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, antidiareal, 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; Bahtiar 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).

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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., 20017; 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 kiessel gel 60 F254 20 x 20 cm, silica gel G 60 70-230 mesh. Organic solvents such as *n*-hexane, ethyl acetate and methanol.

2.2 Source of Endophytic Fungi

Syncephalastrum racemosum of *Ageratum conyzoides* from stock fungus (Elfita et al., 2019). The fungal was identified molecularly in the biological research center-LIPI Cibinong. The *Ageratum conyzoides* was collected in Juli 2018 from Indralaya, Ogan Ilir, South Sumatra.

2.3 Cultivation and Extraction of Endophytic Fungi *S. racemosum*

The suspension of endophytic fungus (which was previously isolated) was prepared by taking 6 ose of endophytic fungi, then inoculated into 200 mL of PDB (in 5x 1 liter bottles). They were incubated at room temperature and static conditions for 6 weeks. Furthermore, endophytic fungal cultures are harvested by separating biomass. the liquid culture was extracted using ethyl acetate in a separating funnel. then the extract was evaporated using a 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. Eluate is collected in a vial (5 mL volume) and analyzed by TLC. Eluat 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 give a EtOAc extract (4.1 g). The extract (4.1 g) was separated over a silica gel column (70.230 mesh, 40 g) with gradient solvent system of n-hexane/EtOAc/MeOH as the eluent to give five fractions (A1–A5). Fraction A1 (2.34 g) was subjected to column chromatography (CC) eluted with *n*-hexane-EtOAc (10:0→0:10) as to give four sub-fractions (A11–A14). Subfraction A12 (1.1 g) was subjected to CC over a silica gel (70-230 mesh, 20 g) eluted with with *n*-hexane-EtOAc (9:1) to give compound 1 (479 mg). Fraction A3 (709 mg) was separated to CC eluted with *n*-hexane-EtOAc (7:3→0:10) as to give five sub-fractions (A31–A35). Subfraction A31 (91 mg) was subjected to CC over a silica gel (70-230 mesh, 10 g) eluted with with *n*-hexane-EtOAc (5:5) to give compound **2** (21 mg). Subfraction A35 (56 mg) was subjected to CC eluted with *n*-hexane-EtOAc (4:6) to give Compound **3** (11 mg, not identified).

3.2 Identification of Chemical Compounds

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

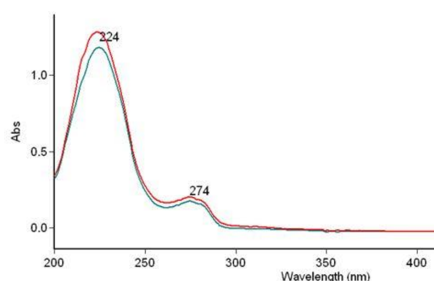

Figure 1: The UV spectrum of compound **1.**

The FTIR spectrum (Figure 2) showed the presence of characteristic absorption bands at υ 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}.

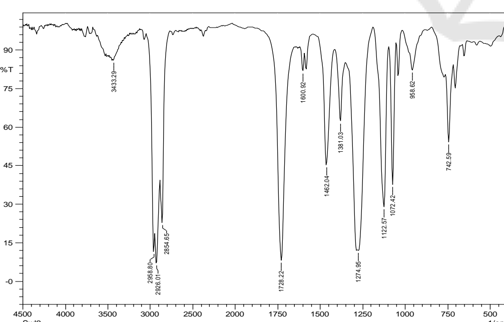

Figure 2: The FTIR spectrum of compound 1.

Figures 3 showed the presence of 6 proton signals. The signal at δ_H 0,89 ppm (12H, *m*) for 4 methyl groups. Furthermore, the signal that accumulates in the area around δ_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 signal at δ_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 δ_H 7,52 and δ_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 disubstitution (Habib and Karim, 2009).

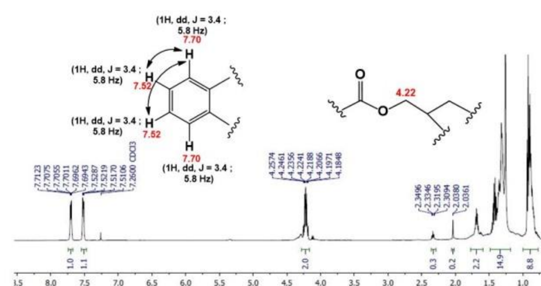

Figure 3: The ^{1}H-NMR spectrum of compound 1 (500 MHz ^{1}H- in $CDCL_3$.

The ^{13}C-NMR spectrum (Figure 4) showed the presence of 12 signals consisting of 8 C sp3 signals that appear below 100 ppm (δ_C 11.1; 14.2; 23.1; 23.9; 29.1; 30.5; 38.9; and 68.3 ppm) and 4 other signals that appear above 100 ppm (δ_C 128.9; 131.0; 132.6; and 167.8 ppm). are signals for C sp^2.

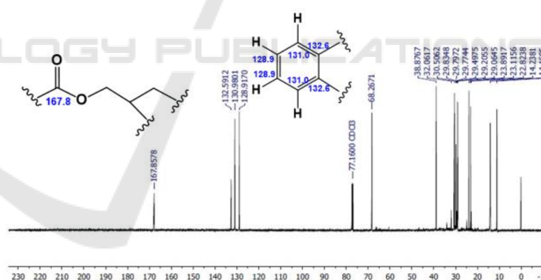

Figure 4: The ^{13}C-NMR spectrum of compound 1 (125 MHz ^{13}C- in $CDCL_3$).

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 δ_H 7,53 dan δ_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 δ_H 4,22 ppm (2H, *m*) for O-CH_2 group (2 CH_2 groups) and at δ_H 0.5-2.00 ppm is a long chain of aliphatic protons. The difference is the appearance of a proton signal at

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δ_H 3.66 ppm which is a methoxyl proton (Habib and Karim, 2009; Muharni et al., 2014).

Figure 5: The ^{1}H-NMR spectrum of compound 2 (500 MHz ^{1}H- in $CDCL_3$).

4 CONCLUSIONS

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

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