±0.00       | 0.00±0.00       | 0.00±0.00       | 0.00±0.00       | 0.00±0.00       |
| Potato 10     | 5.7 mg/ml       | 3.8 mg/ml       | 1.9 mg/ml       | 5.7 mg/ml       | 3.8 mg/ml       |
|              | 0.00±0.00       | 0.00±0.00       | 0.00±0.00       | 0.00±0.00       | 0.00±0.00       |
| Amoxan       | 7.63±0.27       | 7.67±0.16       | 7.65±0.20       | 7.63±0.22       | 7.56±0.26       |
| Methanol     | 0.00±0.00       | 0.00±0.00       | 0.00±0.00       | 0.00±0.00       | 0.00±0.00       |
| Potato 15     | 5.7 mg/ml       | 3.8 mg/ml       | 1.9 mg/ml       | 5.7 mg/ml       | 3.8 mg/ml       |
|              | 0.00±0.00       | 0.00±0.00       | 0.00±0.00       | 0.00±0.00       | 0.00±0.00       |
| Amoxan       | 7.67±0.16       | 7.56±0.26       | 7.63±0.22       | 7.56±0.26       | 7.63±0.22       |
| Methanol     | 0.00±0.00       | 0.00±0.00       | 0.00±0.00       | 0.00±0.00       | 0.00±0.00       |
| Potato 20     | 5.7 mg/ml       | 3.8 mg/ml       | 1.9 mg/ml       | 5.7 mg/ml       | 3.8 mg/ml       |
|              | 0.00±0.00       | 0.00±0.00       | 0.00±0.00       | 0.00±0.00       | 0.00±0.00       |
| Amoxan       | 7.63±0.22       | 7.56±0.26       | 7.63±0.22       | 7.56±0.26       | 7.63±0.22       |
| Methanol     | 0.00±0.00       | 0.00±0.00       | 0.00±0.00       | 0.00±0.00       | 0.00±0.00       |
| Potato 25     | 5.7 mg/ml       | 3.8 mg/ml       | 1.9 mg/ml       | 5.7 mg/ml       | 3.8 mg/ml       |
|              | 0.00±0.00       | 0.00±0.00       | 0.00±0.00       | 0.00±0.00       | 0.00±0.00       |
| Amoxan       | 7.63±0.22       | 7.56±0.26       | 7.63±0.22       | 7.56±0.26       | 7.63±0.22       |
| Methanol     | 0.00±0.00       | 0.00±0.00       | 0.00±0.00       | 0.00±0.00       | 0.00±0.00       |
| Sweet potato 5 | 5.7 mg/ml       | 3.8 mg/ml       | 1.9 mg/ml       | 5.7 mg/ml       | 3.8 mg/ml       |
|              | 7.56±0.33       | 6.60±0.22       | 6.45±0.21       | 11.49±0.53      | 10.54±0.32      |
| Amoxan       | 11.49±0.53      | 12.77±0.13      | 11.77±0.21      | 11.77±0.21      | 10.54±0.32      |
| Methanol     | 0.00±0.00       | 0.00±0.00       | 0.00±0.00       | 0.00±0.00       | 0.00±0.00       |
| Sweet potato 10 | 5.7 mg/ml      | 3.8 mg/ml       | 1.9 mg/ml       | 5.7 mg/ml       | 3.8 mg/ml       |
|              | 9.96±0.21       | 7.71±0.29       | 6.45±0.21       | 7.56±0.33       | 8.45±0.40       |
| Amoxan       | 12.77±0.13      | 11.77±0.21      | 11.77±0.21      | 11.49±0.53      | 10.54±0.32      |
| Methanol     | 0.00±0.00       | 0.00±0.00       | 0.00±0.00       | 0.00±0.00       | 0.00±0.00       |
| Sweet potato 15 | 5.7 mg/ml      | 3.8 mg/ml       | 1.9 mg/ml       | 5.7 mg/ml       | 3.8 mg/ml       |
|              | 10.54±0.32      | 8.45±0.40       | 6.71±0.06       | 11.77±0.21      | 8.45±0.40       |
| Amoxan       | 11.77±0.21      | 11.77±0.21      | 11.49±0.53      | 10.54±0.32      | 8.45±0.40       |
| Methanol     | 0.00±0.00       | 0.00±0.00       | 0.00±0.00       | 0.00±0.00       | 0.00±0.00       |
| Sweet potato 20 | 5.7 mg/ml      | 3.8 mg/ml       | 1.9 mg/ml       | 5.7 mg/ml       | 3.8 mg/ml       |
|              | 8.65±0.33       | 7.06±0.69       | 6.56±0.31       | 11.37±0.46      | 7.42±0.36       |
| Amoxan       | 11.37±0.46      | 11.77±0.21      | 11.77±0.21      | 11.49±0.53      | 10.54±0.32      |
| Methanol     | 0.00±0.00       | 0.00±0.00       | 0.00±0.00       | 0.00±0.00       | 0.00±0.00       |
| Sweet potato 25 | 5.7 mg/ml      | 3.8 mg/ml       | 1.9 mg/ml       | 5.7 mg/ml       | 3.8 mg/ml       |
|              | 7.42±0.36       | 6.44±0.21       | 6.44±0.21       | 7.42±0.36       | 6.44±0.21       |
Means were calculated by one-way ANOVA and separated by Duncan least Significant Difference at 5% level. Value with same alphabets does not differ significantly. Amoxsan as positive control, and methanol as negative control.

Based on antibacterial activity from overall extract metabolites isolated from *Fusarium oxysporum* against *E.coli* showed that corn as carbon source for 15 day fermentation indicated highest antibacterial activity with inhibition zone diameter 10.54 mm at 5.7 mg/ml. Inhibition zone diameters against *Staphylococcus aureus* is showed in Table 3.

**Table 3. Ethyl acetate extract from Fusarium oxysporum metabolite and inhibition zone diameters against S. aureus**

| Carbon source | Fermentation (Day) | Concentration assay | Averages |
|---------------|--------------------|---------------------|----------|
| Corn 5        | Extract 5.7 mg/ml  | 7.09±0.09b          |
|               | Extract 3.8 mg/ml  | 6.68±0.22c          |
|               | Extract 1.9 mg/ml  | 0.00±0.00d          |
|               | Amoxan             | 17.51±0.42a         |
|               | Methanol           | 0.00±0.00d          |
| Corn 10       | Extract 5.7 mg/ml  | 8.68±0.29b          |
|               | Extract 3.8 mg/ml  | 7.56±0.32c          |
|               | Extract 1.9 mg/ml  | 6.72±0.25d          |
|               | Amoxan             | 17.44±0.32a         |
|               | Methanol           | 0.00±0.00e          |
| Corn 15       | Extract 5.7 mg/ml  | 15.24±0.42b         |
|               | Extract 3.8 mg/ml  | 11.78±0.29c         |
|               | Extract 1.9 mg/ml  | 10.86±0.13d         |
|               | Amoxan             | 16.09±0.39a         |
|               | Methanol           | 0.00±0.00e          |
| Corn 20       | Extract 5.7 mg/ml  | 12.54±0.29b         |
|               | Extract 3.8 mg/ml  | 10.26±0.29c         |
|               | Extract 1.9 mg/ml  | 9.03±0.24d          |
|               | Amoxan             | 15.53±0.38a         |
|               | Methanol           | 0.00±0.00e          |
| Corn 25       | Extract 5.7 mg/ml  | 9.98±0.53b          |
|               | Extract 3.8 mg/ml  | 8.01±0.21c          |
|               | Extract 1.9 mg/ml  | 6.77±0.25d          |
|               | Amoxan             | 16.63±0.29a         |
|               | Methanol           | 0.00±0.00e          |
| Potato 5      | Extract 5.7 mg/ml  | 11.34±0.49b         |
|               | Extract 3.8 mg/ml  | 9.57±0.09c          |
|               | Extract 1.9 mg/ml  | 7.93±0.08d          |
|               | Amoxan             | 15.83±0.14a         |
|               | Methanol           | 0.00±0.00e          |
| Potato 10     | Extract 5.7 mg/ml  | 11.63±0.34a         |
|               | Extract 3.8 mg/ml  | 9.64±0.39e          |
|               | Extract 1.9 mg/ml  | 8.18±0.20d          |
|               | Amoxan             | 15.76±0.36a         |
|               | Methanol           | 0.00±0.00e          |
| Potato 15     | Extract 5.7 mg/ml  | 12.20±0.57b         |
|               | Extract 3.8 mg/ml  | 8.74±0.27c          |
|               | Extract 1.9 mg/ml  | 7.36±0.19d          |
|               | Amoxan             | 15.64±0.34a         |
Means were calculated by one-way ANOVA and separated by Duncan least Significant Difference at 5% level. Value with same alphabets does not differ significantly. Amoxsan as positive control, and methanol as negative control.

Based on antimicrobial activity from overall extract metabolites were isolated from Fusarium oxysporum toward S. aureus, corn as carbon source for 15 day fermentation posses highest antibacterial activity with inhibition zone diameter 15.24 mm at 5.7 mg/ml..

The result of metabolite screening was carried out toward overall ethyl acetate extract isolated from Fusarium oxysporum (Table 4).

| Table 4. Screening metabolit test of ethyl acetate extract isolated from Fusarium oxysporum |
| --- |
| **Carbon source** | Fermentation (day) | Alkaloid | Phenolic | Flavonoid | Saponin | Terpenoid |
| Corn | 5 |  |  |  |  | + |
| 10 |  |  |  |  |  | + |
| 15 |  |  |  |  |  | + |
| 20 |  |  |  |  |  | + |
| 25 |  |  |  |  |  | + |
| Potato | 5 |  |  |  |  | + |

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# Table 4. Screening metabolit test of ethyl acetate extract isolated from *Fusarium oxysporum*
Metabolites isolated from *Fusarium oxysporum* achieved maximum production at 15 day fermentation and decreased at 20 and 25 day fermentation, which was assumed to have started death phase [12]. Production of secondary metabolites by microorganisms was often influenced by primary metabolism. The composition and medium concentration were closely linked with the metabolic producing capacities by microorganism and greatly influenced the biosynthesis of bioactive molecules. There were many factors playing important to produce secondary metabolite such as carbon source and fermentation time [13]. Overall, metabolite extract with corn as carbon source and 15 day fermentation (C15) showed better activity than other extracts. Inhibition zone diameter lower than triterpenoid saponin was isolated from endophytic fungi *Fusarium* sp from *Panax notogingseng* plant [14].

Based on screening metabolite *Fusarium oxysporum* have the ability to produce secondary metabolite, overall comprise terpenoid group through mevalonate pathway. Mostly, crude extract from *Fusarium oxysporum* showed antimicrobial activity against Gram positive bacteria of genera *Staphylococcus aureus*. Previously Ibrahim et al (2016) reported endophytic fungus *Fusarium* sp isolated from the roots *Mentha longifolia* have the ability to produce two new tetracyclic terpenoid, and antibacterial activity [15]. *Fusarium chlamydosporium* isolated from leaves *Anvillea garcinii* produced aminobenzamide derivative with potent cytotoxic and antimicrobial agent (Ibrahim et al., 2018) [16].

C15 metabolite has been analyzed both thin layer chromatography and high performance liquid chromatography and depicted 4 component with Rf 0.3; 0.7; 0.84; dan 0.93 respectively using ethyl acetate 100% as mobile phase for TLC and water and methanol for HPLC with *t*<sub>R</sub> 16.86, 17.62, 18.23 and 21.54 min. Every peak confirm one compound, it was showed at Fig 1.

![Chromatogram HPLC C15](image_url)

**Figure 1.** Chromatogram HPLC C15.
4. Conclusion

*Fusarium oxysporum* had ability to produce secondary metabolites. Based on optimization of carbon source and time of fermentation, corn is better carbon source than other medium for 15 day fermentation. Screening metabolite showed that C15 comprised terpenoid. Chromatogram analyzed using TLC and HPLC achieved 4 component.

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Reference

[1] Simarmata, Rumella, Sylvia Lekatompessy, and Harmastini Sukiman. 2007. *Berkala Penelitian Hayati*. 13(April):85–90.

[2] Zhao, J., T. Shan, Y., Mou, &. Zhou, L. 2011. *Mini Review in Medicinal Chemistry* 11:159–68.

[3] Vagelas, I., & Gowen, S. R. 2012. *Pakistan Journal Phytophatol*. 24 (1): 32-38.

[4] Yasinok., Eryasin, A., Sahin, F. I., & Haberal, M. 2008. *Tarim Bilimeri Dergisi*. 14(4):374–80.

[5] Radji, Maksum. 2005. *Majalah Ilmu Kefarmasian*. 2(3):113–26.

[6] Saryono., Finna, P., Nurmala, S., Wahyu, P. W., & Aulia, A. 2018. *Research Journal of Chemistry and Environment*.

[7] Devi, S., Luthin, I., Ardhi, A., Pratiwi, N. W., & Saryono. 2018. *Research Journal of Chemistry and Environment*.

[8] Lorenita, M., Haryani, M., Puspita, F & Trihartomo, D. 2013. *Journal of Agricultural Technology*. 9(3):585–90.

[9] Saryono, Hendris, S., Fitryah, D., Jose, C., Nugroho, T. T. & Ardhi. 2015. *Journal of Chemical and Pharmaceutical Research*. 7(9):201–8.

[10] Fitriyah, D., Christine, J., & Saryono. 2013. *Journal Indonesia. Acta* 3(2).

[11] Shinta, D. Y., Yusmarini., Sonata, H., Teruna, H. Y., & Saryono. *Journal of Chemical and Pharmaceutical Research*. 7(9S): 239-245.

[12] Pavani, M., Sankar, G., Prabhakar, T., Bhavani, A., & Sravani, P. 2014. *International Journal of Pharmaceutical Science Review and Research*. 33: 192-196.

[13] Khatab, A. I., Babiker, E. H., & Saeed, H. A. 2014. *International Current Pharmaceutical Journal*. 5(3): 27-32.

[14] Jin, Z., Gao, L., Zhang, L., Liu, T., Yu, F., Zhang, Z., Guo, Q., & Wang, B. 2016. *Natural Product Research*. 31(22): 2700-2703.

[15] Ibrahim, S. R. M., Mohamed, G. A., & Ross, S. A. 2016. *Phytochemistry Letters*. 15: 125-130.

[16] Ibrahim, S. R. M., Mohamed, G. A., Alhaidari, R. A., Zayed, M. F., Elkholy, A. A., Elkhayat, E. S., & Ross, S. A. 2018. *Bioorganic and Medicinal Chemistry*. 26: 786-790.