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

Soerya Dewi Marliyana a,*, Fajar Rakhman Wibowo b, Desi Suci Handayani c, Triana Kusumaningsih a, Venty Suryanti a, Maulidan Firdaus a, Ema Nur Annisa a

a Department of Chemistry, Universitas Sebelas Maret, Surakarta, Indonesia

*corresponding author: msoerya@staff.uns.ac.id

https://doi.org/10.14710/jksa.24.4.108-113

**Abstract**

Stigmasterol and Stigmastereone 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 was 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 stigmastereone.

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 stigmaster and β-sitosterol [2, 4]. Also, there are also xanthones groups, such as soulatrin, caloxanthone-B, caloxanthone C, macluraxanthone, phylatrinin, brasixanthone, and trapeziofloxanthone [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, phylatrinin, 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, anti-diabetic [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 UV254 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 (aluminium coated silica gel 60 F254 0.25 mm (Merck; Darmstadt, Jerman)) were the adsorbent for chromatography. Cerium(IV) (Merck; Darmstadt, Jerman) and H2SO4 (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 (365 nm) then sprayed with spotting reagent Ce(SO4)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, 1H NMR, and 13C 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 hydroxyl 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 CH2 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 1H NMR and 13C NMR data was carried out.

![Figure 1. FTIR spectrum of F19 compound](image)

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     |

F19* measured in KBr

F19*: reference compound measured in KBr [9]

Identification of F19 with NMR, including 1H NMR and 13C NMR, was carried out in CDCl₃ solvent. The 1H NMR spectrum analysis data (Figure 2) shows the presence of 48 proton signals. The proton signal in the chemical shift (δH) from 0.67 to 2.3 ppm is a signal from the proton sp³ consisting of methine (CH₃), methylene (CH₂), and methyl (CH₃). The presence of 6 methyl groups is indicated by the proton signals at δH (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 (CH₂) groups are indicated by the presence of proton signal at δH (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); 2.01 (2H, m, H-12); 1.52 (2H, m, H-15); 1.72 (2H, m, H-16) and 1.45 (2H, m, H-28). In addition, there are also 11 methine groups indicated by the presence of a proton signal at δH (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, s, 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 1H NMR data analysis is supported by 13C NMR data.
Table 2. NMR data comparison of F19 compound with literature (F19*)

| No. | C | δH (multiplicity, Hz) | δC (ppm) |
|-----|---|---------------------|----------|
| 1   |   | 1.84 (m)            | 36.6     |
| 2   |   | 2.83 (m)            | 37.9     |
| 3   |   | 3.52 (m)            | 71.9     |
| 4   |   | 2.3 (m)             | 42.3     |
| 5   |   | -                   | 140.9    |
| 6   |   | 5.35 (m)            | 121.8    |
| 7   |   | 1.97 (m)            | 31.8     |
| 8   |   | 1.46 (m)            | 29.0     |
| 9   |   | 0.92 (m)            | 50.2     |
| 10  |   | -                   | 36.3     |
| 11  |   | 1.50 (m)            | 24.4     |
| 12  |   | 2.00 (m)            | 39.8     |
| 13  |   | -                   | 40.6     |
| 14  |   | 1.01 (m)            | 56.9     |
| 15  |   | 1.56 (m)            | 24.5     |
| 16  |   | 1.72 (m)            | 28.4     |
| 17  |   | 1.15 (d)            | 56.1     |
| 18  |   | 0.69 (s)            | 12.0     |
| 19  |   | 1.01 (s)            | 19.1     |
| 20  |   | 2.06 (m)            | 39.9     |
| 21  |   | 1.02 (m)            | 23.2     |
| 22  |   | 5.17 (dd, 15.2, 8.65)| 138.4   |
| 23  |   | 5.03 (dd, 15.2, 8.65)| 129.4   |
| 24  |   | 1.54 (m)            | 51.3     |
| 25  |   | 1.55 (m)            | 34.1     |
| 26  |   | 0.85 (m)            | 21.2     |
| 27  |   | 0.80 (m)            | 21.3     |
| 28  |   | 1.44 (m)            | 25.5     |
| 29  |   | 0.81 (m)            | 12.1     |

F19* measured in CDCl₃ 500 MHz (H) and 125 MHz (C)
F19**: measured in CDCl₃ 400 MHz (H) and 100 MHz (C) [9, 10]

Based on the analysis of FTIR, ¹H NMR, and ¹³C 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_{30}H_{48}O_{3}, 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 2. ¹H NMR spectrum of F19 compound

The ¹³C NMR spectrum (Figure 3) shows the presence of 29 carbon atom signals, each signal having a distinctive shift. The chemical shift of carbon (δC) 12.0-71.9 ppm shows a signal from C.₉ which consists of six methyl groups (CH₃) at δC 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 (CH₂) groups at δC 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 δC 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 δC 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).

Figure 3. ¹³C NMR spectrum of F19 compound

Based on the analysis of FTIR, ¹H NMR, and ¹³C 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_{30}H_{48}O_{3}, 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 \(^1\)C NMR data was carried out.

![Figure 5. The FTIR spectrum of the F28 compound](image)

| 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 F28: measured in KBr
Compound F28*: measured in KBr \([9, 11]\)

Identification of F28 was carried out in CDCl\(_3\) solvent using NMR spectroscopy, including \(^1\)C NMR and \(^1\)H NMR. The \(^1\)H NMR spectrum (Figure 6) reveals the presence of 52 proton signals. The proton signal in chemical shift (\(\delta_\text{H}\)) 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 \(\delta_\text{H}\) 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 \(\delta_\text{H}\) 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). The methine group is indicated by a proton signal at \(\delta_\text{H}\) 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 \(^1\)H NMR data analysis is supported by \(^1\)C NMR data.

![Figure 6. The \(^1\)H NMR spectrum of the F28 compound](image)

The \(^1\)C NMR spectrum (Figure 7) shows a carbon signal at \(\delta_\text{C} \approx 12.0-56.2\) ppm which is a signal from \(sp^3\) consisting of 6 methyl groups (CH\(_3\)) at \(\delta_\text{C} \approx 12.0\) (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 \(\delta_\text{C} \approx 24.4\) (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 \(\delta_\text{C} \approx 56.1\) (C-14); 56.0 (C-17); 42.4 (C-20); 51.4 (C-24); 34.1 (C-25) and C quaternary (Cq) at \(\delta_\text{C} \approx 42.5\) (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 \(^1\)C NMR spectrum of the F28 compound](image)
Table 4. NMR data comparison of F28 and literature (F28*) compounds

| No. | δH (multiplicity, Jn Hz) | δC (ppm) |
|-----|-------------------------|-----------|
|     | C  | 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 |
| 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) | - | 18.8 | 23.1 |
| 22  | 5.47 (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 CDCl3, 500 MHz (H) and 125 MHz (13C) |
| F28* measured in CDCl3, 400 MHz (H) and 100 MHz (13C) |

Based on the overall results of FTIR, 1H NMR, and 13C NMR data analysis, and compared with literature data, the suggested compound structure for compound F28 is similar to stigmasterone with the molecular formula C29H46O [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 stigmastene, 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 bark of C. soulattri, which are identical compounds to stigmasterol and stigmastene. Based on the literature review, stigmasterone was isolated for the first time in extracting the stem bark of C. soulattri.

Acknowledgment

This research was funded by the PB2018 UNS 2020 with the scheme Hibah Grup Riset with contract number: 652/UN2.71/PN/2020.

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, Gwendolene Cheng Lian Ee, Mawardi Rahmani, Yun Hin Taufig–Yap, Mohd Aspolah Sukari, Soek Sin Teh, A New Pyranoxanthone from Calophyllum soulattri, Molecules, 16, 5, (2011), 3999–4004. https://doi.org/10.3390/molecules16053999

[3] Gwendolene Cheng Lian Ee, Siau Hui Mah, Soek Sin Teh, Mawardi Rahmani, Rusea Go, Yun Hin Taufig–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, Gwendolene Cheng Lian Ee, Soek Sin Teh, Mawardi Rahmani, Yang Mool Lim, Rusea Go, Phylatrin, 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, 7, (2017), 204–207. https://doi.org/10.15466/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/10.3907/IJPSR.0975-6232.2(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 \( \beta \) -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. Kővér, 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′-\( \alpha \)-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, Stigmacerol 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 Malliki, 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 Lídia 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 Rúrural*, 43, 8, (2013), 1478–1484 https://doi.org/10.1590/0103-84782013005000098

[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 Odynendya gabonensis, *Zeitschrift für Naturforshung 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, Djelliga Ait Eldjoudi, Yousf Ramadan Farrag AbdElHafez, Alfonso Cordero-Barreal, Jesús Pino, Francisca Lago, Manuel Campos-Tooimi, Glauceimeir Rocha Carvalho, Thiago Melo Costa Pereira, Oreste Guállilo, Pharmacological Extracts and Molecules from Virola Species: Traditional Uses, Phytochemistry, and Biological Activity, *Molecules*, 26, 4, (2021), 792 https://doi.org/10.3390/molecules26040792