Stigmasterol and Stigmasterone from Methanol Extract of *Calophyllum soulattri* Burm. F. Stem Bark

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Article Info

Abstract

Stigmasterol and Stigmasterone from Methanol Extract of *Calophyllum soulattri* Burm. F. Stem Bark. *Calophyllum soulattri* Burm. F. has been widely used for herbal medicine. Phytochemical investigation of *C. soulattri* contains a secondary metabolite of the steroid class. Steroid compounds have various biological activities, such as anti-inflammatory, antioxidant, antiproliferative, antibacterial, antimalarial, and anticancer. Two secondary metabolites steroids have been isolated and identified from the stem bark extract of *C. soulattri*. Isolation was carried out through the extraction (maceration), fractionation, and purification stages. Maceration is carried out using methanol as a solvent. Fractionation was carried out by vacuum liquid chromatography (VLC), and purification was by flash column chromatography. Identification of combined fractions and determination of pure isolates were used through thin-layer chromatography (TLC). The solvent used in the chromatography methods was a mixture of n-hexane and ethyl acetate. The structure isolates were identified by FTIR, ¹H NMR, and ¹³C NMR and compared with literature data. Secondary metabolites steroids that have been isolated are identical compounds to stigmasterol and stigmasterone.

1. Introduction

The *Calophyllum* genus is a type of plant that is often found in the tropical forests of Indonesia. The community has used this plant as herbal medicine, including a diuretic, blood pressure, rheumatism, malaria, sexually transmitted diseases, varicose veins, hemorrhoids, infections of the skin, nephritis, and anti-inflammatory drugs [1]. One species of the genus *Calophyllum* is *Calophyllum soulattri*, known as slatri.

Secondary metabolites in *C. soulattri* that have been reported include the terpenoid group, i.e., fridelin [2], soulamarin, a derivate of coumarin [3], the steroid class, such as stigmasterol and β-sitosterol [2, 4]. Also, there are also xanthones groups, such as soulatrin, caloxanthone-B, caloxanthone C, macluraxanthone, phylattrin, brasixanthon, and trapezifolixanthone [5]. Some of these compounds can be found in the stems, leaves, and roots of the *C. soulattri* plant. Whereas the bark of *C. soulattri* contains xanthone groups, including caloxanthone-B, caloxanthone-C, phylattrin, soulatrin, macluraxanthone, and brasixanthone [3], and the steroid group, such as stigmasterol [2].

Exploration of compounds in the stem bark of *C. soulattri* has not been widely carried out, especially the isolation of secondary metabolites of the steroid group. The steroid group has anti-inflammatory, antidiabetic [6], antioxidant, anti-tumor, anti-osteoarthritis, antimutagenic [7], and antibacterial activities [8]. Therefore, it is necessary to explore *C. soulattri*, especially in the stem bark section, contributing to adding a database of steroid group compounds. Furthermore, the database can be used as a source for potential medicinal compounds.

2. Materials and Methods

FTIR spectrophotometer Shimadzu (Kyoto, Jepang), NMR 500 MHz Agilent (Santa Clara, USA), vacuum liquid chromatography (VLC), flash column chromatography,

and UV$_{254}$ lamp were the instruments and tools employed in this research. Methanol, *n*-hexane, ethyl acetate, and acetone were technical grades. Silica gel 60 G (Merck; Darmstadt, Jerman), Silica gel 60 (0,04-0,063 mm) 230-400 mesh ASTM (Merck; Darmstadt, Jerman), Silica gel 60 (0,2-0,5 mm) (Merck; Darmstadt, Jerman) and TLC plate (alumunium coated silica gel 60 F$_{254}$ 0,25 mm (Merck; Darmstadt, Jerman)) were the adsorbent for chromatography. Cerium(IV) (Merck; Darmstadt, Jerman) and H$_2$SO$_4$ (Mallinckrodt) were used as spotting reagents.

The *C. soulattri* stem bark powder (2.5 kg) was macerated using methanol (10 L) solvent for 3 x 24 hours. The filtrate was evaporated to give 385 g of thick blackish brown extract. The 13 g methanol extract was fractionated using VLC with a solvent mixture of n-hexane: ethyl acetate (10: 0; 9: 1; 8: 2; 7: 3; 6: 4; 5: 5; 4: 6; 3: 7; 2: 8; 1: 9; 0:10) with a grading system that produced 20 fractions. The selection of fractions to be purified based on TLC analysis. TLC results were seen with a UV lamp (λ_{254}) then sprayed with spotting reagent Ce(SO$_4$)$_2$. The fractions having the same TLC profile were combined and purified by flash column chromatography.

Based on the TLC profile, fractions 1-9 were further purified using a mixture of eluent n-hexane: ethyl acetate with a ratio of 9.5: 0.5 (200 mL); while each ratio of 9:1, 8:2, 7:3, 6:4, and 5:5 in a volume of 100 mL, and 150 mL of 100% acetone, which gives 72 fractions. The fractions F19-21 and F28 were chosen to be tested for purity because they showed one spot. The fraction F19-21 hereinafter referred to as F19, and F28 were identified for their structure using FTIR, ^{1}H NMR, and ^{13}C NMR spectroscopy methods.

3. Results and Discussion

3.1. Identification of F19 Compound

The isolation of the stem bark extract of *C. soulattri* resulted in two pure isolates, namely F19 and F28. Analysis of the IR spectrum of F19 shows the presence of hydroxy group (-OH) absorption at around 3400-3500 cm^{-1} (broad). The IR spectrum also shows the presence of aliphatic C-H stretching vibrations at around 2900-2800 cm^{-1} (sharp) and weak absorption of alkenes (C=C) around 1600 cm^{-1} (sharp). The absorption at 1400 cm^{-1} (sharp) is the absorption of CH$_2$ bending, while the absorption at around 1000-1100 cm^{-1} (sharp) is the absorption of cycloalkanes. Based on FTIR results, and compared with literature data, isolate F19 is a steroid compound [9]. The FTIR absorption data are shown in Figure 1 and Table 1. Further analysis of the ^{1}H NMR and ^{13}C NMR data was carried out.
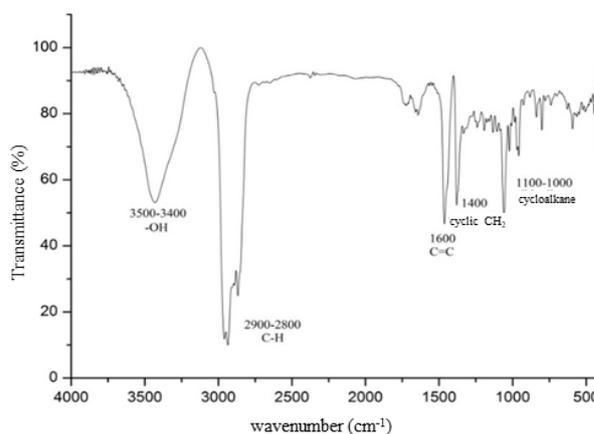
Figure 1. FTIR spectrum of F19 compound

Table 1. FTIR data comparison of F19 compound with literature(F19*)

F19 (cm^{-1})	F19* (cm^{-1})	Vibrations
3427	3547	-OH stretching, broad
2891	2857	C-H bending, sharp
1643	1638	C=C weak, sharp
1463	1462	CH2 bending, sharp
1058	1071	cycloalkanes, sharp

F$_{19}$: measured in KBr
F$_{19}$*: reference compound measured in KBr [9]

Identification of F19 with NMR, including ^{13}C NMR and ^{1}H NMR, was carried out in CDCl$_3$ solvent. The ^{1}H NMR spectrum analysis data (Figure 2) shows the presence of 48 proton signals. The proton signal in the chemical shift (δ_H) from 0.67 to 2.3 ppm is a signal from the proton *sp^3* consisting of methine (CH), methylene (CH$_2$), and methyl (CH$_3$). The presence of 6 methyl groups is indicated by the proton signals at δ_H (ppm) 0.69 (3H, s, H-18); 1.01 (3H, s, H-19); 1.02 (3H, m, H-21); 0.85 (3H, m, H-26); 0.80 (3H, m, H-27) and 0.81 (3H, m, H-29). The 9 methylene (CH$_2$) groups are indicated by the presence of proton signal at δ_H (ppm) 1.84 (2H, m, H-1); 1.83 (2H, m, H-2); 2,3 (2H, m, H-4); 1.97 (2H, m, H-7); 1.50 (2H, m, H-11); 2.00 (2H, m, H-12); 1.56 (2H, m, H-15); 1.72 (2H, m, H-16) and 1.44 (2H, m, H-28). In addition, there are also 11 methine groups indicated by the presence of a proton signal at δ_H (ppm) 3.52 (1H, m, H-3); 1.46 (1H, m, H-8); 0.92 (1H, m, H-9); 1.01 (1H, m, H-14); 1.15 (1H, m, H-17); 2.06 (1H, m, H-20); 1.54 (1H, m, H-24) and 1.55 (1H, m, H-25). There is an alkene proton signal at a chemical shift of 5.35 (1H, m, H-6); 5.04 (1H, ss, J = 8.65; 15.2 Hz, H-22); 5.1 (1H, dd, J = 8.65; 15.15 Hz, H-23) which is the main feature of the steroid framework. This ^{1}H NMR data analysis is supported by ^{13}C NMR data.
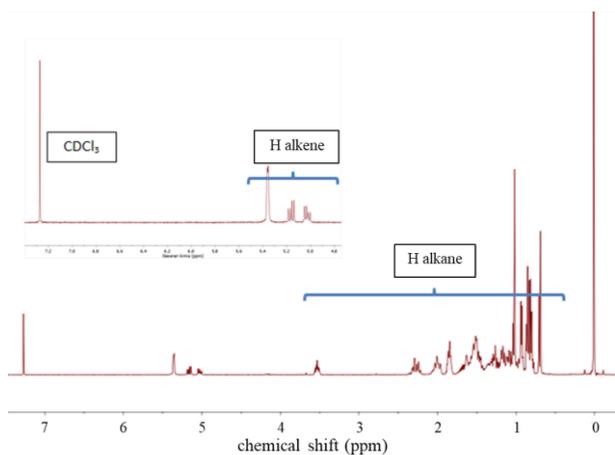
Figure 2. ^{1}H NMR spectrum of F19 compound

The ^{13}C NMR spectrum (Figure 3) shows the presence of 29 carbon atom signals, each signal having a distinctive shift. The chemical shift of carbon (δ_C) 12.0-71.9 ppm shows a signal from C sp^3 which consists of six methyl groups (CH$_3$) at δ_C 12.0 (C-18); 19.1 (C-19); 23.2 (C-21); 21.2 (C-26); 21.3 (C-27); 12.1 (C-29) ppm. In addition, nine methylene (CH$_2$) groups at δ_C 36.6 (C-1); 29.3 (C-2); 42.3 (C-4); 31.8 (C-7); 24.4 (C-11); 39.8 (C-12); 24.5 (C-15); 28.4 (C-16); 25.5 (C-28) ppm was also identified. The presence of 11 metine (CH) groups is shown at δ_C 71.9 (C-3); 29.0 (C-8); 50.2 (C-9); 56.9 (C-14); 56.1 (C-17); 39.9 (C-20); 51.3 (C-24); 34.1 (C-25) and 3 C quaternary (Cq) at δ_C 140.9 (C-5); 36.3 (C-10); 40.6 (C-13) ppm. The alkene C signal is found at 121.8 (C-6); 138.4 (C-22); and 129.4 (C-23). The obtained NMR data were then compared with literature data [9, 10] (Table 2).
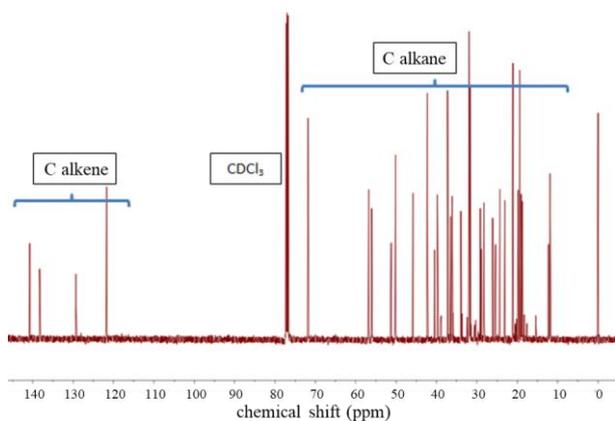
Figure3.^{13}C NMR spectrum of F19 compound

Based on the analysis of FTIR, ^{1}H NMR, and ^{13}C NMR data, as well as the results of comparisons with literature data, compound F19 is a compound identical to stigmasterol (F19*) with the molecular formula $C_{29}H_{48}O$, as shown in Figure 4. Stigmasterol which has been isolated from the stem bark of *C. soulattri*, is also found in many other plants, such as *Neocarya macrophylla* [11], *Ficus hispida* [12], and *Terminalia schimperiana* [13].

Table 2. NMR data comparison of F19 compound with literature (F19*)

No. C	δ_H (multiplicity, *J* in Hz)		δ_C (ppm)	
	F19	F19*	F19	F19*
1	1.84 (m)	1.84 (m)	36.6	36.7
2	1.83 (m)	1.83 (m)	29.3	29.7
3	3.52 (m)	3.51 (m)	71.9	71.9
4	2.3 (m)	2.3 (m)	42.3	42.3
5	–	–	140.9	140.9
6	5.35 (m)	5.34 (m)	121.8	121.3
7	1.97 (m)	1.97 (m)	31.8	31.7
8	1.46 (m)	1.46 (m)	29.0	29.2
9	0.92 (m)	0.94 (m)	50.2	50.0
10	–	–	36.3	36.1
11	1.50 (m)	1.50 (m)	24.4	24.3
12	2.00 (m)	2.00 (m)	39.8	39.8
13	–	–	40.6	40.4
14	1.01 (m)	1.01 (m)	56.9	56.9
15	1.56 (m)	1.56 (m)	24.5	24.3
16	1.72 (m)	1.72 (m)	28.4	28.9
17	1.15 (m)	1.15 (q)	56.1	56.0
18	0.69 (s)	0.70 (s)	12.0	12.0
19	1.01 (s)	1.01 (s)	19.1	19.0
20	2.06 (m)	2.06 (m)	39.9	39.8
21	1.02 (m)	1.03 (m)	23.2	23.1
22	5.17 (dd, 15.2; 8.65)	5.16 (dd, 15.08; 8.62)	138.4	138.4
23	5.03 (dd, 15.15; 8.65)	5.03 (dd, 15.03; 8.62)	129.4	129.3
24	1.54 (m)	1.54 (m)	51.3	51.2
25	1.55 (m)	1.55 (m)	34.1	34.1
26	0.85 (m)	0.85 (d)	21.2	21.1
27	0.80 (m)	0.80 (d)	21.3	22.8
28	1.44 (m)	1.43 (m)	25.5	25.3
29	0.81 (m)	0.81 (t)	12.1	12.0

F$_{19}$: measured in CDCl$_3$, 500 MHz (^{1}H) and 125 MHz (^{13}C)
F$_{19}$*: measured in CDCl$_3$, 400 MHz (^{1}H) dan 100 MHz (^{13}C) [9, 10]

Based on the analysis of FTIR, ^{1}H NMR, and ^{13}C NMR data, as well as the results of comparisons with literature data, compound F19 is a compound identical to stigmasterol (F19*) with the molecular formula $C_{29}H_{48}O$, as shown in Figure 4. Stigmasterol which has been isolated from the stem bark of *C. soulattri*, is also found in many other plants, such as *Neocarya macrophylla* [11], *Ficus hispida* [12], and *Terminalia schimperiana* [13].
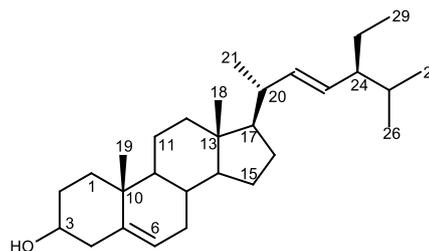
Figure 4. Stigmasterol structure.

3.2. Identification of F28 compound

Analysis of the F28 compound IR spectrum shows the absorption at around 3400-3500 cm^{-1} (broad), which is characteristic of the hydroxy (-OH) group. The presence of aliphatic C-H stretching vibration and alkene (C=C) vibration was shown at around 2900-2800 cm^{-1} (sharp) and around 1600 cm^{-1} (sharp). The presence of a carbonyl group (C=O) is indicated by absorption at around 1700 cm^{-1} (sharp). The absorption at 1400 cm^{-1} (sharp) is the CH$_2$ bending vibration. Besides, there is cycloalkane absorption at around 1000-1100 cm^{-1} (sharp). The IR spectrum of F28 compared with the literature [9, 14] are shown in Figure 5 and Table 3. Further analysis of the ^{1}H NMR and ^{13}C NMR data was carried out.
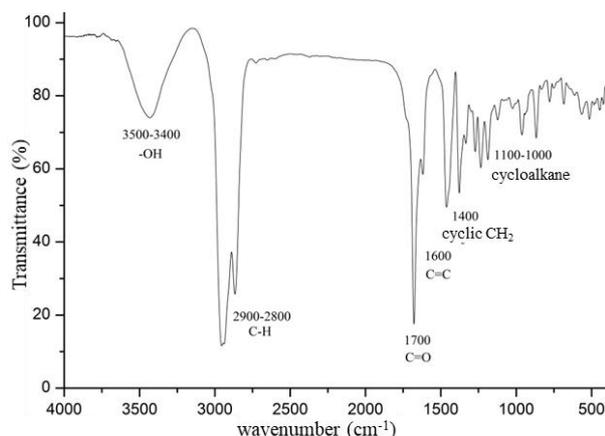
Figure 5. The FTIR spectrum of the F28 compound

Table 3. FTIR data comparison of F28 and Literature (F28*)

F28 (cm^{-1})	F28* (cm^{-1})	Vibrations
3430	3547	–OH *stretching, broad*
2867	2857	C-H *sharp*
1678	1715	C=O *sharp*
1619	1638	C=C *weak*
1462	1462	CH$_2$ *bending, sharp*
1122	1071	cycloalkane, *sharp*

Compound F$_{28}$: measured in KBr
Compound F$_{28}$*: measured in KBr [9, 11]

Identification of F28 was carried out in CDCl$_3$ solvent using NMR spectroscopy, including ^{13}C NMR and 1H NMR. The 1H NMR spectrum (Figure 6) reveals the presence of 52 proton signals. The proton signal in chemical shift (δ_H) from 0.70 to 2.38 ppm is a signal from the proton sp^3 consisting of methyl (CH$_3$), methylene (CH$_2$), and methine (CH). The proton signal for 6 methyl groups is shown at δ_H (ppm) 0.71 (3H, s, H-18); 1.01 (3H, s, H-19); 1.03 (3H, m, H-21); 0.85 (3H, m, H-26); 0.80 (3H, m, H-27) and 0.82 (3H, m, H-29). The presence of a methylene group (CH$_2$) is indicated by a proton signal with δ_H (ppm) 1.50 (2H, m, H-11); 2.00 (2H, m, H-12); 1.57 (2H, m, H-15); 1.72 (2H, m, H-16) and 1.43 (2H, m, H-28). While the methine group is indicated by a proton signal at δ_H (ppm) 1.01 (1H, m, H-14); 1.15 (1H, m, H-17); 2.03 (1H, m, H-20); 1.54 (1H, m, H-24) and 1.55 (1H, m,

H-25). There is an alkene proton signal on chemical shift 5.17 (1H, dd, J = 8.55; 15.1 Hz, H-22); 5.04 (1H, dd, J = 8.6; 15.1 Hz, H-23), which is the main feature of the steroid framework. This 1H NMR data analysis is supported by ^{13}C NMR data.
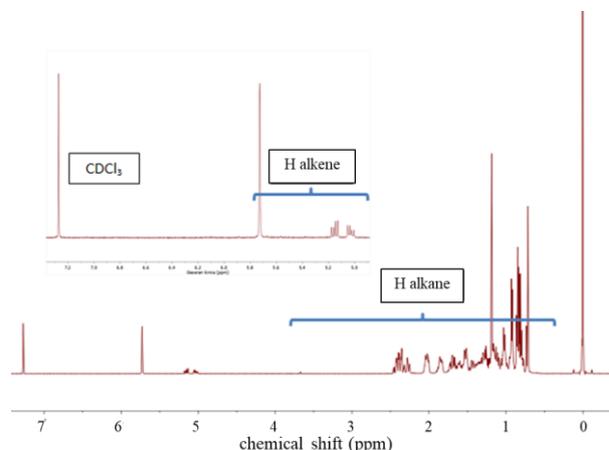
Figure 6. The ^{1}H NMR spectrum of the F28 compound

The ^{13}C NMR spectrum (Figure 7) shows a carbon signal at δ_C 12.0-56.2 ppm which is a signal from C sp^3 consisting of 6 methyl groups (CH$_3$) at δ_C 12.0 (C-18); 19.1 (C-19); 18.8 (C-21); 21.1 (C-26); 21.2 (C-27); 12.1 (C-29) ppm. In addition, methylene (CH$_2$) groups are also appeared at δ_C 24.4 (C-11); 39.7 (C-12); 24.3 (C-15); 28.3 (C-16); 25.5 (C-28) ppm. The presence of a methine (CH) group is indicated at δ_C 56.1 (C-14); 56.0 (C-17); 42.4 (C-20); 51.4 (C-24); 34.1 (C-25) and C quaternary (Cq) at δ_C 42.5 (C-13)ppm.The alkene signal is identified at 138.2 (C-22); and 129.5 (C-23) ppm. The carbonyl group is denoted by the C signal at 200.0 ppm, which is a signal from the ketone group, while the appearance of the signal at 178 ppm is a signal of the carboxylate group from the impurity. Furthermore, the NMR analysis data were compared with literary data [9, 10], as shown in Table 4.
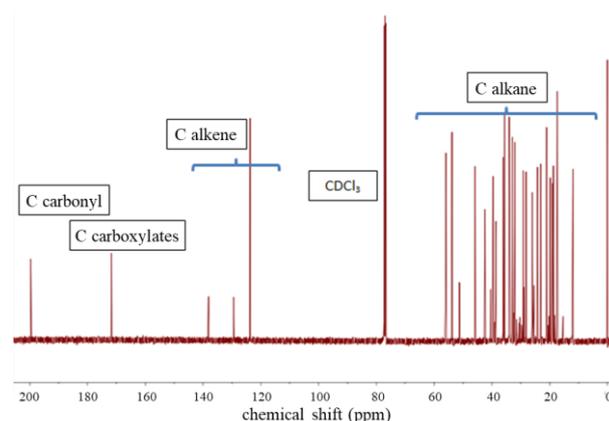
Figure 7. The ^{13}C NMR spectrum of the F28 compound

Table 4. NMR data comparison of F28 and literature (F28*) compounds

No. C	δ_H (multiplicity, J in Hz)		δ_C (ppm)	
	F28	F28*	F28	F28*
11	1.50 (m)	1.50 (m)	24.4	24.3
12	2.00 (m)	2.00 (m)	39.7	39.8
13	–	–	42.5	40.4
14	1.01 (m)	1.01 (m)	56.1	56.9
15	1.57 (m)	1.56 (m)	24.3	24.3
16	1.72 (m)	1.72 (m)	28.3	28.9
17	1.15 (m)	1.15 (q)	56.0	56.0
18	0.71 (s)	0.70 (s)	12.0	12.0
19	1.01 (s)	1.01 (s)	19.1	19.0
20	2.03 (m)	2.06 (m)	42.4	39.8
21	1.03 (m)	1.03 (m)	18.8	23.1
22	5.17 (dd, 15.1; 8.55)	5.17 (dd, 22.2; 15.2)	138.2	138.4
23	5.04 (dd, 15.1; 8.6)	5.04 (dd, 23.2; 8.6)	129.5	129.3
24	1.54 (m)	1.54 (m)	51.4	51.2
25	1.55 (m)	1.55 (m)	34.1	34.1
26	0.85 (m)	0.85 (d)	21.1	21.1
27	0.80 (m)	0.80 (d)	21.2	22.8
28	1.43 (m)	1.43 (m)	25.5	25.3
29	0.82 (m)	0.81 (t)	12.1	12.0

F28: measured in CDCl$_3$, 500 MHz (^{1}H) and 125 MHz (^{13}C)
F28*: measured in CDCl$_3$, 400 MHz (^{1}H) and 100 MHz (^{13}C) [9, 10]

Based on the overall results of FTIR, ^{1}H NMR, and ^{13}C NMR data analysis, and compared with literature data, the suggested compound structure for compound F28 is similar to stigmasterone with the molecular formula C$_{29}$H$_{46}$O [15], the structure of stigmasterone is shown in Figure 2. Based on a literature review, stigmasterone was first discovered in the bark of *C. soulattri*.
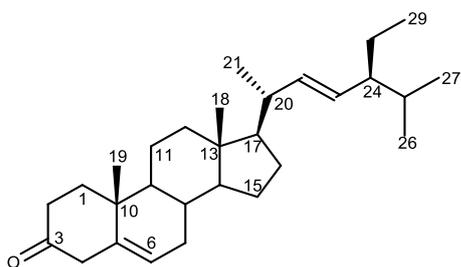
Figure 8. Stigmasterone structure.

Two secondary metabolites of the steroid class that have been isolated from the bark of *C. soulattri*, namely stigmasterol and stigmasterone, contribute to increasing the database of *C. soulattri*, which can then be used as a source of medicinal compounds. Based on the literature review, stigmasterone was isolated from *C. soulattri* for the first time. Apart from *C. soulattri* stigmasterone, it is found in *Amaranthus spinosus* [16] and *Virola surinamensis* [17].

4. Conclusion

Two secondary metabolites of the steroid class have been isolated from the stem bark of *C. soulattri*, which are identical compounds to stigmasterol and stigmasterone. Based on the literature review, stigmasterone was isolated for the first time in extracting the stem bark of *C. soulatrri*.

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