Chemical Compounds from Fungus Syncephalastrum racemosum Isolated as Endophytic from Ageratum conyzoides

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Abstract: Ageratum conyzoides known as bandotan is a plant widely grown in Indonesia. This plant is used for the treatment of various diseases such as antibacterial, anti-diabetic, anti-inflammatory, antioxidant, and analgesic. The active compounds contained in this plant include alkaloids, flavonoids, tannins, glycosides, minerals and other compounds. Plants that have ethno medicine history are promising candidates to obtain bioactive compounds from their endophytic fungi. In the present study, chemical compounds were isolated from endophytic fungus Syncephalastrum racemosum from the stem of Ageratum conyzoides with the chromatography method. The structures of the compounds were determined by spectroscopy analysis. The compounds are aromatic group.

1 INTRODUCTION

Ageratum conyzoides is known as bandotan. In some countries, bandotan is considered a weed plant and its growth is very fast. This plant comes from tropical America, especially Brazil. Most of the A. conyzoides plants are found in Mexico, Central America, the Caribbean Islands, and Florida. But now bandotan is also found in several sub-tropical and tropical countries, including in Indonesia. Bandotan plants are now widespread in various parts of Indonesia. Ageratum conyzoides often grow in yards, roadside fields, dry rice fields, river banks, and areas with a lot of shrubs. This plant has a long history in its use for traditional medicine in several countries. This plant has medicinal bioactive properties. Therefore bandotan plants can be classified as herbal plants (Soerjani et al., 1987; Darma, 1987; Singh et al., 2013; Odeleye et al., 2014; Janarthanan et al., 2016).

In general A. conyzoides contains a variety of bioactive compounds including flavonoids, alkaloids, coumarins, essential oils, tannins, chromene, benzofuran and terpenoids. All parts of this plant have the ability to be anti-inflammatory and anti-allergic. In addition, anti-diareal, nematoside, anticoagulant, smooth muscle relaxant, hemostatic, analgesic, antifungal, antibacterial, and hypothermic factors are also reported (Kamboj and Saluja, 2008; Ndip et al., 2009; Awad et al., 2013; Baltiari et al., 2017).

In Bogor, A. conyzoides is widely known as a wound medicine. According to Heyne, these plant leaves are squeezed, mixed with lime, applied to fresh wounds. Decoction of leaves is also used to treat chest pain, while extracting the leaves for eye drops. Mashed roots are applied to the body to treat fever, the extract can be drunk. bandotan also to treat stomach ache and to cure broken bones (Heyne, 1987; Darma, 1987).

Endophytic fungi are microorganisms that live to form colonies in plant tissues without endangering their host plants. Each high-level plant contains several endophytic microbes which produce secondary metabolites as a result of coevolution or genetic transfer (genetic recombination) from the host plant to endophytic fungi. The ability of endophytic fungi to produce phytochemical compounds that are also produced by their host plants may be related to the presence of genetic recombination of endophytic fungi with hosts during the time of their evolution (Elfita et al, 2013; Sandhu et al., 2014; Golinska et al., 2015).
Endophytic fungi are a source of genetic diversity with various possible new species that have not been described. Therefore, the need for natural products for new antibiotics, chemotherapy and agrochemicals that have high activity, low toxicity, but do not disturb environmental ecology can be expected to be obtained from this endophytic fungus (Rajamanikyam et al., 2001; Kaur et al., 2018; Santoyo et al., 2016; Elfita et al., 2015).

Previous studies have reported six endophytic fungi isolated from the leaves and stems of Ageratum conyzoides (Elfita et al., 2019). Screening the antibacterial activity of ethyl acetate extract from liquid culture showed that the Syncephalastrum racemosum fungus had the highest activity. In this paper, the chemical compounds contained in the antibacterial active extract of the S. racemosum fungus are reported.

2 MATERIALS AND METHODS

2.1 Chemicals

The materials used in this study include endophytic fungi, Syncephalastrum racemosum which have been previously isolated, Potato Dextrose Agar (PDA), Potato Dextrose Broth (PDB), alcohol 70%, KLT kiesel gel 60 230 mesh, silica gel (70–230 mesh, 10 g) eluted with gradient solvent system (n-hexane-EtOAc-methanol). The eluates was collected in a vial (10 mL) and analyzed by TLC under UV lamp. The eluate with the same stain pattern was combined into one fraction. The major and fluorinated stain fractions were then separated and purified. Sub-fractions were separated again using column chromatography over silica gel (70-230 mesh) with gradient eluents. Eluates was collected in a vial (5 mL volume) and analyzed by TLC. Eluates with the same stain pattern were combined into one fraction. The subfraction re-purified column chromatography to obtain the pure compounds. The compounds were analyzed for their chemical structure using spectroscopic methods.

3 RESULT AND DISCUSISON

3.1 Isolation of Chemical Compounds

The filtrate was evaporated by rotary evaporator to obtain concentrated ethyl acetate extract (Marcellano et al., 2017; Elfita et al., 2012).

2.4 Isolation of Secondary Metabolites and Identification of Structures

The ethyl acetate concentrate extract of endophytic fungi was separated by chromatography column. The sample was prepared by preadsorption and put into the column over silica gel 70-230 mesh. separation was carried out using using eluents with gradient system (n-hexane-EtOAc-methanol). The eluates was collected with a vial (10 mL) and analyzed by TLC under UV lamp. The eluate with the same stain pattern was combined into one fraction. The major and fluorinated stain fractions were then separated and purified. Sub-fractions were separated again using column chromatography over silica gel (70-230 mesh) with gradient eluents. Eluates was collected in a vial (5 mL volume) and analyzed by TLC. Eluates with the same stain pattern were combined into one fraction. The subfraction re-purified column chromatography to obtain the pure compounds. The compounds were analyzed for their chemical structure using spectroscopic methods.
3.2 Identification of Chemical Compounds

Compound 1  The UV spectrum of compound 1 in methanol solvents (Figure 1) showed absorption at $\lambda_{\text{max}}$ 224 and 274 nm. Addition of NaOH does not cause a bathochromic shift. This indicates the absence of a free hydroxyl group on the aromatic ring (Muharni et al., 2014).

The FTIR spectrum (Figure 2) showed the presence of characteristic absorption bands at $\nu$ 3344.3 cm$^{-1}$ which is absorption for OH, while the absorption of 2854.7-2958.8 cm$^{-1}$ is a typical for aliphatic C-H. In addition there is absorption in the area of 1728.22 cm$^{-1}$ which is absorption for C = O bonds. The presence of aromatic C = C is characterized by absorption at 1600.9 and 1462.04 cm$^{-1}$ and typical absorption of C-O ester in the area of 1274.9 cm$^{-1}$.

The signal at $\delta_H$ 4.22 ppm (2H, m) showed the presence of O-CH$_2$ group (2 CH$_2$ groups). Furthermore, the signal in the area of $\delta_H$ 7.52 and $\delta_H$ 7.70 ppm (2H, dd J = 3.4 and 5.8 Hz) respectively showed the presence of four aromatic protons (coupled meta and ortho). Each signal represented two protons. The compound 1 as an aromatic ring in the form of symmetrical dissubstitution (Habib and Karim, 2009).

Figures 3 showed the presence of 6 proton signals. The signal at $\delta_H$ 0.89 ppm (12H, m) for 4 methyl groups. Furthermore, the signal that accumulates in the area around $\delta_H$ 1.30-140 ppm (18H, m) showed the presence of aliphatic CH$_2$ groups. In the $^1$H-NMR spectrum also shows that the signal in the area of 1.68 ppm (2H, m) is a signal for two CH groups coupled by protons through three bonds.

The $^1$H-NMR spectrum of compound 2 (Figure 5) showed a group of protons similar to compound 1. It appears that compound 2 also has signals in the regions $\delta_H$ 7.53 dan $\delta_H$ 7.71 ppm (2H, and J = 3.3 and 5.7Hz) respectively showed the presence of four aromatic protons (coupled meta and ortho). Each signal represented two protons. The compound 2 as an aromatic ring in the form of symmetrical disubstitution. The next similar signal, at $\delta_H$ 4.22 ppm (2H, m) for O-CH$_2$ group (2 CH$_2$ groups) and at $\delta_H$ 0.5-2.00 ppm is a long chain of aliphatic protons. The difference is the appearance of a proton signal at

Figure 1: The UV spectrum of compound 1.

Figure 2: The FTIR spectrum of compound 1.

Figure 3: The $^1$H-NMR spectrum of compound 1 (500 MHz $^1$H- in CDCl$_3$).

Figure 4: The $^{13}$C-NMR spectrum of compound 1 (125 MHz $^{13}$C- in CDCl$_3$).
\( \delta_H 3.66 \text{ ppm which is a methoxyl proton} \) (Habib and Karim, 2009; Muharni et al., 2014).

Figure 5: The \( ^1\text{H}-\text{NMR} \) spectrum of compound 2 (500 MHz \( ^1\text{H} \) in CDCl₃).

4 CONCLUSIONS

Chemical compounds isolated from the ethyl acetate extract of liquid cultured of endophytic fungi Syncephalastrum racemosum were identified as phthlate derivatives.

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