Chemical Compounds From The Antibacterial Active Fraction Of 
*Cordyline fruticosa* (L)

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Abstract. *Cordyline fruticosa* (L) known as dehuang, belongs to the family Agavaceae. The chemical 
constituents of this plant includes steroids, terpenoids, flavonoids, phenolic compounds, and saponins. The 
secondary metabolites of the genus *Cordyline* have various biological activities, one of which is antibacterial. 
The antibacterial compounds can be applied to many fields such as food, drinks, and medicines. The stem of *C. 
fruticosa* was extracted by maceration in a gradient solvent namely n-hexane, ethyl acetate, and methanol. Each 
fraction was evaporated using a rotary evaporator and their antibacterial activity was determined using the Kirby 
Bauer method against *Escherichia coli* and *Salmonella typhi* as Gram negative, *Staphylococcus aureus* and 
*Bacillus subtilis* as Gram positive. The chemical compounds were isolated from antibacterial active fractions 
by chromatographic techniques and their chemical structures were identified by spectroscopic methods. Two 
compounds were obtained from the ethyl acetate fraction as a steroid and triterpenoid.

1. Introduction

Traditional medicinal plants have long been the target of new drug discovery. The development of traditional 
medicine, especially from plants, for assist increasing standard of public health is quite widespread. One of the 
advantages of using medicine from plants in humans is as antibiotic. Antibiotics are substances that kill or weaken 
microorganisms (bacteria, fungi, and parasites). There are many diseases due to pathogenic bacteria infection such as 
*Escherichia coli*, *Salmonella typhimurium*, *Staphylococcus aureus* and *Bacillus subtilis*, that can be cured by several 
antibacterial agents. The most important of bioactive compounds from medicinal plants are, alkaloids, tannin, steroid, 
terpenoid, flavonoid and phenolic compounds [1]-[4].

In the tropical countries, infectious diseases caused by microbes still have a high prevalence. Besides that, the 
excessive use of antibiotics may cause a tendentious to occur microorganism resistance against existing antibiotics. 
Therefore, the discovery and development of new antibiotics remain an essential target of new drug discovery. 
Although research or efforts to find antibiotic drugs are currently focused on biotechnology, explorative drug research 
is a viable alternative. Besides proper economic and safety factors consideration, the utilization of the drugs from 
nature has been proven and tested [5]-[8].

One of natural medicine that used as a treatment of infectious disease is the decoction of *Cordyline fruticosa* 
stem. *C. fruticosa* consists of more than 480 species spread in tropical and subtropical regions throughout the world 
[9]. This plant is known as a traditional medicinal plant that has been used to treat various disease, including bloody 
cough, bloody urine, diarrhea, cardiovascular disease, digestive disorders, skin infections, liver cancer, arthritis, and 
neuritis [10]-[12].

*Cordyline fruticosa* (L) A Chev, has synonyms are *Cordyline terminalis* (L) Kunth and *Cordyline fruticosa* 
Backer [13], is a plant of the monolyedoneae class that is usually planted as ornamental plants in the forest and used 
as hedges or borders of tea plantations [14]. *Cordyline fruticosa* has several compounds, are saponins, tannins, 
flavonoids, polyphenols, steroids, polysaccharides, calcium oxalates, and iron [14]. Based on research from 
Fouedjouetal in [15], the *C. fruticosa* has been reported the three new steroid chemical compounds are spirossta-5,25
fruticoside \(H\), \(5\alpha\)-spirost-25 (fruticoside I), and (22S) (fruticoside J). The second compound also exhibits moderate antibacterial activity against Gram-positive \textit{Enterococcus faecalis}.

In this paper, the antibacterial activity of \textit{C. fruticosa} fraction is reported. Also, the chemical content of the active antibacterial fraction, which was isolated by chromatographic techniques and identified by spectroscopic methods, is reported.

2. Material and Method

2.1. Plant Materials
The stem of \textit{C. fruticosa} was collected in Northern Inderalaya sub-district, Ogan Ilir, South Sumatra. The plant was identified by Mrs. Nita Aminasih, a botanist at the Department of Biology, Faculty of Mathematics and Natural Sciences, University of Sriwijaya.

2.2. Preparation and Extraction of Plant Materials
The fresh stem of \textit{C. fruticosa} (1 kg) was washed with running water and dried under room temperature for one week. Approximately 500 g of the air-dried stems were ground into powdered form. The dry powders (500 g) were extracted by maceration with a gradient solvent in n-hexane, ethyl acetate, and methanol at the room temperature. The extracts were filtered and evaporated to obtain the concentrated fraction.

2.3. Preparation of Tested Bacterial Suspension
The tested bacteria (subcultured) 24 - 48 hours old are made a suspension by transferring an ose needle to a test tube containing a physiological saline solution (0.85% NaCl/MHB). The suspension was homogeneous using vortex and measured the absorbance with spectronic at 625 nm wavelength; the measured absorbance value must be equal to the standard of McFarland 0.5. If the absorbance is higher than standard, dilute the suspension by adding physiological saline solution or MHB, and if the absorbance is lower than standard, add the bacteria using an ose needle until the absorbance is equal to McFarland 0.5. In this circumstance, the bacterial suspension is equivalent to 1.5 x 10^8 bacterial cells/mL. The final suspension density at used to be tested is 5 x 10^5 bacterial cells/mL. The final density is obtained by diluting the test bacteria suspension, equivalent to McFarland 0.5, with 1:150 diluent and the result is diluted again with a ratio of 1:2 [16].

2.4. Antibacterial Activity of Fraction from Stem of \textit{C. fruticosa}
The concentrated fractions namely n-hexane, ethyl acetate, and methanol fractions were tested for their antibacterial activity by the disk diffusion method. The tested bacterial suspensions (i.e \textit{Salmonella typhi} (IPCCB.11.669), \textit{Escherichia coli} (ATCC 25922), \textit{Staphylococcus aureus} (ATCC 25923), and \textit{Bacillus subtilis} (ATCC 6633)) were standardized based on 0.5 McFarland Standard. 100 μl of the microorganism suspensions at a density of 5 x 10^5 cells/mL were seeded onto corresponding medium plates containing 20 ml Mueller Hinton Agar (MHA). The concentrated fractions were dissolved in EtOH to a concentration of 4 mg/ml (400 μg/disk). The pure compounds were serially diluted in EtOH from 250 μg/ml to 3.9 μg/ml. 10 μl of the solutions were added to paper disks (6.0 mm). The positive control for bacteria are tetracycline (30 μg/disk), streptomycin (10 μg/disk), and penicillin (10 units/disk) [17], [18].

2.5. Isolation and Identification of Pure Compounds
The antibacterial active fraction was continued with separation by column chromatography with silica gel (70-230 mesh) as stationary phase using a gradient solvent system. The eluate is collected in vials every 10 mL and analyzed by thin layer chromatography (KLT) to group column fractions. The KLT plates were observed under a UV lamp at λ 254 nm and sprayed with sulfuric acid. Sub fractions that show major stains are followed by separation on column chromatography and purified by recrystallization. The pure compounds were identified by spectroscopic methods which included IR, and NMR 1 D, and compared with data from the literature. Pure compounds were tested for antibacterial activity by determining of MIC values.
3. Results and Discussion

3.1. Antibacterial Activity of Fractions from Stem of C. Fruticosa

Antibacterial activity was classified into three groups: strong, moderate, and weak/inactive. This grouping based on the relative activity of substances tested against standard antibiotics (strong: ≥70 % inhibition; moderate: 50-70% inhibition; weak: <50% inhibition). Antibiotics can be used as a positive control if they have intermediate to sensitive inhibition categories (inhibition zone diameter <15 mm) [19].

Antibacterial activity of C. fruticosa stem fractions compared to standard antibiotics is shown in Table 1. The result showed that ethyl acetate fraction is the most active with strong activity against the two test bacteria (S. typhi and S. aureus) compared to three standard antibiotics. The methanol fraction showed strong activity against S. typhi test bacteria compared to three standards of antibiotics. However, the methanol fraction against E. coli only showed strong activity against Penicillin, whereas against two antibiotics (tetracycline and streptomycin) showed moderate activity. The hexane fraction did not have antibacterial activity. Ethyl acetate and methanol fraction, in general, have broad-spectrum antibacterial activity, which is strong to moderate activity against four test bacteria gram-positive and negative types. The ethyl acetate fraction is continued to the separation and purification of pure compounds.

3.2. Chemical Compounds from Ethyl Acetate Fraction

Extraction and isolation – The dry powders (500 g) of C. fruticosa were macerated with n-hexane at room temperature (1 x 24 h) and filtered. The powder is dried-wind and macerated up to three times. The filtrate was evaporated under reduced pressure to give an n-hexane fraction (11.4 g). Maceration continued with ethyl acetate and methanol, each done with three replications. The filtrate was evaporated, and 19.8 g of the ethyl acetate fraction and 17.7 g of the methanol fraction were obtained. The ethyl acetate fraction (5 g) was separated by column chromatography with silica gel (70-230 mesh), eluting with a gradient solvent system (n-hexane-ethyl acetate = 10:00:10 and ethyl acetate-methanol = 10:00:10), yielded five subfractions (A-E). Fraction A (0.81 g) was separated over a silica gel column (70-230 mesh, 30 g) with a solvent system of n-hexane-ethyl acetate = 10:00:10 as the eluent to give three fractions (A1–A3). Fraction A2 (240 mg) was subjected to column chromatography over a silica gel (70-230 mesh, 30 g) eluted with a solvent system of n-hexane-ethyl acetate = 8:2 to obtain compound 1 (42 mg). Fraction B (0.74 g) was subjected to column chromatography over a silica gel (70-230 mesh, 30 g) eluted with a solvent system of n-hexane-ethyl acetate = 10:00:10 to give four sub-fractions (B1–B4).

Subfraction B3 (0.46 g) was purified with a column chromatography eluted with n-hexane-ethyl acetate = 7:3 to obtain compound 2 (28.6 mg).

**Compound 1.** Stigmasterol. White crystal. IR (KBr) νmax cm⁻¹: 3428 (O-H), 2938; 2868 (C-H aliphatic), 1661 (C=O), 1462 (cyclic CH₂); 1377 (–CH₂(CH₃)₂γ); 1049 (cycloalkane). ¹H NMR (in CDCl₃, 500 MHz) δ ppm: 3.53 (1H, m), 5.34 (1H, t), 1.00 (3H, s), 0.69 (3H, s), 0.92 (3H, d), 5.03 (1H, m), 5.19 (1H, m), 0.80 (3H, d), 0.82 (3H, d), dan 0.84 (3H, t). ¹³C NMR (in CDCl₃, 125 MHz) δ ppm: 37.3 (C-1), 31.8 (C-2), 71.9 (C-3), 71.9 (C-3), 42.4 (C-4), 140.9 (C-5), 121.8 (C-6), 31.8 (C-7), 37.4 (C-8), 50.3 (C-9), 36.3 (C-10), 21.2 (C-11), 39.2 (C-12), 42.4 (C-13), 56.9 (C-14), 24.4 (C-15), 29.1 (C-16), 56.1 (C-17), 12.2 (C-18), 19.6 (C-19), 40.6 (C-20), 21.2 (C-21), 138.4 (C-22), 129.4 (C-23), 51.4 (C-24), 31.8 (C-25), 21.3 (C-26), 19.2 (C-27), 25.6 (C-28), and 12.4 (C-29).

**Compound 2.** Oleanolic acid. White powder. IR (KBr) νmax cm⁻¹: 3427 (O-H), 2932 ; 2851 (C-H aliphatic), 1730 (C=O). ¹H NMR (in CDCl₃, 500 MHz) δ ppm: 1.67 (2H, m); 1.65 (2H, m); 3.52 (1H, m), 1.52 (1H, m), 5.35 (1H, d, J=5.1), 0.93 (2H, m), 2.85 (1H, t, J=7.1), 1.00 (3H, s), 0.79 (3H, s), 0.80 (3H, s), 0.79 (3H, s), 0.79 (3H, s), 1.03 (3H, s), 0.80 (3H, s), and 0.80 (3H, s). ¹³C NMR (in CDCl₃, 125 MHz) δ ppm: 39.8 (C-1), 28.3 (C-2), 72.0 (C-3), 39.6 (C-4), 56.1 (C-5), 19.1 (C-6), 32.1 (C-7), 39.9 (C-8), 50.3 (C-9), 37.4 (C-10), 23.2 (C-11), 121.9 (C-12), 141.0 (C-13), 42.4 (C-14), 28.4 (C-15), 23.9 (C-16), 46.0 (C-17), 42.5 (C-18), 46.0 (C-19), 29.9 (C-20), 34.1 (C-21), 32.1 (C-22), 28.4 (C-23), 18.8 (C-24), 18.9 (C-25), 19.1 (C-26), 25.6 (C-27), 174.0 (C-28), 32.1 (C-29), and 24.5 (C-30).
Table 1. Antibacterial activity of fractions from stem of *c. Fruticosa* compare to standard antibiotics

| Sample          | Fraction     | Tested bacteria | Inhibition zone (mm) | Antibacterial activity percentage (%) | Antibioclin zone of Tetracycline (mm) | Inhibition zone of Streptomycin (mm) | Inhibition zone of Penicillin (mm) |
|-----------------|--------------|-----------------|----------------------|---------------------------------------|--------------------------------------|--------------------------------------|----------------------------------|
|                 | (400 μg/disk)|                |                      |                                       | S. tiphi (19.8)                       | S. tiphi (19.7)                        | S. tiphi (18.2)                    |
|                 |              | B. subtilis     | 6.8 ± 1.6            |                                       |                                      |                                      |                                  |
| Stem of C.      |              | E. coli        | 33.3*                |                                       | S. tiphi (19.4)                       | S. subtilis (19.4)                    |                                  |
| fruticosa       | n-hexane     | S. aureus      | -                    |                                       | E. coli (20.4)                        | S. aureus (19.9)                      |                                  |
|                 | Ethyl acetate| S. tiphi       | 13.9 ± 3.2           | 70.2***                               | 70.6***                              | 76.4***                              |                                  |
|                 |              | B. subtilis    | 12.7 ± 2.4           | 59.3**                                | 65.3**                               | 66.5**                               |                                  |
|                 |              | E. coli        | 11.8 ± 3.1           | 57.8**                                | 63.1**                               | 64.8**                               |                                  |
|                 |              | S. aureus      | 14.2 ± 3.6           | 72.1***                               | 71.4***                              | 73.2***                               |                                  |
|                 | Methanol     | S. tiphi       | 15.2 ± 2.8           | 76.8***                               | 77.2***                              | 83.5***                               |                                  |
|                 |              | B. subtilis    | 12.5 ± 1.7           | 58.4**                                | 64.4**                               | 65.4**                               |                                  |
|                 |              | E. coli        | 13.0 ± 3.3           | 63.7**                                | 69.5**                               | 71.4***                               |                                  |
|                 |              | S. aureus      | 13.5 ± 2.9           | 68.5**                                | 67.8**                               | 69.6**                               |                                  |

Note: a Inhibition zone (mm) of fraction/ inhibition zone (mm) of standard antibiotics

*** strong: inhibition of ≥ 70% ** moderate: inhibition 50-70% * weak: inhibition < 50%

The identification of compound 1 was carried out by the spectroscopic method, including 1H-NMR, 13C-NMR, and DEPT (Table 2). Based on the 1H-NMR spectrum (500 MHz, CDCl3), the isolated compound showed characteristic signals for steroid compounds, where there is no signal above the chemical shift of 5.5 ppm and visible signals that accumulate in areas below 2.0 ppm are typical for steroids. Furthermore, there is a vinyl proton at 5.03 (m, 1H); 5.19 (m, H); and 5.34 ppm (t, 1H), which indicates two double bonds, one of the double bonds is tied to C sp3 quaternary. Besides, there are protons bound C binds OH in an area about 3.53 ppm (m, 1H), which is commonly found in steroid compounds groups. Furthermore, a signal with high intensity can be seen at 0.6-1.1 ppm, is thought to be a signal for the methyl group. Other signals that cover areas below 2 ppm are signals for cyclohexane protons.

Analysis of 1H NMR spectrum data showed the presence of 28 signals. Four signals for carbon sp2 appear in areas above 100 ppm (121.84; 129.42; 138.45; and 140.90 ppm), which strengthens the existence of two double bonds in compounds 1. Furthermore, a signal at 71.94 ppm is also visible, which is characteristic for C sp3 binding to OH. These signals are typical for steroid groups that have two double bonds and a hydroxyl group at C-3. Other signals that accumulate in areas below 60 ppm are signals for C sp3 in the form of C, CH, CH2, and CH3, from the basic skeleton of steroids.
Table 2. The $^1$H and $^{13}$C nmr data of compound 1 $^a$ ($^1$H-500 mhz, $^{13}$C-125 mhz, in cdcl$_3$) dan Stigmasterol 1 $^b$ ($^1$H-400 mhz, $^{13}$C-100 mhz, in cdcl$_3$, ppm) [20].

| No. | C   | $^{13}$C-NMR $^a$ | $^1$H-NMR $^a$ | $^{13}$C-NMR $^b$ | $^1$H NMR $^b$ | DEPT $^a$ |
|-----|-----|------------------|----------------|------------------|----------------|-----------|
| 1   | 37.3| 37.15            |                |                  |                | CH$_2$    |
| 2   | 31.8| 31.56            |                |                  |                | CH$_2$    |
| 3   | 71.9| 3.53 (1H, m)     | 71.71          | 3.51 (1H, m)     | CH             |
| 4   | 42.4| 42.19            |                |                  |                | CH$_2$    |
| 5   | 140.9| 140.8           |                |                  |                | C=C      |
| 6   | 121.8| 5.34 (1H, t)   | 121.62         | 5.31 (1H, t)     | C=CH          |
| 7   | 31.8| 31.56            |                |                  |                | CH$_2$    |
| 8   | 37.4| 31.79            |                |                  |                | CH        |
| 9   | 50.3| 50.02            |                |                  |                | CH        |
| 10  | 36.3| 36.16            |                |                  |                | C         |
| 11  | 21.2| 21.12            |                |                  |                | CH$_2$    |
| 12  | 39.2| 39.57            |                |                  |                | CH$_2$    |
| 13  | 42.4| 42.10            |                |                  |                | C         |
| 14  | 56.9| 56.76            |                |                  |                | CH        |
| 15  | 24.4| 24.27            |                |                  |                | CH$_2$    |
| 16  | 29.1| 28.83            |                |                  |                | CH$_2$    |
| 17  | 56.1| 55.84            |                |                  |                | CH        |
| 18  | 12.2| 1.00 (3H, s)     | 12.15          | 1.03 (3H, s)     | CH$_3$        |
| 19  | 19.6| 0.69 (3H, s)     | 19.88          | 0.71 (3H, s)     | CH$_3$        |
| 20  | 40.6| 40.40            |                |                  |                | CH        |
| 21  | 21.2| 0.92 (3H, d)     | 20.99          | 0.91 (3H, d)     | CH$_3$        |
| 22  | 138.4| 5.03 (1H, m)   | 138.23         | 4.98 (1H, m)     | C=C          |
| 23  | 129.4| 5.19 (1H, m)   | 129.16         | 5.14 (1H, m)     | C=C          |
| 24  | 51.4| 51.13            |                |                  |                | CH        |
| 25  | 31.8| 31.94            |                |                  |                | CH        |
| 26  | 21.3| 0.80 (3H, d)     | 21.23          | 0.80 (3H, d)     | CH$_3$        |
| 27  | 19.2| 0.82 (3H, d)     | 19.01          | 0.82 (3H, d)     | CH$_3$        |
| 28  | 25.6| 25.40            |                |                  |                | CH$_2$    |
| 29  | 12.4| 0.84 (3H, t)     | 12.25          | 0.83 (3H, t)     | CH$_3$        |

The $^{13}$C NMR spectrum data of compound 1 was a comparison with stigmasterol described in the literature [20]. The compound 1 was confirmed as stigmasterol (Fig. 1).
| No. | C   | δ\text{H} (ppm) | δ\text{H} (ppm) | δ\text{C} (ppm) | δ\text{H} (ppm) |
|-----|-----|----------------|----------------|----------------|----------------|
| 1   |     | 39.8          | 1.67 (2H, m)   | 38.7           | 39.1           |
| 2   |     | 28.3          | 1.65 (2H, m)   | 27.8           | 27.9           |
| 3   |     | 72.0          | 3.52 (1H, m)   | 77.8           | 79.7           |
| 4   |     | 39.6          |                | 39.1           | 40.0           |
| 5   |     | 56.1          |                | 55.5           | 55.9           |
| 6   |     | 19.1          |                | 18.7           | 19.1           |
| 7   |     | 32.1          |                | 33.0           | 33.4           |
| 8   |     | 39.9          |                | 39.5           | 40.2           |
| 9   |     | 50.3          | 1.52 (1H, m)   | 49.4           | 48.3           |
| 10  |     | 37.4          |                | 37.1           | 37.8           |
| 11  |     | 23.2          |                | 23.6           | 24.0           |
| 12  |     | 121.9         | 5.35 (1H, d, J=5.1) | 122.3 | 123.3 |
| 13  |     | 141.0         |                | 144.6           | 143.3           |
| 14  |     | 42.4          |                | 41.9           | 42.4           |
| 15  |     | 28.4          |                | 28.1           | 28.4           |
| 16  |     | 23.9          | 0.93 (2H, m)   | 23.6           | 24.1           |
| 17  |     | 46.0          |                | 47.9           | 46.6           |
| 18  |     | 42.5          | 2.85 (1H, J=7.1) | 41.8           | 41.9           |
| 19  |     | 46.0          |                | 46.2           | 46.6           |
| 20  |     | 29.9          |                | 30.7           | 30.4           |
| 21  |     | 34.1          |                | 34.0           | 34.5           |
| 22  |     | 32.1          |                | 32.9           | 31.3           |
| 23  |     | 28.4          | 1.00 (3H, s)   | 28.5           | 28.8           |
| 24  |     | 18.8          | 0.79 (3H, s)   | 16.6           | 16.2           |
| 25  |     | 18.9          | 0.82 (3H, s)   | 15.4           | 16.0           |
| 26  |     | 19.1          | 0.79 (3H, s)   | 17.7           | 17.7           |
| 27  |     | 25.6          | 1.03 (3H, s)   | 25.9           | 26.6           |
| 28  |     | 174.0         |                | 180.0          | 172.0          |
| 29  |     | 32.1          | 0.80 (3H, s)   | 33.0           | 33.7           |
| 30  |     | 24.5          | 0.84 (3H, s)   | 23.6           | 24.2           |
Fig. 1 Structure of compound 1 (stigmasterol)

In the $^1$H NMR spectrum also seen a signal pattern that is typical for triterpenoids, where visible signals that are attached in the area 2 ppm for aliphatic carbon. In the spectrum there are seven methyl signals each with a singlet peak with 3H integration at $\delta^H$ 0.79; 0.79; 0.80; 0.82; 0.84; 1.00; and 1.03 ppm. It also shows the signal for the vinyl proton at $\delta^H$ 5.35 (1H, d, J = 5.1) which is coupled by a neighboring proton so that it appears as the peak of the doublet. Furthermore, the proton signal at $\delta^H$ 3.52 (1H, m) is a signal from the proton at C which also binds OH which is coupled with two neighboring protons that are not chemical environment. In the $^{13}$C NMR spectrum there are 30 carbon atom signals that are typical for triterpenoid compounds. The 30 signals, only three signals appear above 100 ppm (C sp$^3$), namely at $\delta^C$ 121.9; 141.0; and 174.0 ppm. This signal indicates that the compound has a C = C double bond and a carbonyl group of carboxylic acids.

The $^{13}$C NMR spectrum data of compound 2 was a comparison with oleanolic acid described in the literature [21], [22]. The $^1$H and $^{13}$C NMR data of compound 2 see Table 3. The compound 2 was confirmed as oleanolic acid (Fig. 2).

Fig. 2 Structure of compound 2 (oleanolic acid)

3.3. MIC Value of Chemical Compounds

MIC values of compounds 1 and 2 are listed in Table 4. Compound 2 showed good antibacterial activity against S. typhi (MIC 62.5 µg / mL) and S. aureus (MIC 62.5 µg / mL); and moderate against E. coli (MIC 125 µg / mL), whereas against B. subtilis (MIC > 250 µg / mL) was a weak category. Compound 1 has a MIC value > 250 µg / mL against all test bacteria so that it is categorized as weak. MIC values are categorized in three groups: strong (<50 µg / mL), moderate (50-100 µg / mL), and weak (> 100 µg / mL) [23]. According to Pinheiro in [24], MIC values of the pure compound is a good activity if they have MIC values < 100 µg/mL.
Table 4. Mic values of the compound 1, 2 and positive control (antibiotic) at concentration series from 250 μg/ml to 3.9 μg/ml against tested bacteria.

| Compound      | MIC values (μg/mL) | S. typhi | B. subtilis | E. coli | S. aureus |
|---------------|--------------------|----------|-------------|---------|-----------|
| Compound 1    | > 250              | > 250    | > 250       | > 250   | > 250     |
| Compound 2    | 62.5               | > 250    | 125         | 62.5    |           |
| Tetracycline  | 3.9                | 3.9      | 3.9         | 3.9     |           |
| Streptomycin  | 3.9                | 3.9      | 3.9         | 3.9     |           |
| Penicillin    | 3.9                | 3.9      | 3.9         | 3.9     |           |

Note: The inhibition zone > 9 mm indicated that the compound has antibacterial activity at the corresponding concentration.

By the antibacterial compound's discovery in C. fruticosa stems, this plant extract has the potential to be developed as a source of active ingredients that can be applied in food, drink, and medicine. The literature provides numerous data that Oleanolic acid have many important pharmacological effects such as antimicrobial, antifungal, anti-inflammatory, hepatoprotective, anti-hyperlipidemic, antitumor, anti-HIV, anti cancer, and anti cancer. Oleanolic acid is relatively non-toxic and have been used in cosmetics and some health products [25], [26]. The C. fruticosa plant is widely used as an alternative treatment for Indonesian people, but it is not competitive enough to compete with chemical drugs. In Indonesia, the availability of C. fruticosa raw is abundant. This potential must be developed with ongoing research series so that it becomes a mainstay for the future pharmaceutical industry in Indonesia.

4. Conclusions
The ethyl acetate fraction of C. fruticosa stem has the highest antibacterial activity compared to other fractions. In the fraction, two pure compounds were isolated namely stigmasterol and oleanolic acid. The results of the antibacterial activity test showed that oleanolic acid had a role in providing antibacterial activity in the fraction. This research shows the potential of C. fruticosa stem to be developed in the fields of food, drink, and medicine, through a series of further studies.

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