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

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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, ^{1}H NMR, and ^{13}C 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_2SO_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.

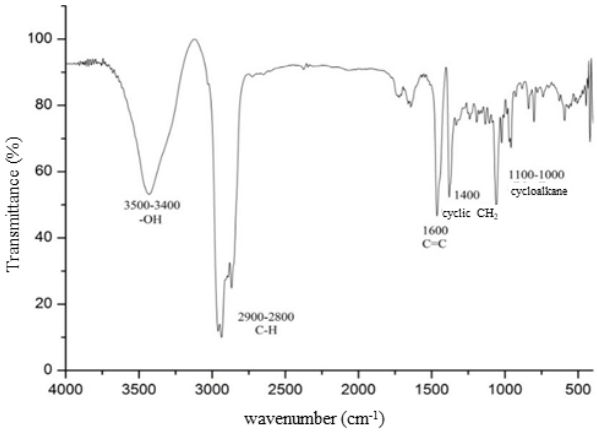

Figure 1. FTIR spectrum of F19 compound

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.

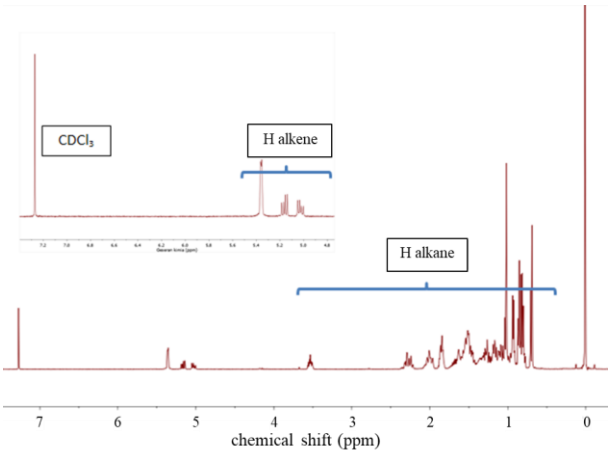

Figure 2. ^{1}H NMR spectrum of F19 compound

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).

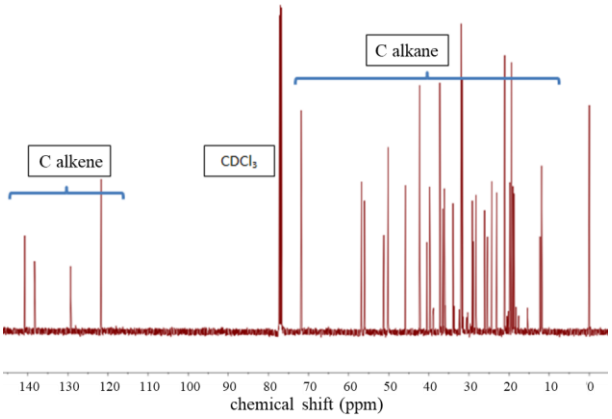

Figure3.^{13}C NMR spectrum of F19 compound

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].

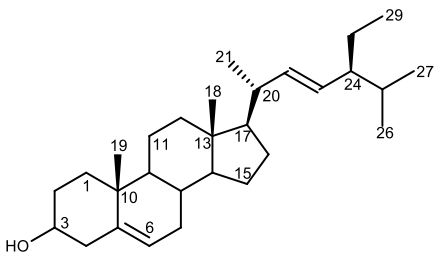

Figure 4. Stigmasterol structure.

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 ^1H NMR and ^{13}C NMR data was carried out.

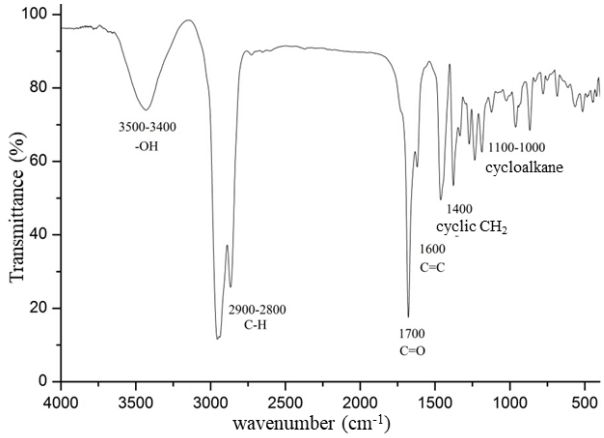

Figure 5. The FTIR spectrum of the F28 compound

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.

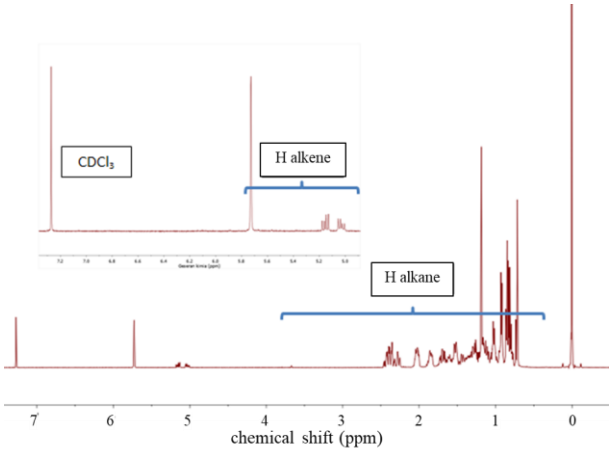

Figure 6. The ^1H NMR spectrum of the F28 compound

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.

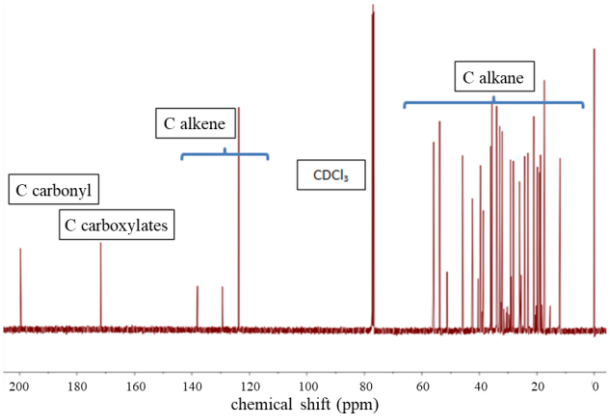

Figure 7. The ^{13}C NMR spectrum of the F28 compound

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

F_{28}: measured in $CDCl_3$, 500 MHz (^1H) and 125 MHz (^{13}C)
F_{28}*: measured in $CDCl_3$, 400 MHz (^1H) and 100 MHz (^{13}C) [9, 10]

Based on the overall results of FTIR, ^1H 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*.

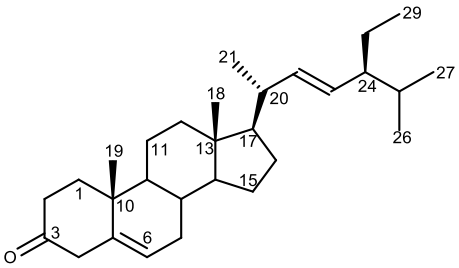

Figure 8. Stigmasterone structure.

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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References

[1] Xiao-Hui Su, Man-Li Zhang, Li-Geng Li, Chang-Hong Huo, Yu-Cheng Gu, Qing-Wen Shi, Chemical Constituents of the Plants of the Genus Calophyllum, *Chemistry & Biodiversity*, 5, 12, (2008), 2579-2608 https://doi.org/10.1002/cbdv.200890215

[2] Siau Hui Mah, Gwendoline Cheng Lian Ee, Mawardi Rahmani, Yun Hin Taufiq-Yap, Mohd Aspollah Sukari, Soek Sin Teh, A New Pyranoxanthone from Calophyllum soulattri, *Molecules*, 16, 5, (2011), 3999-4004 https://doi.org/10.3390/molecules16053999

[3] Gwendoline Cheng Lian Ee, Siau Hui Mah, Soek Sin Teh, Mawardi Rahmani, Rusea Go, Yun Hin Taufiq-Yap, Soulamarin, a New Coumarin from Stem Bark of Calophyllum soulattri, *Molecules*, 16, 11, (2011), 9721-9727 https://doi.org/10.3390/molecules16119721

[4] Siau Hui Mah, Gwendoline Cheng Lian Ee, Soek Sin Teh, Mawardi Rahmani, Yang Mooi Lim, Rusea Go, Phylattrin, a New Cytotoxic Xanthone from Calophyllum soulattri, *Molecules*, 17, 7, (2012), 8303-8311 https://doi.org/10.3390/molecules17078303

[5] J. C. Gómez-Verjan, K. D. Rodríguez-Hernández, R. Reyes-Chilpa, Chapter 8 - Bioactive Coumarins and Xanthones From Calophyllum Genus and Analysis of Their Druglikeness and Toxicological Properties, in: R. Atta ur (Ed.) *Studies in Natural Products Chemistry*, Elsevier, 2017 https://doi.org/10.1016/B978-0-444-63930-1.00008-9

[6] Muhammad Aurang Zeb, Saadat Ullah Khan, Taj Ur Rahman, Muhammad Sajid, Shah Seloni, Isolation and Biological Activity of β-Sitosterol and Stigmasterol from the Roots of Indigofera heterantha, *Pharmacy & Pharmacology International Journal*, 5, 5, (2017), 204-207 https://doi.org/10.15406/ppij.2017.05.00139

[7] Navpreet Kaur, Jasmine Chaudhary, Akash Jain, Lalit Kishore, Stigmasterol: A Comprehensive Review, *International Journal of Pharmaceutical Sciences and Research*, 2, 9, (2011), 2259-2265 https://doi.org/10.13040/IJPSR.0975-8232.2(9).2259-65

[8] Geone Corrêa, V. G. C. Abreu, D. A. Martins, Jacqueline Takahashi, Humberto Fontoura, Denise Carmona Cara, Dorila Piló-Veloso, A. F. A. Carvalho, Anti-inflammatory and antimicrobial activities of steroids and triterpenes isolated from aerial parts of Justicia acuminatissima (Acanthaceae), *International Journal of Pharmacy and Pharmaceutical Sciences*, 6, 6, (2014), 75-81

[9] Luhata Lokadi Pierre, Munkombwe Namboole Moses, Isolation and Characterisation of Stigmasterol and B -Sitosterol from Odontonema Strictum (Acanthaceae), *Journal of Innovations in Pharmaceuticals and Biological Sciences*, 2, 1, (2015), 88-95 https://doi.org/10.13140/RG.2.1.3689.7365

[10] Peter Forgo, Katalin E. Kövér, Gradient enhanced selective experiments in the 1H NMR chemical shift assignment of the skeleton and side-chain resonances of stigmasterol, a phytosterol derivative, *Steroids*, 69, 1, (2004), 43-50 https://doi.org/10.1016/j.steroids.2003.09.012

[11] A. J. Yusuf, M. I. Abdullahi, A. K. Haruna, A. Y. Idris, A. M. Musa, Isolation and Characterization of Stigmasterol and Bis-(5, 7-diacetyl-catechin-4'-α-rhamnopyranoside) from the Stem bark of Neocarya macrophylla (Sabine) Prance (Chrysobalanaceae), *Nigerian Journal of Basic and Applied Sciences*, 23, 1, (2015), 15-22 https://doi.org/10.4314/njbas.v23i1.3

[12] P. Muthukrishnan, P. Prakash, B. Jeyaprabha, K. Shankar, Stigmasterol extracted from Ficus hispida leaves as a green inhibitor for the mild steel corrosion in 1M HCl solution, *Arabian Journal of Chemistry*, 12, 8, (2019), 3345-3356 https://doi.org/10.1016/j.arabjc.2015.09.005

[13] Muluh Emmanuel Khan, Lodiya Maxwell Bala, Muniratu Maliki, Phytochemical analyses of Terminalia schimperiana (Combretaceae) root bark extract to isolate stigmasterol, *Advanced Journal of Chemistry-Section A*, 2, 4, (2019), 327-334 https://doi.org/10.33945/SAMI/AJCA.2019.4.6

[14] Josiane Aparecida de Lima, Ivana Lícia de Campos Gavioli, Cristina Maria Pacheco Barbosa, Alexandre Berndt, Flávia Maria de Andrade Gimenes, Claudia Cristina de Paro Paz, Eduardo Antonio da Cunha, Soybean silage and sugarcane tops silage on lamb performance, *Ciência Rural*, 43, 8, (2013), 1478-1484 https://doi.org/10.1590/S0103-84782013005000098

[15] Emmanuel Ngeufa Happi, Simone Véronique Fannang, Marie Fomani, Suzye Mireille Moladje Donkwe, Nkoungou Yomzak Carine Nicaise, Jean Duplex Wansi, Norbert Sewald, Steroids and Ceramide from the Stem Bark of Odyendyea gabonensis, *Zeitschrift für Naturforschung B*, 68, 8, (2013), 924-930 https://doi.org/10.5560/znb.2013-3132

[16] Anjali Ganjare, Nishikant Raut, Nutritional and medicinal potential of Amaranthus spinosus, *Journal of Pharmacognosy and Phytochemistry*, 8, 3, (2019), 3149-3156

[17] María González-Rodríguez, Clara Ruiz-Fernández, Vera Francisco, Djedjiga Ait Eldjoudi, Yousof Ramadan Farrag AbdElHafez, Alfonso Cordero-Barreal, Jesus Pino, Francisca Lago, Manuel Campos-Toimil, Glaucimeire Rocha Carvalho, Thiago Melo Costa Pereira, Oreste Gualillo, Pharmacological Extracts and Molecules from Virola Species: Traditional Uses, Phytochemistry, and Biological Activity, *Molecules*, 26, 4, (2021), 792 https://doi.org/10.3390/molecules26040792