Triacylglycerols Produced by Biomass of Endophytic Fungus Neopestalotiopsis Surinamensis from the Scurrula Atropurpurea Leaves

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Abstract

In Indonesia, Scurrula atropurpurea is a medicinal plant known as *benalu*. Triacylglycerol can be obtained from plants, animals, algae, and microorganisms such as endophytic fungi. Triacylglycerol can be used in cosmetics, food, and medicine because they have biological activities such as antitumor, antibacterial, and cytotoxic. Besides, the compound can be used as a biodiesel substitute for triacylglycerols sourced from oil palm. This study aims to isolate and characterize triacylglycerol from biomass of endophytic fungal *N. surinamensis* from the *S. atropurpurea* leaves. The compound was isolated and purified by the column chromatography method. The structure of the compound was determined by spectroscopic data (FTIR, 1H-NMR, and 13C-NMR). Analysis of the spectrum and compared with the literature, the isolated compound is a triacylglycerol.

Keywords: triacylglycerol, endophytic, isolated, structure, FTIR, 1H-NMR, 13C-NMR

INTRODUCTION

Endophytic fungi are microorganisms that live in plant tissue without causing harm to the host. Endophytic fungi have a high level of biodiversity. They can produce new bioactive compounds that are potentially exploited in the fields of medicine, food, agriculture, and industry [1-3]. Endophytic fungi are an excellent bioactive source due to its ability in producing bioactive in a short period and does not require a large growing area [4].

The lipid group that is commonly stored in plants is triacylglycerol (TAG), a fatty acid of triester bound to glycerol. Lipids serve as a carbon and energy reserves and are precursors for membrane lipids and steroid biosynthesis in plants. Besides, the microorganisms, such as endophytic fungi, also have a high ability to produce triacylglycerol, which is named with single cell oil. The triacylglycerol has potential commercial value as a food supplement, pharmaceutical, and biodiesel [5-7].

The fatty acids of triacylglycerol can be distinguished from long-chain alkyl (R) groups. The difference is in the chain's length, the number of double bonds, and the carbon position of the double bonds. There are two groups of fatty acids namely saturated fatty acids (SFA) and unsaturated fatty acids (mono and poly double bonds) [8]. Yinghua et al. [2] examined clinical trials on female and male subjects that consumed medium and long-chain triacylglycerol. The results show that in male hypertriglyceridemia subjects, the triacylglycerol can reduce body weight and body fat and increase blood lipid profile. [9]. Xue et al. [5] have carried out Previous similar studies on Chinese hypertriglyceridemia subjects. The result of consuming medium and long chain triacylglycerol can reduce body fat and blood triglycerides [5]. Triacylglycerol is also reported to inhibit triple negative mammary breast cancer cell proliferation [10]. Another study reported that the triacylglycerol of endophytic fungi as a source of biofuel precursors [11]. Fungal lipids containing polyunsaturated fatty acids (PUFAs) are valuable products because of their health promoting roles. The production of fungal lipids has many advantages, such as a short life cycle, less labor...
needed, less affection by place, season aclimateasier to
scale up [12].
Previous studies have isolated the compound
Quercetin-3-O-α-L-rhamnopyranoside from the liquid
culture of fungus N. surinamensis from the S.
atropurpurea leaves [13]. Besides, the biomass of
dendophytic fungi is a source of lipid fungal which has
different the fatty acid composition. This study
reported the stages of isolation and identification of
fungal lipids from biomass of N. surinamensis. The
triacylglycerol of lipid fungal produced by endophytic
gungi is included in the raw material of non-edible oil
that can be used as raw material for the pharmaceutical,
food, cosmetics, and biodiesel industries.

MATERIALS AND METHODS

Materials
The sterilization and medium for fungal growth
used in this study include: alcohol 70%, distilled water,
potato dextrose agar (PDA), and potato dextrose broth
(PDB). The chemicals for the isolation of pure
compounds include various organic solvents
(methanol, ethyl acetate, n-hexane), for thin layer
chromatography analysis (TLC, kiessel gel 60 F254 20
x 20 cm), stationary phase on column chromatography
(CC, silica gel G 60 70-230 mesh).

Endophytic fungal
Neopestalotiopsis surinamensis of Scurrula
atropurpurea from stock fungus [13]. The fungal was
identified molecularly in the biological research
center-LIPI Cibinong.

Cultivation and extraction of N. surinamensis
The mycelia agar plugs (six pieces, 0.5 x 0.5 cm)
were inoculated with ose needles into 0.2 L potato
dextrose broth (PDB) medium. The cultures were made
in 3 L of PDB medium placed into 15 erlenmeyers.
Incubation was carried out for three weeks at ± 27°C
under static conditions. Furthermore, biomass was
filtered using filter paper to separate from the broth
cultures. The biomass is rinsed with distilled water and
dried to a constant weight in an oven at 60°C. Dry
biomass was ground and extracted by soxletation with
methanol. Then the methanol extract was evaporated in
the rotary evaporator to get concentrated extract [14,
15].

Isolation of triacylglycerol and identification of
chemical structure
The concentrated MeOH extract (5.31 g) was
separated by chromatographic techniques (CC) over
stationary phase (silica gel, 50 g) and eluted with n-
hexane-ethyl acetate (10:0&0:10). The eluates (10 mL)
were collected in bottles and analyzed by TLC to
obtain three subfractions (A-C). Fraction A (1.21 g) in
the form of a mixture of oil was separated by CC (silica
gel, 40 g) and eluted by n-hexane-ethyl acetate
(10:0&9:1) to obtain a pale yellow oil (0.72 g). The oil
was identified by IR, 1H-NMR, and 13C-NMR
spectrum, and compared with triacylglycerols
spectrum from the literature.

RESULT AND DISCUSSION

Fungal lipids production
The fungal lipids (0.72 g) were obtained from the
separation of 5.31 g dry of biomass by column
chromatography. The yield obtained is 13.6%. The
environment and the host of fungus are very influential
in their ability to produce fungal lipids. Fungi that live
in oily environments and oil-producing host plants
have a higher ability to produce fungal lipids that reach
35% of the biomass's dry weight. The research shows
that the fungal lipids from the biomass of endophytic
fungal can be increased again by providing the right
nutritional ratio for fungal growth media. The fungal
needs a source of nutrients, including C, N, and P, for
growth. These three nutrients can be varied by
comparing to produce more fungal lipids [16].

The dry weight of biomass and fungal lipids
content were obtained at different optimal incubation
period for each fungus [17]. Beside, researched the
highest fungal lipids content of the dry weight of
biomass whose time varies for each fungus. The lipid
was produced the highest at the optimal time and
decreases the next day. Sources of carbon and nitrogen
contained in the medium are reduced, then the lipids
produced by fungi will be broken again into organic
components that can be used as a source of nutrition
for growth [18].

Identification of fungal lipids as triacylglycerol
The IR spectrum of fungal lipids from N.
surinamensis and the spectrum of triacylglycerol from
literature shown in Figure 1. A comparison of the two
spectra was showed high similarity [19]. The
significant peaks were appeared in signals 1747 - 1855
cm⁻¹, which confirmed the presence of carbonyl
groups, and signals at 2855 - 2924 cm⁻¹ as the methyl
groups.
Figure 1. The IR spectrum of fungal lipids from N. surinamensis (A) and triacylglycerol from literature (B)

Figure 2. The 1H-NMR spectrum of fungal lipids from N. surinamensis (1H-500 MHz, in CD3Cl)
The 1H-NMR spectrum of fungal lipids (Figure 2) showed the presence of the signals at the most downfield proton. The signal at 5.31 ppm (4H, m) confirmed the presence of unsaturated carbon (vinylic proton) in the long chain of the fungal lipids, followed by a signal for glyceryl methine proton at 5.25 ppm (1H, m). A signal at 4.27 ppm (2H, dd, 4.25 Hz) and a signal at 4.12 (2H, m) are two methylene groups attach at the glyceryl. The methylene groups attach to the ester carbonyl appear on the signal at 2.74 ppm (1H, t, J = 6.45 Hz), whereas the signal at 2.28 ppm (6H, m), 2.01 (6H, m), 1.59 (6H, m), represents the fatty acids moieties of fungal lipids. The terminal methyl protons were showed at 0.86 ppm (9H, m).

Harry-O’kuru et al. [20] reported the 1H-NMR spectrum of triacylglycerol from Maclura pomifera L. The chemical shift values of triacylglycerol at the most downfield signals as the vinylic proton at 5.4 ppm (9−10H), followed by a signal at 5.3 ppm (1H, m), which is the glyceryl methine proton. A signal at 4.3 ppm (2H, dd, J = 4.3 Hz) and a signal at 4.1 (2H, m) are the glyceryl CH2s. The signal at 2.75 ppm (4H, t, J = 6.6 Hz, 6.5 Hz) represents the methylene groups attach to the ester carbonyl, whereas the signal at 2.30 ppm (6H, m), 2.03 (11H, m), 1.6 (6H, m), represents the fatty acids moieties of triacylglycerol. The terminal methyl protons are the overlapping triplets (multiplet) at 0.87 ppm [20]. The 1H-NMR spectrum of triacylglycerol from osage orange (M. pomifera L.) is similar to the 1H-NMR spectrum of fungal lipids from N. surinamensis.

The spectrum of 13C-NMR (Figure 3) showed the presence of three signals of glyceryl carbon (−CH−O−) at δC 69.0 ppm, (−CH2−O−) glyceryl at δC 64.5, 62.1 ppm and three ester carbonyls carbon at δC 173.2, 173.19, and 172.8 ppm, which is a characteristic of fungal lipids. Furthermore, the signal at δC 126-131 ppm indicated eight unsaturated carbon in the fatty acids moieties of fungal lipids, whereas the carbon at δC 10-35 ppm is saturated carbon from the fatty acids moieties.

Harry-O’kuru et al. [20] reported the 13C-NMR spectrum of triacylglycerol from osage orange (M. pomifera L.). The chemical shift values of triacylglycerol showed the presence of three signals of glyceryl carbon (−CH−O−) at δC 68.9 ppm, (−CH2−O−)
glyceryl at δC 62.0, 60.3 ppm, three ester carbonyls carbon at δC 173.17, 173.1, 172.7 ppm, which is a characteristic of triacylglycerol. The signal at δC 120-135 ppm indicated the presence of unsaturated carbon in the fatty acids moieties [20]. The 13C-NMR spectrum of triacylglycerol from osage orange (M. pomifera L.) is similar to the 13C-NMR spectrum of fungal lipids from N. surinamensis.

Based on the analysis of the spectroscopic data and compared with the literature, fungal lipids have a chemical structure as triacylglycerol. The structure of triacylglycerol from N. surinamensis is shown in Figure 4.

**Figure 4.** The structure of triacylglycerol

The study of literature shows that an endophytic fungus can be developed as a source of fungal lipids if yield ≥ 20%. The yield of triacylglycerol from biomass of N. surinamensis needs to be improved through further research to find the optimum conditions, including the type of media, nutritional composition, pH, temperature, and incubation period [16, 19]. The research will open up opportunities in the supply of raw materials for the pharmaceutical, food, cosmetics, and biodiesel industries. The advantages of raw materials derived from endophytic fungi are that it does not require a large area of land, can be produced in a short time, low cost, and the same quality as raw materials from plants such as palm oil and castor oil.

**CONCLUSION**

Separation of the biomass of N. surinamensis by column chromatography yielded the fungal lipids 13.6%. Based on the analysis by the spectroscopy method and compare by literature, the fungal lipids as a triacylglycerol. The research can be developed as raw material for the pharmaceutical, food, cosmetics, and biodiesel industries through further research to find the optimum conditions.

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