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Steroids Produced by Endophytic Fungi (*Fusarium phaseoli*) Isolated from *Chisocheton macrophyllus* and their Antibacterial Activity against *Escherichia coli* and *Staphylococcus aureus*

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ABSTRACT. Steroids are secondary metabolic derivatives of terpenes containing the tetracyclic ring system known to exhibit fascinating pharmacological activity. Steroids are distributed in various genera of endophytic fungi including *Fusarium* genus which lives inside a higher tree such as *Chisocheton macrophyllus*. The purpose of this research is to identify and characterize the chemical structure of steroids generated by *F. phaseoli*, an endophytic fungus obtained from *C. macrophyllus* roots, as well as to assess their antibacterial activity against *Escherichia coli* and *Staphylococcus aureus*. The brown rice medium was fermented with *F. phaseoli* for six weeks before extraction with ethyl acetate. The extracts yielded four compounds, identified using spectroscopic methods such as FTIR, HRTOF-MS, 1D, and 2D NMR, and then compared to previously described compounds. Compounds **1**-**4** were identified as ergosterol (**1**), ergosterol peroxide (**2**), atroside (**3**), and cerevisterol (**4**). The four isolated compounds were evaluated for antibacterial activity against *Staphylococcus aureus* ATCC 6538 and *Escherichia coli* ATCC 11229 and displayed activity with MIC_{50} values of 500 µg/mL.

Keywords: Antibacterial activity; *Chisocheton macrophyllus*; *Fusarium phaseoli*; steroids.

INTRODUCTION

Terpenoid group is the popular compounds that have been isolated from Meliaceae and have several bioactivities (Hidayat et al., 2017). Steroid is one of terpene derivatives which usually consists of four rings (A, B, C have six membered and D has five-membered) (Happi & Teufel, 2024). Previous researchers have reported that steroids are compounds with fascinating bioactivities such as anticancer, antibacterial, anti-inflammatory, antioxidant, antimalarial, antiproliferative, and cytotoxic activity (Mayanti et al., 2022; Lindsay et al., 2023). The largest source of steroids is plant and fungi. Steroid compounds isolated from endophytic fungi have demonstrated potent biological activity with distinctive structure (Ji et al., 2021). Endophytic fungi are microorganisms that interact with plants where endophytes provide better absorption of nutrients, energy and protection against environmental stress and lead to the production of various secondary metabolites (Basit et al., 2021; Lu et al., 2021). Steroids are distributed in different genera of endophytic fungi namely *Chaetomium, Xylaria, Aspergillus, Phomopsis, Penicillium, Glomerella, Melochia, Eurotium* and *Fusarium*. These genera of endophytic fungus, especially *Fusarium,* are widely distributed in the Meliaceae family (Li et al., 2017; Khan et al., 2018; Suzuki et al., 2019; Hu et al., 2017). The Meliaceae family, an angiospermous family, has 575 species and 51 genera (Yadav et al., 2015). One of its genera is *Chisocheton*, which is the largest plants in Meliaceae family and is widely spread in tropical regions such as Asia.

Previous research on the *Chisocheton* plant has identified several steroid chemicals, including stigmasterol, stigmast-5-en-3β-ol (β-sitosterol), and β-sitosterol-13-*O*-acetate (Katja et al., 2017) found that β-sitosterol has antibacterial action against *S. aureus* and *E. coli* (MIC: 20 µg/mL) (Ododo et al., 2016). *S. aureus* and *E. coli* are gram-positive and negative

bacteria that often infect humans and cause several diseases such as diarrhoea, abdominal cramps, bloody urine, urinary tract infection and loss of appetite. These two bacteria are also resistant to several antibiotics, so the discovery of new antibiotic compounds must be carried out (Paul, 2024). The *Fusarium* genus live in the *C. macrophyllus* plant (Mulyani et al., 2023). *C. macrophyllus*, a species of the genus *Chisocheton*, usually grows in tropical rainforests (Bailly, 2024). Little studies have been published on the phytochemical analysis of *F. phaseoli*, an endophytic fungus isolated from *C. macrophyllus*, and its antibacterial property against *S. aureus* and *E. Coli* (Sari et al., 2022). Therefore, it is important for further study to explore the chemicals produced by endophytic fungi identified from *C. macrophyllus* and their potential as antibacterial agents to prevent antibiotic resistance in the future (Azhari & Supratman, 2021). In the previous paper, a new ergostane-type sterol, ergost-5,22*E*-dien-3*β*-oleate-20-ol, produced by *F. phaseoli* from the host plant *C. macrophyllus* was described (Sari et al., 2022). In the present communication, isolation and structural identification of the other steroids produced by *F. phaseoli* from the host plant *C. macrophyllus* along with their antibacterial activity will be discussed.

EXPERIMENTAL SECTION

General Experiment Procedure

The instruments used are common laboratory glasses in the natural products chemistry laboratory, autoclave, laminar airflow, and column chromatography. The macerate and fraction concentrations were carried out using a rotary evaporator type R-215 Buchi with vacuum system V-700 Buchi. The detection of spots for TLC analysis was irradiated under ultraviolet-visible light (254 and 365 nm). The characterization of pure isolate was carried out based on measurement of infrared (IR) spectrum using Thermo Scientific Nicolet Summit FTIR and HRTOF-MS were measured using Waters Xevo QTOF MS. Using a JEOL ECA-500 and Bruker Topspin, the NMR spectra were measured at 500 MHz for ^{1}H NMR and 125 MHz for ^{13}C NMR with tetramethyl silane (TMS) as an internal standard. Antibacterial activity was tested by using incubator, 96 well microplate, and spectrophotometer at λ_{max} 600 nm.

Plant Material

Samples are part of roots, stem barks and leaves of *C. macrophyllus*, which were gathered and characterized at Bogor Botanical Garden Conservation Center, West Java. The endophytic fungi were then isolated from each part of the specimens as reported in the previuosly publication (Sari et al., 2022)

Fermentation and Isolation

Fermentation of *C. macrophyllus* sample and isolation of steroids (**1**-**4**), had already described in the previously paper (Sari et al., 2022).

Compound **1**: White crystalline; m.p 159°C; IR (KBr) ν_{max} 3387, 2952, 2868, 1689, 1605, 1365, 1025 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz): 1.29 (2H, m), 1.86 (2H, m), 3.62 (1H, m), 2.26 (1H, m), 2.45 (1H, m), 5.55 (1H, dd, *J*=5.5, 3.0 Hz), 5.37 (1H, dd, *J*=5.4, 2.5 Hz), 1.96 (1H, m), 1.67 (2H, m), 1.46 (2H, m), 1.88 (1H, m), 1.70 (2H, m), 1.28 (2H, m), 1.25 (1H, m), 0.93 (3H, s), 0.61 (3H, s), 2.04 (1H, m), 1.02 (3H, d, *J* = 7 Hz), 5.20 (1H, m), 5.16 (1H, m), 1.84 (1H, m), 1.58 (1H, m), 0.83 (3H, d, *J*=7.5 Hz), 0.81 (3H, d, *J*=7.5 Hz), 0.91 (3H, d, *J*=7.0 Hz); and ^{13}C NMR ($CDCl_3$, 125 MHz) Table 1; HR-TOFMS (positive ion mode) *m/z* 397.3500 $[M+H]^+$ (calcd. for $C_{28}H_{45}O^+$, *m/z* 397.3470).

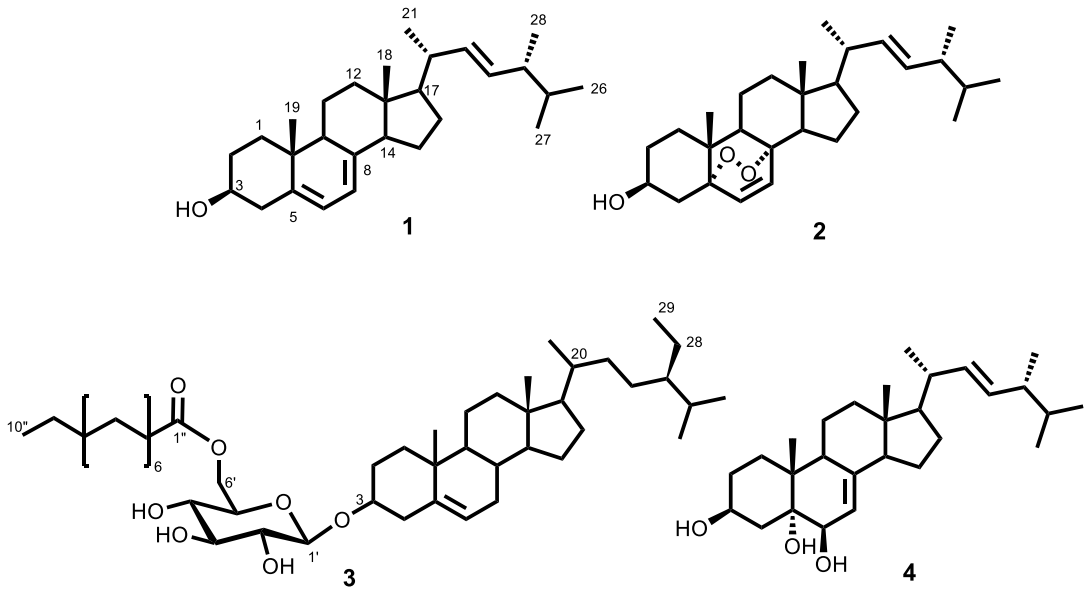

Figure 1. Chemical structure of compounds **1**-**4**.

Compound **2**: White crystalline; m.p 181-183°C; IR (KBr) ν_{max} 3299, 2954, 2920, 2853, 1722, 1458, 1377, 1082 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz): 1.7 (1H, m), 1.95 (1H, m), 1.54 (1H, m), 1.84 (1H, m), 3.95 (1H, m), 1.91 (1H, m), 2.28 (1H, m), 6.22 (1H, d, *J*=8.4 Hz), 6.43 (1H, d, *J*=8.8 Hz), 1.5 (1H, m), 1.23 (1H, m), 1.53 (1H, m), 1.23 (1H, m), 1.96 (1H, m), 1.56 (1H, m), 1.41 (2H, m), 1.60 (1H, m), 1.35 (1H, m), 1.75 (1H, m), 1.22 (1H, m), 0.80 (3H, s), 0.86 (3H, s), 2.03 (1H, ddd, *J*=2.0, 5.2, 14.0 Hz), 0.98 (3H, d, *J*=7.0 Hz), 5.21 (1H, dd, *J*=7.2, 15.2 Hz), 5.11 (1H, dd, *J*=8.4, 15.2 Hz), 1.84 (1H, m), 1.46 (1H, m), 0.79 (3H, d, *J*=6.8 Hz), 0.82 (3H, d, *J*=6.4 Hz), 0.92 (3H, d, *J*=6.8 Hz); ^{13}C NMR ($CDCl_3$, 125 MHz) Table 1; HR-TOFMS (positive ion mode) *m/z* 451.3179 $[M+Na]^+$ (calcd. for $C_{28}H_{44}O_3Na^+$, *m/z* 451.3188).

Compound **3**: Pale yellow oil, $[\alpha]_D^{20}$ +23.3 (*c* 1.0, $CHCl_3$); IR (KBr) ν_{max} 3309, 2930, 2850, 1731, 1639, 1466, 1376, 1051, 1026 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz): 1.34 (1H, m), 1.71 (1H, m), 3.32 (1H, m), 5.29 (1H, m), 0.60 (3H, s), 0.93 (3H, s), 0.85 (3H, d, *J*=6.3 Hz), 0.74 (3H, d, *J*=6.5 Hz), 0.74 (3H, d, *J*=6.5 Hz), 0.77 (3H, t , *J*=7.0 Hz), 4.32 (1H, d, *J*=7.6 Hz), 3.51 (1H, m), 3.48 (1H, m), 3.38 (1H, m), 3.37 (1H, m), 4.35 (1H, dd, *J*=11.0, 4.0 Hz), 4.22 (1H, br d, *J*=11.6 Hz), 1.18 (br s), 0.80 (3H, t, *J*=6.7 Hz); ^{13}C NMR ($CDCl_3$, 125 MHz) see **Table 1**; HR-TOFMS (negative ion mode) *m/z* 729.5656 $[M-H]^-$ (calcd. for $C_{45}H_{77}O_7^-$, *m/z* 729.5669).

Compound **4**: White crystalline; m.p 186-189°C; IR (KBr) ν_{max} 3368, 2952, 2866, 1456, 1369, 1040 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz): 4.07 (1H, qt, *J*=6.5 Hz), 3.62 (1H, d, *J*=5.0 Hz), 5.34 (1H, m), 0.58 (3H, s), 1.07 (3H, m), 1.01 (3H, d, *J*=6.5 Hz), 5.14 (1H, dd, *J*= 15.2, 7.5 Hz), 5.21 (1H, dd, *J*= 15.2, 7.5 Hz), 0.81 (3H, d, *J*=7.5 Hz), 0.81 (3H, d, *J*=7.5 Hz), 0.91 (3H, d, *J*=6.8 Hz); ^{13}C NMR ($CDCl_3$, 125 MHz) see **Table 1**; HR-TOFMS (positive ion mode) *m/z* 453.3325 $[M+Na]^+$ (calcd. for $C_{28}H_{46}O_3Na^+$, *m/z* 453.3345)

Antibacterial Activity Evaluation

The antibacterial activity was done following the method stated by (Odongo et al., 2023). The first step was both bacteria stock (*S. aureus* and *E. coli*) were planted on Mueller Hinton Agar medium using Ose needle and the process of incubation at 37°C was done for 18-24 hours. The bacteria culture was moved to Mueller Hinton Broth medium and incubated at 37 °C for 18-24 hours. The turbidity was checked and compared to the turbidity of *McFarland* standard. Suspension of bacteria culture that matches with the standard then inserted into the reaction tube. The positive control used is ciprofloxacin. Bacteria stock without any treatment was inserted to the reaction tube labeled negative control (-). The next step was sample diluted with variation of volume to have variation of concentration. Samples was measured the absorbance using a UV-Vis spectrophotometer to have initial absorbance. Variation of concentration of sample inserted bacteria and temperature at 37°C was used for the incubation process as long as 24 hours then the absorbance to have final absorbance. The initial and final absorbance of samples was calculated to give.

RESULTS AND DISCUSSION

Steroids **1-4** were found on the *n*-hexane fraction of *F. phaseoli* with a purification process with several column chromatography methods as mentioned in the previous paper (Sari et al., 2022).

Compound **1** was gained as white crystalline from an *n*-hexane extract of *F. phaseoli*. Compound **1** was soluble in chloroform and acetone, which showed a light blue spot following spraying with H_2SO_4 in an ethanol 10% accompanied by heating. Compound **1** did not give any fluorescent under UV light 254 nm nor 265 nm. UV spectrum revealed no absorption at a wavelength above 200 nm indicating no conjugated double bond. Compound **1** has $C_{28}H_{44}O$ molecular formula based on HR-TOFMS (positive ion mode) *m/z* 397.3500 $[M+H]^+$ (calc. for $C_{28}H_{45}O^+$, *m/z* 397.3470) with seven degrees of unsaturation. The spectrum of FTIR **2** showed a specific absorption band which was ascribed to stretching of OH (3384 cm^{-1}), stretching of C-H sp^3 (2952 and 2868 cm^{-1}), showed the stretching of C=C (1689 and 1605 cm^{-1}), *gem*-dimethyl (1365 cm^{-1}), and C-O (1025 cm^{-1}).

This prediction was supported by proton NMR (**Table 1**) which denoted the existence of the steroid-characteristic with four CH_3 doublet at 1.02 (3H, d, *J* = 7.0 Hz), 0.83 (3H, d, *J*=7.5 Hz), 0.81 (3H, d, *J*=7.5 Hz), 0.91 (3H, d, *J*=7.0 Hz) and two CH_3 singlet at 0.93 (3H, s), 0.61 (3H, s) (Marliyana et al., 2021). One O-CH at 3.62 (1H, m) supported the prediction of FTIR result. Olefinic methine resonating at δ_H 5.55 (1H, dd, *J*=5.5, 3.0 Hz, H-6), 5.37 (1H, dd, *J*=5.4, 2.5 Hz, H-7), 5.20 (1H, m), and 5.16 (1H, m). The results presented that this compound was steroid-type. Based on ^{13}C NMR combined with DEPT 135° spectra suggested a total of 28 carbons that include six CH_3, seven CH_2, six CH, a single O—CH at δ_C 70.5 (C-3), four CH=CH, and two olefinic Cq at δ_C 139.8 (C-5) and 141.4 (C-8), which was near to the chemical shift of the olefinic Cq of ergostane-type steroid. Compound **1** presented the similarity of chemical shift with compound ergostane known as an ergosterol afforded from *Laetiporus* sp. (Martinez et al., 2015), therefore compound **1** was identified as an ergosterol. Comparison the ^{13}C-NMR data of compound **1** to those of ergosterol was shown in **Table 1.**

Compound **2** was obtained in white crystalline from *n*-hexane extract of *F. phaseoli* which was soluble in chloroform and acetone, which gave a dark green color spot after spraying with H_2SO_4 in ethanolic solution followed by heating at 120 °C. Compound **2** did not give any fluorescent under UV light both in 254

nm nor 265 nm. UV spectrum revealed no absorption at wavelength above 200 nm indicating there is no conjugated double bond. HR-TOFMS *m/z* 451.3179 $[M+Na]^+$ (calc. for $C_{28}H_{44}O_3Na^+$, *m/z* 451.3188) presented that this compound was determined as $C_{28}H_{44}O_3$ (seven degrees of unsaturation). FTIR of compound **2** showed a specific absorption band which was ascribed to stretching of OH (3299 cm^{-1}), C-H sp^3 (2954, 2920 cm^{-1}), C=C (1722 cm^{-1}), bending of C-H sp^3 (1458 cm^{-1}), *gem*-dimethyl (1377 cm^{-1}), and C-O (1082 cm^{-1}). This analysis was validated with NMR spectra. The existence of four doublet peaks denoted methyl protons with δ_H 0.98 (3H, d, *J*=7.0 Hz), 0.79 (3H, d, *J*=6.8 Hz), 0.82 (3H, d, *J*=6.4 Hz), 0.92 (3H, d, *J*=6.8 Hz) and two methyl singlets.

According to analysis data of ^{1}H-NMR and ^{13}C-NMR, compound **2** had similarity to compound **1** (ergosterol). After all, compound **2** had only one additional olefinic pair at δ_C 135.4/C-6 (6.22, 1H, d, *J*=8.4 Hz) and 130.8/C-7 (δ_H 6.43, d, *J*= 8.8 Hz) and two quaternary oxygenated carbons at δ_C 82.2/C-5 and 79.5/C-8. The peroxide bridge connecting C-5 and C-8 was formed by two remaining oxygen atoms and an additional ring to satisfy the unsaturation requirement, while the two olefinic methines (-CH_6=CH_7-) were detected by the large coupling constant of the cis relationship of H-6/H-7 (*J*6/7=8.4 and 8.8 Hz). The NMR results (**Table 1**) of **2** were consistent with previously reported data of ergosterol-5,8-peroxide (Kobori et al., 2006). Thus, **2** was identified as an ergosterol-5,8-peroxide. Comparison the ^{13}C-NMR data of compound **2** to those of an ergosterol-5,8-peroxide was shown in the **Table 1**.

Pale-yellow oil of compound **3** had been afforded from the *n*-hexane extract of *F. phaseoli*. Compound **3** was soluble in chloroform and acetone, which gave a dark green color spot after spraying with H_2SO_4 in 10% ethanol followed by heating at 120 °C. Compound **3** did not give any fluorescent under UV light both of 254 nm nor 265 nm. UV spectrum revealed no absorption at a wavelength above 200 nm indicating no conjugated double bond. HR-TOFMS spectrum presented molecular formula $C_{45}H_{78}O_7$ with *m/z* 729.5656 $[M-H]^-$ (calcd. for $C_{45}H_{77}O_7^-$, *m/z* 729.5669) and discovered seven degrees of unsaturation. The FTIR spectrum of compound **3** showed a specific absorption band which was ascribed to stretching of OH (3309 cm^{-1}), C-H sp^3 (2930 and 2850 cm^{-1}), C=C (1731 and 1639 cm^{-1}), C-H sp^3 (1466 cm^{-1}), *gem*-dimethyl (1376 cm^{-1}), and stretching of C-O ether (1051 and 1026 cm^{-1}).

The ^{1}H-NMR spectrum denoted four methyls at δ_H 0.85, 0.74, 0.74, and 0.77 ppm, along with two quaternary methyl signals at δ_H 0.60 and 0.93 ppm which indicated the characteristic of steroid compound. There was a signal at δ_H 0.80 (3H, t, *J*=6.7 Hz, H-10") presented of fatty acid methyl signal. A peak at δ_H 3.32 (1H, m, H-3) recommended belongs to H-3 of steroid core skeleton, while the rest of six oxygenated methine signal at δ_H 4.32 (1H, d, *J*=7.6 Hz, H-1'), 3.51 (1H, m, H-2'), 3.48 (1H, m, H-3'), 3.38 (1H, m, H-4'), 3.37 (1H, m, H-5'), 4.35 (1H, dd, *J*=11.0, 4.0 Hz, Ha-6'), 4.22 (1H,*br* d, *J*=11.6 Hz, Hb-6') are typical for proton chemical shift of sugar substituent, namely glucose that attach through H-3 of steroid skeleton. The chemical shift is typical for an acylated steroid glucoside (Ali et al., 2001) and a significant signal at δ_H 1.22-1.277 indicated the existence of a fatty acid component.

The spectra of ^{13}C NMR and DEPT 135° revealed 29 carbon signals of steroid, 6 signals of glucose, and the rest of the signals belong to fatty acid substituents. The chemical shift of 29 carbon signal of steroid including six methyls, ten methylene, seven methine, one oxygenated methine, one olefinic methine, and one olefinic quaternary carbon which indicates the possibility of C_{29} stigmastane-type steroid skeleton. The olefinic methine at δ_C 122.1 paired with olefinic quaternary carbon at δ_C 140.3 were identical to the chemical shift at olefinic C-5 and C-6 of steroid compound. The presence of sugar chemical shift at δ_C 101.2 (C-1'), 73.6 (C-2'), 76.0 (C-3'), 70.2 (C-4'), 73.9 (C-5'), 63.2 (C6') supported the existence of glucose substituent attach to C-3 of steroid. The existence of ester carbonyl at δ_C 174.5 (C-1") and high signal of methylene at δ_C indicated the fatty acid unit attached to glucose. The results of this analysis described three degrees of unsaturation where four degrees of unsaturation were related to the tetracyclic framework of the steroid. These results were compared with data from the literature and it can be concluded that this compound was an atroside, which was first isolated from *Perovskia atriplicifolia* (An et al., 2021). Consequently, compound **3** was identified as an atroside. Comparison the ^{13}C-NMR data of compound **3** to those of an atroside was shown in the **Table 2**.

Compound **4** has the same form with **2** namely white crystalline and after being sprayed with sulfate acid in 10% ethanol it showed a dark green color spot and the HR-TOFMS showed the peak with the molecular formula, $C_{28}H_{46}O_3$ *m/z* 453.3325 $[M+Na]^+$ (calcd. for $C_{28}H_{46}O_3Na^+$, *m/z* 453.3345). The result of the FTIR spectrum also showed the similarity peak with **2,** including (3368 cm^{-1}, OH), (2952 and 2866 cm^{-1}, CH), (1456 cm^{-1}, C-H sp^3), (1369 cm^{-1}, *gem*-dimethyl), and (1040 cm^{-1}, C-O). This prediction was supported by NMR spectra which discovered the characteristics of steroids with four methyl doublets and two methyl singlets. Two oxygenated methine at δ_H 4.06 (1H, qt, *J*=6.5 Hz, H-3) and 3.61 (1H, m, H-6) supported this prediction. A peak at 5.34, 5.14, and 5.21 ppm described that this compound has three olefinic methine and was derivative of an ergostane-steroid type. Furthermore, the coupling constant of H-22/H-23 represented the vicinal olefin proton with *trans*-orientation.

Table 1. Comparison ^{13}C-NMR data of compound **1-2** with ergosterol and an ergosterol-5,8-peroxide

position carbon	δc of **1***	δc of ergosterol**	δc of **2***	δc of ergosterol-5,8-peroxide***
1	38.4	38.4	34.7	34.1
2	32.0	32.0	30.1	30.1
3	70.5	70.4	66.5	66.5
4	40.8	40.8	37.0	37.0
5	139.8	139.8	82.2	82.2
6	119.6	119.6	135.4	135.4
7	116.3	116.3	130.8	130.8
8	141.4	141.3	79.5	79.4
9	46.3	46.2	51.1	51.1
10	37.1	37.0	36.9	37.0
11	21.1	21.1	23.4	23.4
12	39.1	39.1	39.3	39.4
13	42.8	42.8	44.6	44.6
14	54.6	54.6	51.7	51.7
15	23.0	23.0	20.7	20.6
16	28.3	28.3	28.7	28.7
17	55.7	55.7	56.2	56.2
18	12.1	12.0	12.9	12.9
19	16.3	17.6	18.2	18.2
20	40.5	40.4	39.8	39.7
21	21.1	21.1	20.9	20.9
22	135.6	135.6	135.2	135.2
23	132.0	132.0	132.3	132.3
24	42.8	42.8	42.8	42.8
25	33.1	33.1	33.1	33.1
26	19.7	19.6	19.7	19.7
27	20.0	19.9	20.0	20.0
28	17.6	16.2	17.6	17.6

* Kobori et al., (2006); **75 MHz in $CDCl_3$; *** 125 MHz in $CDCl_3$

The spectra of carbon and DEPT-135 NMR proved the number of carbons is 28 with six methyl signals that supported the proton NMR data, seven methylene, six methine, two quaternary carbon, and one oxygenated carbon support the suggestion of steroid skeleton. Peaks appeared at δ_C 135.4 (C-22) and 132.2 (C-23) belong to methine was similar to that of the C28 side ergostane chain. The existence of olefinic methine at δ_C 117.5 (H-7) paired with one quaternary olefinic carbon δ_C 144.1 (H-8) are suspected modified B ring of ergosterol, which double bond opened yield an oxygenated H-5 (δ_C 76.0) and H-6 (δ_C 73.7). These data show that this structure has two degrees of unsaturation, whereas the tetracyclic steroid framework has four degrees of unsaturation. These data illustrated the existence of two degrees of unsaturation and the remaining four belong to the steroid framework, namely tetracyclic. The analysis of NMR spectra was compared to the literature and concluded that **4** was cerevisterol which had been found in *Beauveria* sp. (An et al., 2021). Therefore, compound **4** was identified as a cerevisterol. Comparison the ^{13}C-NMR data of compound **3** to those of cerevisterol was shown in the **Table 2.**

The result has shown no antibacterial activities performed by compound **1-4**. However, the crude extract performed weak antibacterial activities against *S. aureus* ATCC 6538 (MIC:156.0 µg/mL). All the isolated steroids (**1-4**) in comparison with the extract could be related to the synergistic action of the other components. Based on reported study, ergosterol (**1**) known to have antibacterial activity against *S. aureus* with MIC value of 15.63 µg/mL (Hussein et al., 2022). The inactivity of ergosterol in this research might cause by several factor including the stronger *S. aureus* strain and purity of compound.

Table 2. Comparison of ^{13}C-NMR data o compound **3-4** with atroside and cerevisterol.

position carbon	δc of 3*	δc of atroside**	δc of 4*	position carbon (Cont.)		δc of cerevistrol***	position carbon (Cont.)
1	33.0	33.1	37.2	29	11.9	37.1	11.9
2	30.9	31.0	29.3	1'	101.2	29.2	101.2
3	67.8	67.9	79.5	2'	73.6	67.7	73.6
4	39.5	39.5	38.9	3'	76.0	39.5	76.1
5	76.0	76.0	140.3	4'	70.2	76.0	70.2
6	73.7	73.7	122.1	5'	73.9	73.7	73.8
7	117.5	117.5	31.9	6'	63.2	117.6	63.2
8	144.1	144.1	31.8	1"	174.5	144.1	174.4
9	43.5	43.5	50.2	2"	34.2	43.5	34.2
10	37.2	37.1	36.7	3"	24.9	36.8	24.9
11	22.1	22.1	21.0	4"-7"	29.7	21.0	29.6
12	39.2	39.2	39.7	8"	31.8	39.2	31.8
13	43.8	43.8	42.3	9"	22.7	43.8	22.7
14	54.8	54.8	56.7	10"	14.0	54.8	14.0
15	22.9	22.8	24.2			24.1	
16	28.0	28.0	28.2			28.1	
17	56.0	56.0	56.1			56.0	
18	12.4	12.4	11.8			11.8	
19	18.9	18.8	19.3			19.2	
20	40.5	40.5	36.1			37.2	
21	21.2	21.2	18.7			18.8	
22	135.4	135.4	33.9			135.4	
23	132.2	132.2	26.1			132.2	
24	42.8	42.8	48.5			42.8	
25	33.1	33.1	29.2			33.1	
26	20.0	20.0	19.8			20.0	
27	19.7	19.8	19.0			19.7	
28	17.6	17.5	23.0			17.6	

* An et al., (2021); ** 600 MHz in $CDCl_3$; 600 MHz in $CDCl_3$

Table 3. The antibacterial properties of extracts, fractions, and compounds **1-4**

Samples (µg/mL)	MIC µg/mL	
	Staphylococcus aureus ATCC 6538	*Escherichia coli ATCC 11229*
Fermented Extract (EA)	156.0	313.0
n-hexane extract	>500	>500
EA extract	>500	>500
Ergosterol (**1**)	>500	>500
Ergosterol peroxide (**2**)	>500	>500
Atroside (**3**)	>500	>500
Cerevisterol (**4**)	>500	>500
Ampicillin (Control +)	15.6	31.25

The results of antibacterial evaluation of the extract, *n*-hexane extract, ethyl acetate extract and compound **1-4** were evaluated against *S. aureus* ATCC 6538 and *E. coli* ATCC 11229. The antibacterial activity evaluation described at **Table 3**.

CONCLUSIONS

Four isolated steroids produced by fungus *F. phaseoli* from *C. macrophyllus* were identified as ergosterol (**1**), ergosterol-5,8-peroxide (**2**), atroside (**3**), and cerevisterol (**4**) by spectroscopic methods (HRMS, IR, and 1D-NMR) and compared with NMR data from the literature. Antibacterial activity showed that the initial extract resulting from *F. phaseoli* fermentation with ethyl acetate had weak inhibition, while all isolated steroids were inactive against *S. aureus* ATCC 6538 and *E. coli* ATCC 11229. Therefore, the synergistic effect of the active compounds in the extract could affect its antibacterial activity. However, the isolated steroids, which were inactive against *S. aureus* and *E. coli*, need to be evaluated its cytotoxic activity against other bacteria or

other bioactivity potential such as cytotoxic activity and anti-inflammatory activity because steroid compounds are also known for their anticancer and anti-inflammatory activity.

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