



Research article

Isolation of active compounds from *Streptomyces sennicomposti* GMY01 and cytotoxic activity on breast cancer cells lineRifki Febriansah^a, Triana Hertiani^b, Jaka Widada^c, Muhammad Taher^d, Ema Damayanti^e, Mustofa Mustofa^{f,*}^a School of Pharmacy, Faculty of Medicine and Health Sciences, Universitas Muhammadiyah Yogyakarta, Indonesia^b Pharmacognosy and Phytochemistry Laboratory, Pharmaceutical Biology Department, Faculty of Pharmacy, Universitas Gadjah Mada, Yogyakarta, Indonesia 55281^c Department of Agricultural Microbiology, Faculty of Agriculture, Universitas Gadjah Mada, Yogyakarta, Indonesia 55281^d Department of Pharmaceutical Technology, Kulliyah of Pharmacy, International Islamic University Malaysia, Malaysia^e Research Center for Food Technology and Processing, National Research and Innovation Agency (BRIN), Gunungkidul, Yogyakarta, Indonesia 55681^f Department of Pharmacology and Therapy, Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada, Yogyakarta, Indonesia 55281

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ABSTRACT

The occurrence of resistance to anticancer and the emergence of serious side effects due to chemotherapy is one of the main problems in cancer treatment, including breast cancer. The need for effective anticancer with a specific target is urgently required. *Streptomyces* are widely known as the potential producers of new anticancer molecules. Previously reported that the methanol extract of *Streptomyces sennicomposti* GMY01 isolated from Krakal Coast, Gunungkidul had very strong cytotoxic activity against MCF-7 and T47D breast cancer cells with IC₅₀ values of 0.6 and 1.3 µg/mL, respectively. The following study aimed to isolate and identify active compounds of the *S. sennicomposti* GMY01 and evaluate its cytotoxic activity. The study was started by re-culturing and re-fermented optimization of *S. sennicomposti* GMY01 in a larger volume, then the bacteria were extracted using methanol following the bioassay-guided isolation of the extract obtained. The active compounds obtained were then structurally determined using UV/Vis spectroscopy, Fourier Transform-Infrared (FT-IR), Liquid Chromatography-Mass Spectroscopy (LC-MS), ¹H NMR, and ¹³C NMR and analyzed for their cytotoxic activity using MTT assay on MCF-7 and normal Vero cells line. The results showed that the culture of the *S. sennicomposti* GMY01 using Starch Nitrate Broth (SNB) media yields the best results compared to other culture media. An active anticancer compound namely mannotriose was successfully isolated from the methanol extract with an IC₅₀ value of 5.6 µg/mL and 687 µg/mL against the MCF-7 and Vero cells lines, respectively, indicating that this compound showed strong cytotoxic activity with high selectivity.

* Corresponding author.

E-mail addresses: mustofakugm@gmail.com, mustofak@ugm.ac.id (M. Mustofa).

1. Introduction

Cancer is a cellular disease characterized by the disruption or failure of multiplication regulatory mechanisms and other homeostatic functions in multicellular organisms. This results in cells growing and developing uncontrollably, even then spreading and attacking other body tissues. Cancer is still a major health problem in the world due to its high morbidity and mortality. In 2020, it was estimated that 19.3 million new cases of cancer were reported with 10.0 million deaths [1]. Breast cancer is the second most deadly disease after lung cancer. In Indonesia in 2020, the cases of breast cancer were 65,858 with 22,430 deaths [2]. Based on the national health survey, the Yogyakarta Province, one of the 38 provinces of Indonesia, has more than twice the national cancer prevalence. In Yogyakarta, the majority of cases of breast cancer are diagnosed at the late stage, with Yogyakarta City having the highest proportion of diagnoses at stage 4. The trend of increasing breast cancer incidence is fastest in Yogyakarta City with an average percentage change per year of 18.77 % [3].

Several risk factors are associated with the development of breast cancer, namely cigarette smoke, alcohol consumption, age at first menstruation, age at first delivery, dietary fat, and family history [4]. Hormones also play an important role in the occurrence of breast cancer. Estradiol and progesterone in the normal menstrual cycle increase the risk of breast cancer. This occurs in breast cancer that has estrogen receptors, where about 50 % of breast cancer cases are estrogen-dependent cancers [5].

Although the molecular mechanisms that influence the risk of breast cancer and its progression have not been known with certainty, the activation of oncogenes due to genetic modification or the occurrence of epigenetic modifications can lead to the occurrence of breast cancer cell multiplication and migration [6]. Several oncogenes are reported to be involved in breast cancer carcinogenesis, including Ras, c-myc, epidermal growth factor receptor (EGFR, erb-B1), and erb-B2 (HER-2/neu) [7].

To find a new anticancer molecule, the screening was carried out through a combination of PKS I and NRPS gene analysis and TLC densitometry profile of *Actinomycetes* isolates from Krakal coast, Gunungkidul, Yogyakarta, Indonesia. Among 52 isolates tested, 14 isolates have cytotoxic activity, and one isolate showed the most potential activity against MCF-7 and T47D breast cancer cell lines with IC₅₀ values of 0.6 µg/mL and 1.3 µg/mL, respectively [8]. *Actinomycetes* are gram-positive bacteria that have a high G + C DNA. *Actinomycetes* are one of the important sources of microbes in the development of biotechnology and medicine. Almost 70 % of natural antibiotics are produced by these bacteria. *Streptomyces* is the largest antibiotic-producing genus, although the role of other genera in drug discovery cannot be ruled out. The characteristic of most *Actinomycetes* is that the bacteria are mesophilic, i.e., bacteria will grow optimally at temperatures between 25 and 30 °C [9]. Furthermore, this also applies to the isolates of *Streptomyces* sp. GMY01.

Further identification showed that GMY01 isolate belongs to the *Streptomyces* genus and had the closest similarity with *Streptomyces sennicomposti* strain RCPT1-4T with an average nucleotide identity (ANI) and ANI based on BLAST+ (ANIb) values of 98.09 and 97.33 % (>95 %) [10]. In the previous study proved that the methanol fraction of the cultured isolates of the *Streptomyces* sp. GMY01 has potential cytotoxic activity against MCF-7 and T47D breast cancer cells with IC₅₀ values of 0.8 and 127 µg/mL, respectively. The methanol extract was also shown to be non-toxic in normal NIH-3T3 cells with an IC₅₀ value of 3707 µg/mL [8]. Genome mining analysis of *Streptomyces* sp. GMY01 showed that this isolate had 10 NRPS genes encoding various secondary metabolites from the previously known active compounds [11]. An *in vitro* bioassay of the GMY01 bioactive on cervical carcinoma of HeLa cell and lung carcinoma of HTB cells exhibited moderate activity with low toxicity on Vero cells as a normal cell. Metabolite profiling by LC-MS/MS analysis revealed that the active fraction of GMY01 contained carbohydrate-based compounds, C₁₇H₂₉NO₁₄ (471.15880 Da) as a major compound (97.50 %) and mannitriose (C₁₈H₃₂O₁₆; 504.16903 Da, 1.96 %) as a minor compound [10]. It is indicated that the isolate has the potential to be explored to discover new anticancer molecules.

This study was conducted to isolate active compounds from the methanol extract of *S. sennicomposti* GMY01 against the MCF-7 breast cancer cell line. Structure elucidation of the isolated active compound and cytotoxic test against the Vero normal cell line was also conducted. This study is still limited to *in vitro* study, the result of this study needs to be analyzed further by *in vivo* study to confirm the results.

2. Methods

2.1. Culture of *Streptomyces sennicomposti* GMY01 and preparation of extracts from culture fluids

Streptomyces sennicomposti strain GMY01 was isolated from a marine sediment sample collected from Krakal Beach (8°8'44"S 110°35'59"E), Gunungkidul Regency, Daerah Istimewa Yogyakarta Province, Indonesia [10]. *S. sennicomposti* GMY01 was deposited at the Indonesian Culture Collection, (World Data Center for Microorganisms, WDCM 769), Indonesian Institute of Sciences (LIPI) as InaCC A147 and NITE Biological Research Center (NBRC, WDCM 825) Japan with registration number NBRC 110111. *S. sennicomposti* GMY01 was maintained using International *Streptomyces* Project-2 (ISP-2) media and incubated at 30 °C. For initial fermentation, Tryptic Soy Broth (Difco, Sparks, MD) and Starch Nitrate Broth (SNB) were used as fermentation medium for 3–5 days. For final fermentation, GMY01 was cultured at 30 °C and agitated at 180 rpm for 3 days in a 250 mL Erlenmeyer flask containing 100 mL of TSB as the inoculum. A total of 30 mL (10 % v/v) of 3 days inoculum culture was inoculated in 300 mL of Starch Nitrate Broth (SNB) media using a 500 mL Erlenmeyer tube, then incubated on a rotary shaker incubator at 200–250 rpm for 11 days at room temperature (25–30 °C). The SNB medium contained 0.5 g of NaCl, 1 g of KNO₃, 0.5 g of K₂HPO₄, 0.5 g of MgSO₄·7H₂O, 0.01 g of FeSO₄·7H₂O, and 20 g of soluble starch in 1000 mL of distilled water [12]. The secondary metabolite was obtained by separating the cell biomass from the liquid culture using centrifugation at 3000 rpm for 15 min. The cell biomass obtained was then macerated using methanol for 1 h. After completion, it was filtered, and the supernatant was evaporated to obtain a solid extract for bioassay and active compound isolation.

2.2. Isolation of the active compound from the culture extract of *Streptomyces sennicomposti* GMY01 with bioassay-guided isolation method

The culture extract of *S. sennicomposti* GMY01 isolated its active compounds by bioassay-guided isolation method [13]. The culture of *S. sennicomposti* GMY01 was extracted using a maceration method with methanol as solvent. The methanol extract obtained from *S. sennicomposti* GMY01 was dissolved in methanol combined with Celite (Merck, Germany), with the ratio of extract: Celite (1: 3) (w/w), then dried using rotary evaporator. The dried extract was fractionated using flash chromatography (Reveleris™, Buchi, Switzerland) and stationary phase column C-18 with water-acetonitrile as the mobile phase to obtain separate fractions. Flash chromatography was carried out based on the procedure manual for dry-loading samples [13]. The results of the active fraction obtained were then separated using the chromatotron method. From the results of the chromatotron separation, active isolates were obtained, and the results were observed using TLC densitometry and LC-MS spectroscopy methods.

2.3. Cytotoxic activity test of the active fraction on cancer cell culture

Cytotoxic activity test against MCF-7 breast cancer cells was carried out on all isolated fractions or active compounds. A total of 100 μ L of MCF-7 cell culture suspension with a density of 1×10^5 cells/mL and 100 μ L of the test fraction or compound at various concentrations were put into a 96-well plate and incubated for 24 h at 37 °C. All treatments were performed in triplicate. After the incubation period was complete, the culture medium was discarded and replaced with 100 μ L of the new complete medium and 10 μ L of MTT solution [A 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] (Sigma, Aldrich). The well plates were incubated again for 4 h at 37 °C. After the incubation, 100 μ L of 10 % SDS solution was added in 0.01 M HCl and incubated again for 24 h. Then the absorbance was read using an ELISA reader with a wavelength of 595 nm. The percentage of cell viability was calculated by comparing the absorbance value of the wells treated with the test compound with the absorbance of the negative control wells. The percentage of cell viability was then used to calculate the percentage of growth inhibition and to determine the concentration of the test compound that could inhibit cell growth up to 50 % (IC₅₀). Cytotoxic activity test for the isolated active compound was also conducted against the Vero cell line. Statistical analysis using one-way ANOVA followed by posthoc test Tukey.

2.4. Structure elucidation of the active compound

2.4.1. Spectrophotometry UV/Vis method

The active compound isolated from *Streptomyces* sp. GMY01 obtained was carefully weighed as much as 0.5 mg, then put in a 5 mL volumetric flask, then added with distilled water to the limit mark and obtained a concentration of 100 mg/mL. The solution was read for absorption at a wavelength between 200 and 800 nm using UV/Vis spectrophotometry to determine the maximum wavelength by looking at the highest and most stable absorption value.

2.4.2. Infra-red spectroscopy (FT-IR) method

A total of 0.1 mg of the active compound sample was mixed with 20 mg of KBr and then pelleted. Measurements were made at wave numbers between 4000 and 400 cm^{-1} . FT-IR spectrophotometer analysis was carried out to see the groups contained in the isolate of the active compound. The instrument used is the Buck Scientific Model 500 Infrared Spectrophotometer with a power supply of 100–240 V 50/60 Hz.

2.4.3. Chromatography LC-MS method

The manufacture of mobile phase water: acetonitrile (3:1) is done by taking 300 mL of water and 100 mL of acetonitrile, then putting it in a 500 mL volumetric flask and adding distilled water to the limit, then filtering using a cellulose nitrate membrane filter. The isolated sample of the active compound was dissolved using methanol and injected into the LC-MS apparatus. The LC-MS apparatus was set in positive mode with a mass range between 50 and 1350 Da and the mobile phase flowed in a gradient for 25 min. The spectral results obtained show the fractional component of the groups contained in the isolate of the active compound.

2.4.4. Spectroscopy NMR method

The ¹H NMR and ¹³C NMR analysis tests were carried out with an NMR spectrometer (500 MHz; Jeol JNM-ECZ500R) in D₂O solvent.

3. Result and discussion

In this study, the isolation of cytotoxic active compounds from *Actinomycetes* against the MCF-7 breast cancer cell line was carried out. Optimization of the *Streptomyces sennicomposti* GMY01 fermentation was carried out to stimulate the optimal production of secondary metabolites.

To be able to produce secondary metabolites, *Streptomyces* must undergo a fermentation process using an appropriate medium. The result of the fermentation process is an extract containing a mixture of primary and secondary metabolites. The organic solvent extract of *S. sennicomposti* GMY01 obtained was then targeted for secondary metabolite profile analysis using TLC densitometry and LC-MS spectroscopy methods. The production of secondary metabolites from *Actinomycetes* is associated with several factors including nutrient media, pH, O₂, temperature, and the addition of inducers or enzyme inhibitors [14,15]. Other environmental factors during fermentation, such as agitation speed can also affect the secondary metabolites production. In addition, the length of fermentation time

used during fermentation also affects the amount of *Streptomyces* biomass formed and the antibiotic activity of the secondary metabolites produced (Narayana and Vijayalakshmi 2008; Kiranmayi et al., 2011; Sengupta et al., 2015).

In this study, the optimization of the culture media and different fermentation containers was carried out to optimize the growth of *S. sennicomposti* GMY01. Meanwhile, in optimizing the length of fermentation time, it was seen that starting on day 3, the *S. sennicomposti* GMY01 growth started to decline. Optimization to produce changes in the type and number of secondary metabolites was carried out by culturing *S. sennicomposti* GMY01 using variations of 2 different media (TSB and SNB), 2 different types of fermentation containers (regular Erlenmeyer and baffled Erlenmeyer), and 2 different fermentation durations (3 and 5 days) (see Table 1). It is known that carbon sources such as fructose, glycerol, starch, and sucrose in culture media can lead to increased production of antibiotics by *Streptomyces* spp [16,17]. while the decrease in secondary metabolite production can be caused by the presence of glucose, ammonia, and phosphate [16,18]. The color change of the extract produced by *S. sennicomposti* GMY01 is an early indicator that supports the changes in nutrients in the fermentation media can cause changes in the secondary metabolites produced. The color change was seen when *S. sennicomposti* GMY01 was cultured using SNB media which showed a more intense color than when cultured using TSB media.

The methanol extract of *Streptomyces* sp. GMY01 had almost the same metabolite profile, both cultured using TSB and SNB media. However, based on the resulting cytotoxic activity, the bacteria *S. sennicomposti* GMY01 cultured with SNB media had the best cytotoxic activity compared to other extracts. This indicates that although they have similar metabolite profiles, there are still differences in the metabolite profiles produced and the cytotoxic activity obtained. Differences in culture media can cause different profiles of secondary metabolites produced by isolates of *S. sennicomposti* GMY01. This study is in accordance with several studies that have been carried out previously, which have found differences in the characteristics of the secondary metabolite profile of 1 isolate cultured using 10 mediums and analyzed using HPLC chromatogram data [19]. In addition to using secondary metabolite profile analysis, biological activity data in this case cytotoxic activity can also be used to obtain unique compounds that are predicted to influence these activities. From the results of the study, it was found that culture media using SNB showed stronger cytotoxic activity on MCF-7 cells than TSB media. SNB media has been widely used in research for the fermentation process of a bacterium. Previous research stated that SNB media was used for the fermentation process of 5 types of bacteria isolated from the rhizosphere of the *Ficus carica* L. plant and an antibacterial test was carried out on ethyl acetate extract from bacterial pellets [20]. From the results of the study, it was found that there was 1 spot in the extract which had an effect as an antibiotic. In addition, the *Bacillus* sp. bacteria which was cultured in SNB media produced sericin compounds that were proven to have cytotoxic activity in several cancer cell cultures, which were Caco-2, MCF-7, and A549 cells [21]. In a previous study, an extract of *S. sennicomposti* GMY01 which was fermented using SNB medium showed anticancer activity on human cancer cell line A549 [12].

Based on the result of the extract separation using the flash chromatography method obtained 9 fractions (Fig. 1). Then the fractionation extract was tested for cytotoxic activity to choose the most potent cytotoxicity activity of the fraction.

Based on the result of active compound analysis using the LC-MS spectroscopy method showed that 1 dominant peak at RT: 8.16 min (Fig. 2). From the results of the LC method analysis, it is suspected that the active compound responsible for the cytotoxic effect is the dominant peak at a retention time of 8.16 min. From the results of the LC method analysis, fractions were then observed as active compounds at the dominant peak of RT: 8.16 min using the MS method. From the results of MS analysis, it is known that the most active compound This dominant has a BM value of 334.65 Da. This matter can be the BM of the active compound or a fraction of compounds contained in the isolate.

Based on the result of active compound analysis using the FT-IR spectroscopy method showed several functional groups in the peak (Fig. 3). From the results of FT-IR spectroscopy analysis, there is a peak at a wavelength of 1035–1149 cm⁻¹ with a stretching vibration type which is a typical peak for the alkane group. Furthermore, there is also a peak at wavelength 1388 cm⁻¹ which leads to C–O. The peak is at a wavelength of 1635 cm⁻¹ with a bending vibration type which is the C=O group. The wavelength of 2934 cm⁻¹ is typical of the C–H group and the wavelength of 3388 cm⁻¹ is typical of the –OH group.

Based on the results of the isolation of the active compounds of the selected extracts, it was known that the isolates of the active compounds were in the form of mannatriose compounds. This is shown from the data analysis of ¹H NMR and ¹³C NMR which leads to the structure of these compounds. In addition, the LC-MS/MS analysis data showed that the active fraction of the methanol extract of *S. sennicomposti* GMY01 also contains mannatriose compounds [10]. The results of the 1H NMR analysis show that there is a typical peak of hydrogen atoms in the saccharide ring at a chemical shift of 3.4–4.0 ppm.

In addition, there is also a typical peak of anomeric hydrogen atoms at a chemical shift of 5.0–5.4 ppm. This is in accordance with the previous study conducted in by Yang et al. [22], who conducted an analysis of isolate compounds in the form of polysaccharides

Table 1

The best IC₅₀ value of the extract optimization of *Streptomyces sennicomposti* GMY01 on MCF-7 breast cancer cell line.

No. Sample	Medium	Fermentor	Duration of Fermentation	Extract Part	Solvent	IC ₅₀ (μg/mL)
1	SNB	Baffled	3 days	Pellet	Methanol	858
2	TSB	Erlenmeyer	3 days	Pellet	Methanol	1308
3	SNB	Baffled	5 days	Pellet	Methanol	245
4	SNB	Erlenmeyer	5 days	Supernatant	Ethyl acetate	89
5	SNB	Baffled	3 days	Supernatant	Ethyl acetate	893
6	TSB	Baffled	3 days	Supernatant	Ethyl acetate	421
7	TSB	Baffled	5 days	Supernatant	Ethyl acetate	555
8	SNB	Erlenmeyer	5 days	Pellet	Methanol	78

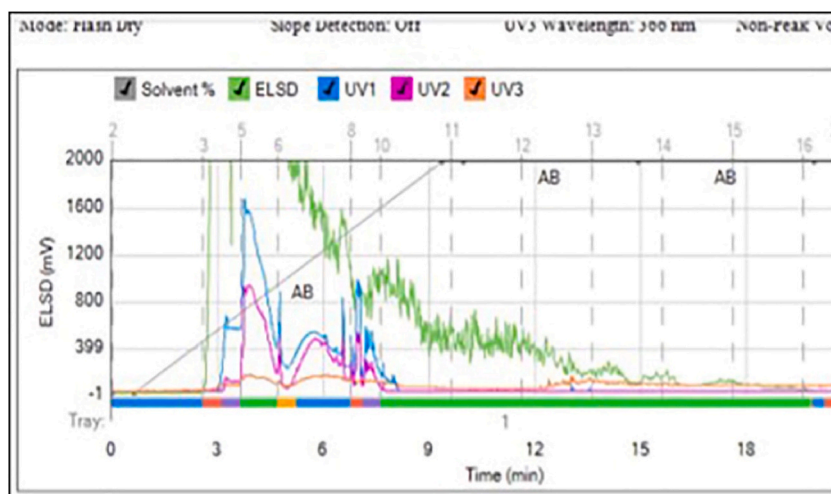


Fig. 1. A fractionation results of methanol extract from *Streptomyces sennicompsti* GMY01 using Flash Chromatography Method.

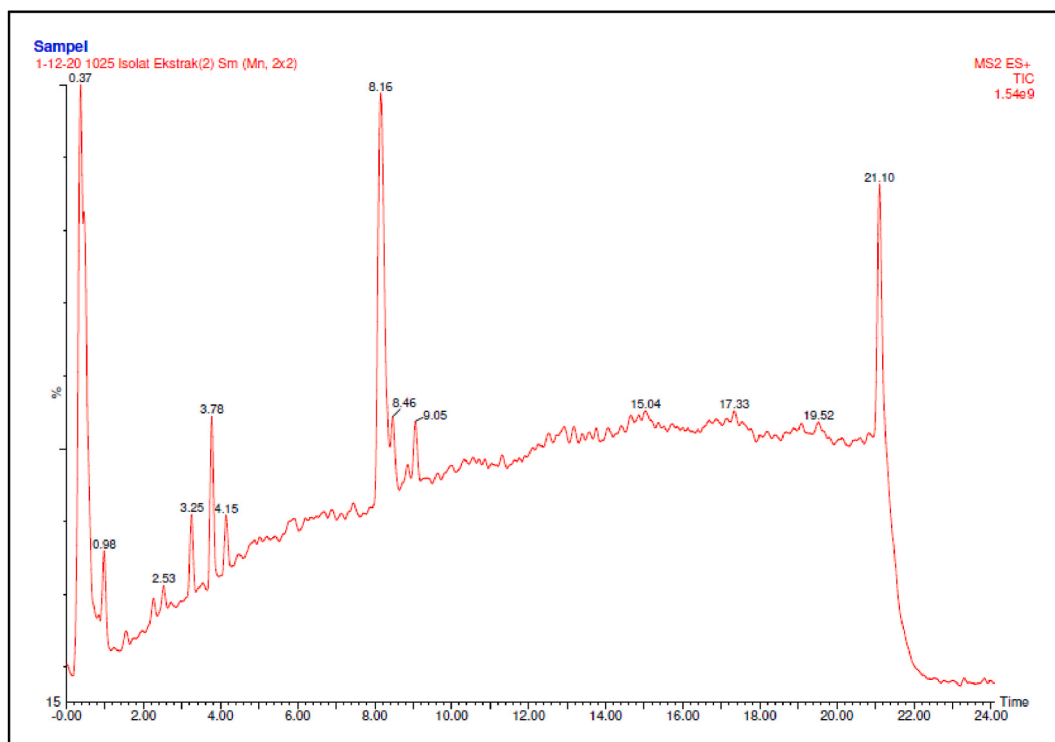


Fig. 2. Active compound of *Streptomyces sennicompsti* GMY01 analysis using LC-MS spectroscopy method.

obtained from the fungus *Phellinus igniarius* (Fig. 4).

The typical peak of hydrogen atoms at a chemical shift of 3.4–4.0 ppm is one of the main characteristics of the presence of saccharide groups in these compounds. Where to determine the number of sugar groups that make up the compound, can be seen from the peak profile of the anomeric hydrogen atoms that appear on the chemical shift between 5.0 and 6.0 ppm. The number of anomeric hydrogen peaks indicates the number of sugar groups that make up the isolated compound. From the observation, it is known that the peak profile of anomeric hydrogen in the isolate of the active compound of the bacterium *Streptomyces* sp. GMY01 contains 3 hydrogen peaks, so it is suspected that the isolate of the active compound has 3 constituent sugar groups. This is also consistent with previous research about the preparation and analysis of mannobiose, mannotriose, and mannotetraose compounds from the yeast *Saccharomyces cerevisiae* [23] (Fig. 5). From the comparison results of the anomeric hydrogen peaks contained in the isolates of the active compound of the bacterium *S. sennicompsti* GMY01 has a close resemblance to the peak of the mannotriose compound in the isolated

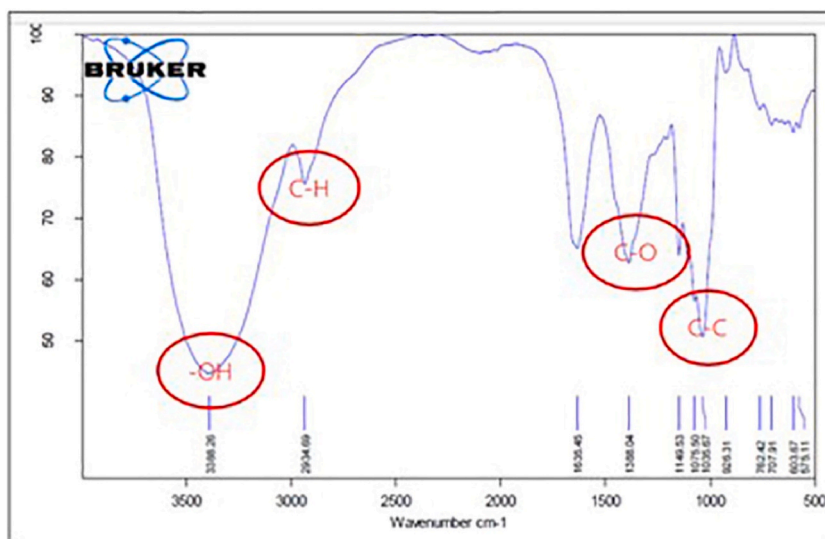


Fig. 3. Active compound of *Streptomyces sennicompsti* GMY01 analysis using FT-IR spectrophotometry method.

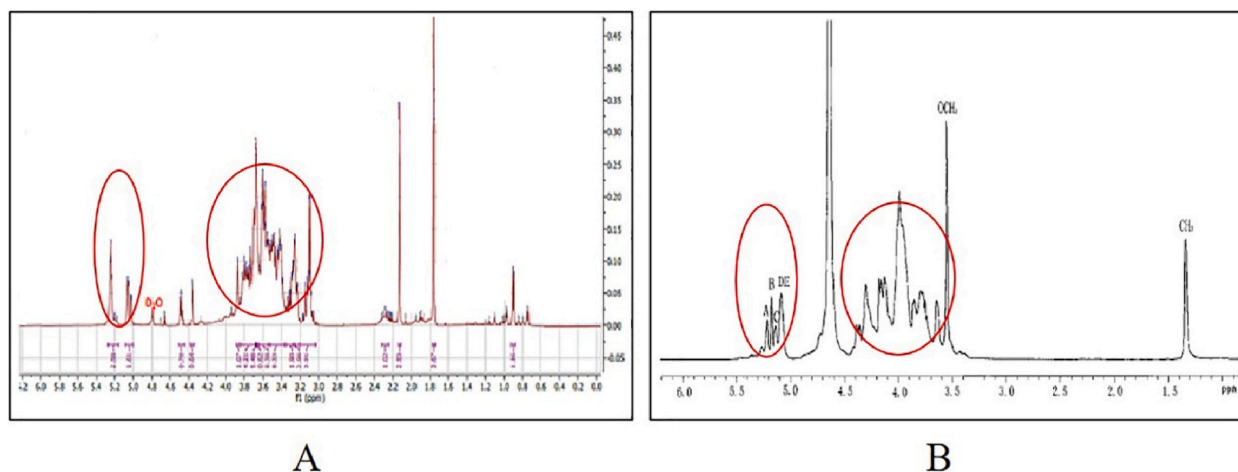


Fig. 4. The ^1H NMR spectral analysis profile of active compound isolates from *Streptomyces sennicompsti* GMY01 with solvent D_2O (A), isolate the fungal compound *Phellinus igniarius* with solvent D_2O (B) [22].

compound from the yeast *Saccharomyces cerevisiae*.

Based on the ^{13}C NMR analysis data, it is known that the active compound isolates of *S. sennicompsti* GMY01 contain a typical carbon atom spectrum for sugar groups in the chemical shift between 60 and 100 ppm. In addition, there is also a typical peak for the anomeric carbon atom in the chemical shift between 140 and 150 ppm (Fig. 6).

Until now, there is still limited research on candidates for anticancer drugs based on polar carbohydrates. Previous studies have shown that antitumor activity can be carried out by increasing solubility by adding hydrophilic functional groups such as sugars (glucose, galactose, and mannose) [24]. In recent years, several carbohydrate compounds have been developed for various medicinal applications ranging from compounds with antibiotic, antiviral, or fungicidal activity to anticancer compounds [25]. Some of them are polysaccharide anticancer compounds (CPP) isolated from *Cyclocarya paliurus* which were tested against the inhibition of HeLa gastric cell human carcinoma [26]. There are 3 novel polysaccharide acids (PRM1, PRM3, and PRM5) isolated from the roots of *Rhynchosia minima* which have anticancer effects on lung cancer A549 and liver cancer cells HepG2 [27]; The original and modified fucoidan fractions that have galactose residues also have anticancer activity against DLD-1 and HCT-116 colorectal carcinoma cells [28]. Galactose is a water-soluble carbohydrate that has high hydrophilic properties. In a previous study, it was shown that the compound paclitaxel (taxol), a diterpenoid originally isolated from the bark of the *Taxus brevifolia* tree, is currently used in the treatment of various types of cancer but has limited water solubility, which hinders its use as an anticancer agent. Attempts at synthesizing the galactose-bound taxoid analog paclitaxel (10-a-GAG-DT) proved to be superior to all other taxoids in water solubility and antitumor

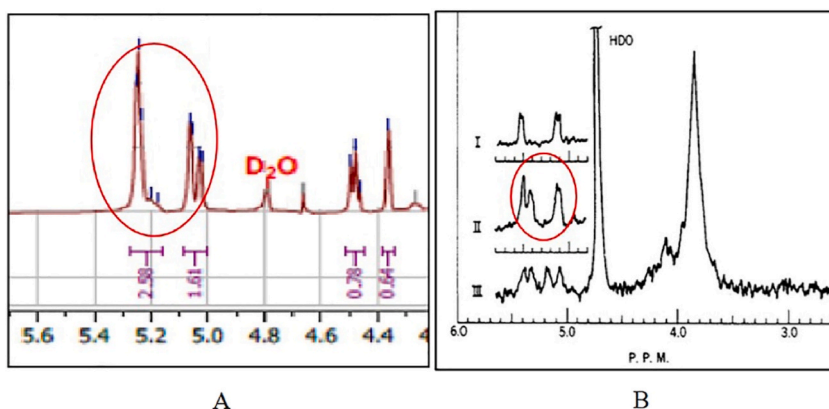


Fig. 5. Analysis of anomeric hydrogen peaks in ^1H NMR spectra of active compound isolates from *Streptomyces sennicompsti* GMY01 with solvent D_2O (A), isolate compounds in the fungus *Phellinus igniarius* with solvent D_2O [23] (B).

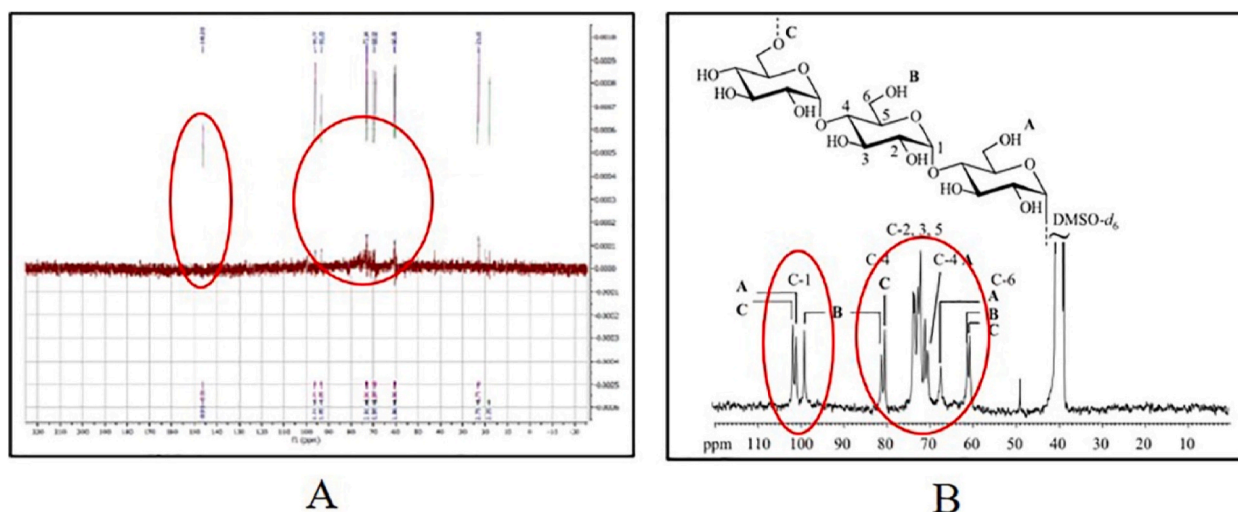


Fig. 6. Analysis profile on ^{13}C NMR spectra of active compound isolates from *Streptomyces sennicompsti* GMY01 with solvent D_2O (A), isolate compound on *Aurerobacidium pullulan* with solvent DMSO-d_6 [32] (B).

activity [24].

From the results of the cytotoxic test, the isolated compound suspected to be a manntriose compound was known to have an IC_{50} value of MCF-7 cancer cells of $5.6 \mu\text{g}/\text{mL}$, while the drug doxorubicin had an IC_{50} value of $4.5 \mu\text{g}/\text{mL}$. Based on research in the previous study showed that the IC_{50} value of a compound fraction or isolate with an IC_{50} value of less than $10 \mu\text{g}/\text{mL}$ is included in the category of the very strong cytotoxic agent [29]. So, it can be concluded that both doxorubicin and manntriose compound isolates are categorized as very strong cytotoxic agents against MCF-7 breast cancer cells. In the selectivity test of the active compound isolate, manntriose was analyzed on normal Vero cells. From the results of the cytotoxic test on normal Vero cells of the active isolate compound manntriose and doxorubicin, the IC_{50} values were 687 and $5.9 \mu\text{g}/\text{mL}$ (Fig. 7). Thus, based on these data, the selectivity index (SI) of the active compound isolate manntriose is 122 times, while for doxorubicin it is 0.8 times. The small SI data for the drug doxorubicin are also in line with those of Wang et al. (2017). From these results, it is known that the drug doxorubicin has an IC_{50} value of $1.0 \mu\text{M}$ in MCF-7 cancer cells, and $0.4 \mu\text{M}$ in normal Vero cells, so it has an SI value of 0.4 times. Based on research from previous study, the SI value of a compound fraction or isolate with an SI value of more than 3 times is included in the category of cytotoxic agents with good selectivity [29]. So based on this statement, it can be concluded that the active isolate compound manntriose has a very good SI value, while the drug doxorubicin has a poor SI value. This very good SI value illustrates that the active isolate compound manntriose has a relatively high degree of safety in normal cells compared to doxorubicin.

It has been reported mechanism of carbohydrate-based compounds is anticancer. Many studies have reported that the bioactivity of polysaccharides is largely related to their physicochemical properties, including polysaccharide content, molecular weight, and type of sugar. Other studies have also reported that the anticancer activity of some polysaccharides is associated with free radical stabilizing activity, specific conformation and affinity for cell surface receptors, and antioxidant enzyme activity [26]. Other polysaccharide

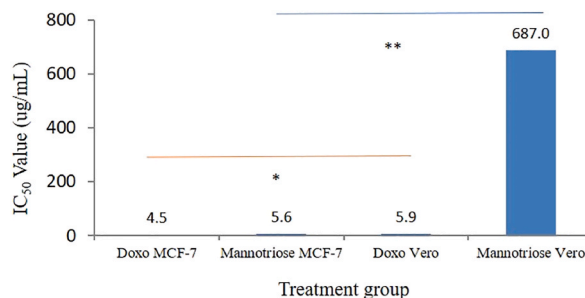


Fig. 7. Comparison of the cytotoxic value of doxorubicin and isolates of the active compound mannotriose in MCF-7 cancer cells and Vero normal cells. All treatments were performed in triplicate. The * sign indicates the data is not significantly different, ** indicates the data is significantly different. Statistical analysis using one-way ANOVA followed by posthoc test Tukey.

anticancer compounds, fucoidans derivatives have biological activities that depend on different structural characteristics such as monosaccharide composition, molecular weight, presence of branches, and content of sulfate and acetyl groups [30]. In vitro anti-tumor studies of the compound prumicin also showed inhibition of protein synthesis and DNA synthesis in HeLa cancer cell cultures [31].

4. Conclusion

The active compounds of mannotriose were isolated from a methanol extract of *Streptomyces sennicomposti* GMY01 which has strong cytotoxic activity against MCF-7 breast cancer cells line with IC₅₀ value of 5.6 µg/mL and has high selectivity index on Vero normal cells line (SI = 122).

CRedit authorship contribution statement

Rifki Febriansah: Conceptualization, Formal analysis, Investigation, Writing – original draft, Writing – review & editing, Visualization. **Triana Hertiani:** Conceptualization, Data curation, Resources, Supervision. **Jaka Widada:** Conceptualization, Methodology, Resources, Supervision. **Muhammad Taher:** Conceptualization, Supervision, Validation. **Emad Damayanti:** Conceptualization, Formal analysis, Methodology, Resources, Writing – original draft, Writing – review & editing. **Mustofa Mustofa:** Conceptualization, Data curation, Funding acquisition, Project administration, Supervision, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

- [1] H. Sung, J. Ferlay, R.L. Siegel, M. Laversanne, I. Soerjomataram, A. Jemal, F. Bray, Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries, *CA. Cancer J. Clin.* 71 (2021) 209–249, <https://doi.org/10.3322/caac.21660>.
- [2] World Health Organization, Source: the Global Cancer Observatory, 2021. <https://gco.iarc.fr/today>.
- [3] B. Ng, H. Puspitaningtyas, J.A. Wiranata, S.H. Hutajulu, I. Widodo, N. Anggorowati, G.Y. Sanjaya, L. Lazuardi, P. Sripan, Breast cancer incidence in Yogyakarta, Indonesia from 2008–2019: a cross-sectional study using trend analysis and geographical information system, *PLoS One* 18 (2023) 1–17, <https://doi.org/10.1371/journal.pone.0288073>.
- [4] K.B. Tran, J.J. Lang, K. Compton, R. Xu, A.R. et al Acheson, The global burden of cancer attributable to risk factors, 2010–19: a systematic analysis for the Global Burden of Disease Study 2019, *Lancet* 400 (2022) 563–591, [https://doi.org/10.1016/S0140-6736\(22\)01438-6](https://doi.org/10.1016/S0140-6736(22)01438-6).
- [5] J.B. Gibbs, Mechanism-based target identification and drug discovery in cancer research, *Science* 287 (2000) 1969–1973, <https://doi.org/10.1126/science.287.5460.1969>.
- [6] F.L. Meyskens, P. Tully, Principles of cancer prevention, *Semin. Oncol. Nurs.* 21 (2005) 229–235, <https://doi.org/10.1016/j.soncn.2005.06.002>.
- [7] H. Masuda, D. Zhang, C. Bartholomeusz, H. Doihara, G.N. Hortobagyi, N.T. Ueno, Role of epidermal growth factor receptor in breast cancer, *Breast Cancer Res. Treat.* 136 (2012), <https://doi.org/10.1007/s10549-012-2289-9>. Role.
- [8] Y. Farida, J. Widada, E. Meiyanto, Combination methods for screening marine Actinomycetes producing potential compounds as anticancer, *Indones. J. Biotechnol.* 12 (2007) 988–997, <https://doi.org/10.22146/ijbiotech.7772>.
- [9] E.A. Barka, P. Vatsa, L. Sanchez, N. Gaveau-Vaillant, C. Jacquard, H.-P. Klenk, C. Clément, Y. Ouhdouch, G.P. van Wezel, Taxonomy, physiology, and natural products of actinobacteria, *Microbiol. Mol. Biol. Rev.* (2016), <https://doi.org/10.1128/mmlr.00019-15>.
- [10] J. Widada, E. Damayanti, M. Mustofa, A. Dinoto, R. Febriansah, T. Hertiani, Marine-derived *Streptomyces sennicomposti* GMY01 with anti-plasmodial and anticancer activities: genome analysis, in: *In Vitro Bioassay, Metabolite Profiling, and Molecular Docking*, 2023, pp. 1–19.

- [11] C. Herdini, S. Hartanto, S. Mubarika, B. Hariwiyanto, N. Wijayanti, A. Hosoyama, A. Yamazoe, H. Nojiri, J. Widada, Diversity of nonribosomal peptide synthetase genes in the anticancer producing actinomycetes isolated from marine sediment in Indonesia, Indones. J. Biotechnol. 20 (2015) 34, <https://doi.org/10.22146/ijbiotech.15266>.
- [12] E. Damayanti, K. Nisa, S. Handayani, R.T. Dewi, R. Febriansah, Mustofa, A. Dinoto, J. Widada, Cytotoxicity and molecular mechanism of marine-derived *Streptomyces* sp. GMY01 on human lung cancer cell line A549, J. Appl. Pharmaceut. Sci. 11 (2021) 46–55, <https://doi.org/10.7324/japs.2021.110605>.
- [13] E. Damayanti, J. Widada, P.D.N. Lotulung, A. Dinoto, M. Mustofa, Bioassay guided fractionation of marine *Streptomyces* sp. GMY01 and antiplasmodial assay using microscopic and flow cytometry method, Indones. J. Pharm. (2020), <https://doi.org/10.22146/ijp.809>.
- [14] H.B. Bode, B. Bethe, R. H'fs, A. Zeeck, Big effects from small changes : possible ways to explore, Nature's Chem. Divers. (2002) 619–627.
- [15] R. Subramani, W. Aalbersberg, Marine actinomycetes: an ongoing source of novel bioactive metabolites, Microbiol. Res. 167 (2012) 571–580, <https://doi.org/10.1016/j.micres.2012.06.005>.
- [16] A.A. Hamza, H.A. Ali, B.R. Clark, C.D. Murphy, A. Elsheikh, Isolation and characterisation of actinomycin D producing *Streptomyces* spp. from Sudanese soil, Afr. J. Biotechnol. 12 (2013) 2624–2632, <https://doi.org/10.5897/AJB12.1066>.
- [17] C. Zhu, F. Lu, Y. He, Regulation of Avilamycin Biosynthesis in *Streptomyces viridochromogenes* : Effects of Glucose , Ammonium Ion , and Inorganic Phosphate, 2007, pp. 1031–1038, <https://doi.org/10.1007/s00253-006-0572-6>.
- [18] M.F.V.Q. Sousa, C.E. Lopes, N.P. Jr, DEVELOPMENT OF A BIOPROCESS FOR THE PRODUCTION OF ACTINOMYCIN-D 19 (2002) 277–285.
- [19] X. Liu, E. Ashforth, B. Ren, F. Song, H. Dai, M. Liu, J. Wang, Q. Xie, L. Zhang, Bioprospecting microbial natural product libraries from the marine environment for drug discovery, J. Antibiot. (Tokyo) (2010) 415–422, <https://doi.org/10.1038/ja.2010.56>.
- [20] N. Sulistyani, I. Narwanti, TLC-bioautography profile of ethyl acetate extract of 5 bacteria isolated from *Ficus carica*, L Rhizosphere 4 (2015) 81–87.
- [21] E.M. El-Fakharany, M.H. Saad, M.S. Salem, N.M. Sidkey, Biochemical characterization and application of a novel lectin from the cyanobacterium *Lyngabya confervoides* MK012409 as an antiviral and anticancer agent, Int. J. Biol. Macromol. 161 (2020) 417–430, <https://doi.org/10.1016/j.ijbiomac.2020.06.046>.
- [22] Y. Yang, L. Bin Ye, J.S. Zhang, Y.F. Liu, Q.J. Tang, Structural analysis of a bioactive polysaccharide, PISP1, from the medicinal mushroom *Phellinus igniarius*, Biosci. Biotechnol. Biochem. 73 (2009) 134–139, <https://doi.org/10.1271/bbb.80546>.
- [23] V. Der Decken, C.E. Ballou, Preparation of Mannobiose, Mannotriose, and a New Mannotetraose from, 1965, p. 4.
- [24] K. Mikuni, K. Nakanishi, K. Hara, K. Hara, W. Iwatani, T. Amano, K. Nakamura, Y. Tsuchiya, H. Okumoto, T. Mandai, In vivo antitumor activity of novel water-soluble taxoids, Biol. Pharm. Bull. (2008), <https://doi.org/10.1248/bpb.31.1155>.
- [25] C. Hartinger, A. Nazarov, S. Ashraf, P. Dyson, B. Keppler, Carbohydrate-metal complexes and their potential as anticancer agents, Curr. Med. Chem. (2008), <https://doi.org/10.2174/092986708785908978>.
- [26] J.H. Xie, X. Liu, M.Y. Shen, S.P. Nie, H. Zhang, C. Li, D.M. Gong, M.Y. Xie, Purification, physicochemical characterisation and anticancer activity of a polysaccharide from *Cyclocarya paliurus* leaves, Food Chem. (2013), <https://doi.org/10.1016/j.foodchem.2012.09.078>.
- [27] X. Jia, C. Zhang, J. Qiu, L. Wang, J. Bao, K. Wang, Y. Zhang, M. Chen, J. Wan, H. Su, J. Han, C. He, Purification, structural characterization and anticancer activity of the novel polysaccharides from *Rhynchosia minima* root, Carbohydr. Polym. (2015), <https://doi.org/10.1016/j.carbpol.2015.05.059>.
- [28] R.V. Usoltseva, S.D. Anastuyuk, I.A. Ishina, V.V. Isakov, T.N. Zvyagintseva, P.D. Thinh, P.A. Zadorozhny, P.S. Dmitrenok, S.P. Ermakova, Structural characteristics and anticancer activity in vitro of fucoidan from brown alga *Padina boryana*, Carbohydr. Polym. (2018), <https://doi.org/10.1016/j.carbpol.2017.12.071>.
- [29] N. Weerapreeyakul, A. Nonpunya, S. Barusux, T. Thitimetharoch, Evaluation of the anticancer potential of six herbs against a hepatoma cell line, Chin. Med. 7 (2012) 1, <https://doi.org/10.1186/1749-8546-7-15>.
- [30] M.T. Ale, J.D. Mikkelsen, A.S. Meyer, Important determinants for fucoidan bioactivity: a critical review of structure-function relations and extraction methods for fucose-containing sulfated polysaccharides from brown seaweeds, Mar. Drugs (2011), <https://doi.org/10.3390/md9102106>.
- [31] K. Otaguro, A. Ishiyama, M. Kobayashi, H. Sekiguchi, T. Izuhara, T. Sunazuka, H. Tomoda, H. Yamada, S. Omura, In Vivo and in Vivo Antimalarial Activities of a Carbohydrate Antibiotic, Prumycin, against Drug-Resistant Strains of Plasmodia, J. Antibiot., Tokyo, 2004, <https://doi.org/10.7164/antibiotics.57.400>.
- [32] Fang Xuanjun, Ying Zhang, Studies on structure of pullulan from *Aureobasidium pullulans* by FTIR and NMR, Chin. J. Biochem. Mol. Biol. 15 (1) (1999) 109–113.