6β-hydroxyipolamiide of *Stachytarpheta jamaicensis* Leaves

Yuliana, F. Auwaliyah, and S. Fatmawati

**Abstract**—*Stachytarpheta jamaicensis* is one species of Verbenaceae family that is used as a traditional medicine. 6β-hydroxyipolamiide is a natural occurring compound that was successfully isolated from *S. jamaicensis* leaves. The identification of compound was analyzed by using UV-Vis, Infra-Red (IR) spectrophotometry and Nuclear Magnetic Resonance (NMR) spectrometry. This is the first report that the compound has isolated from this plant.

**Keywords**—6β-hydroxyipolamiide, *Stachytarpheta jamaicensis*, Isolation.

I. **INTRODUCTION**

The biodiversity of plant species makes Indonesia ranked in the top five in the world with biodiversity. It is reported that around 55% of Indonesia's biodiversity is endemic. The status of Indonesia's biodiversity shows that the number of plant diversity of spermatophytes is around 30,000–40,000 species [1]. Therefore, for decades, researchers focused on plants known to be useful as medicinal plants to treat various diseases [2][3]. Medicinal plants have also been used as natural sources containing bioactive compounds that offer the benefits of good therapy and care for several types of diseases [4][5], and [6]. Therefore, the use of medicinal plants as an alternative to chemical drugs synthesized in the past [19][20], has been going on for thousands of years, before synthetic drugs were discovered and marketed [18][22], and [23]. Previous research on stachytarpheta has shown that there are biological activities of several species, including antioxidants, antimicrobials and antidiabetic [24]. The genus Stachytarpheta is reported to have many biological activities that can cure several diseases. As *Stachytarpheta indica* is reported as antiinflammatory [25], antioxidants [26] and antibacterial [27][28], *Stachytarpheta cayennensis* has compound that active as anti-inflammation [29][30], anti diabetic [31][32], and [33], antibacterial [34][35] and antioxidants [35] *Stachytarpheta angustifolia* as antibacterial [36] and *Stachytarpheta gesnerioides* as antibacterial and antioxidants [37].

*S. jamaicensis* was reported that has been used as a traditional medicine to cure allergies and problems with respiratory conditions, coughs, colds, fever, and digestive complications [38][39]. In northern Nigeria, decoction of leaves is used for dysentery or diarrhea [40]. In traditional medicine in the Caribbean and other tropical countries, these plants are traditionally used as anti-inflammatory, diuretic and analgesic [28], anthelmintics [41], anti diarrheal [42] and antinociceptive [29]. The potential of plants as medicinal plants is due to the secondary metabolites they contain [43][44]. The secondary metabolites content of *S. jamaicensis* includes coumarin, flavonoids, tannins, terpenoids [45] and saponins [46]. Some secondary metabolites that have been found include lanostane phenylacetate (1,3,16β-yl-phenylpropylacetate-lanostan-5,11,14,16,23,25-hexen-22-one) (1), two steroidal glucosides 16 β- (glucopyranosyl β-D, 3,8,22-trihydroxy) Cholesterol-1 β-yl-6-O- (3,4,5trimethoxybenzoyl) β-D (2) and 16-β (β-D – Glucopyranosyl 3,8,22-trihydroxy-cholest-17-en-3β-β-yl) 6-O- (3,4,5-trimethoxybenzoyl) β-D (3) and 16-β (β-D – Glucopyranosyl 3,8,22-trihydroxy-cholest-17-en-3β-β-yl) 6-O- (3,4,5-trimethoxybenzoyl) β-D (4) and 16-β (β-D – Glucopyranosyl 3,8,22-trihydroxy-cholest-17-en-3β-β-yl) 6-O- (3,4,5-trimethoxybenzoyl) β-D (5). The identification of compound was analyzed by using UV-Vis, Infra-Red (IR) spectrophotometry and Nuclear Magnetic Resonance (NMR) spectrometry.
The study aimed to isolate compounds of *S. jamaicensis* leaves. The material used was *S. jamaicensis* leaves from Aru-Maluku, Indonesia.

**II. METHOD**

**A. Preliminary Test**

The preliminary test in this study aims to determine the solvent that is suitable for use in research. The 30 g dry powder of *S. Jamaicensis* was macerated with 200 mL different solvents, *n*-hexane, dichloromethane, ethyl acetate and methanol for 3x24 hours. The maceration results of each solvent were concentrated using a rotary vacuum evaporator and concentrated extract was obtained. The extracts obtained were monitored using the TLC plate with eluent *n*-hexane:chloroform (95:5).

**B. Extract of S. jamaicensis Leaves**

3840 g of dried powder of *S. jamaicensis* leaves macerated with 19 L of methanol for 3x24 hours. The maceration result was concentrated with a rotary vacuum evaporator and concentrated extract was obtained. Subsequently the concentrated extract was monitored by TLC. The stains produced were detected by UV light at λ = 254 and 366 nm then the TLC plate was sprayed with 1.5% cerium sulphate solution in *H*₂*SO*₄ 2N and heated in the oven.

**C. Fractionation and Isolation of Compounds**

100 g crude methanol extract of leaves of *S. jamaicensis* was fractionated using the partitioning method with *n*-hexane: methanol (6:1) eluent. Methanol fraction as much as 40 g then further separation using the KCV method with eluent ethyl acetate: acetone (100:0:100) using the KCV column with a column height of 6.7 cm and a diameter of 11.4 cm. This eluent system uses the principle of increasing polarity. From this fractionation process there are 5 fractions (M1-M5). The M3 fraction with a mass of 26 g was further fractionated to simplify the compounds to be isolated. The M3 fractionation process was carried out by using the KCV method with eluent ethyl acetate: acetone with a polarity enhancement system and *n*-M3A-M3H subfraction was obtained. M3G subfraction (1.6 g) was recrystallized with *n*-hexane: acetone solvent and white solids (Compound) were obtained as much as 448.4 mg which then carried out purity tests using TLC 3 eluent systems, 2D TLC and melting point test. Structure elucidation of compound 1 was carried out using UV-Vis, IR, 1H NMR, 13C NMR, DEPT 135, 2D NMR (HMQC and HMBC) instruments.

**III. RESULTS AND DISCUSSION**

**A. Preliminary test**

Preliminary tests were carried out by extracting leaves of *S. jamaicensis* 30 g each with 200 mL different solvents, namely: *n*-hexane, dichloromethane, ethyl acetate and methanol for 3x24 hours. The maceration results of each solvent were concentrated using a rotary vacuum evaporator and concentrated extract was obtained. The results of the extract obtained were monitored using the TLC plate with eluent n-hexane:
MeOH (1: 9). Stains on the TLC plate were detected with UV lights at \( \lambda = 254 \) and 366 nm, then stains were sprayed with 1.5% cerium sulphate solution in H\(_2\)SO\(_4\) 2N and heated in the oven. The results of TLC monitoring of variations in extract are shown that the methanol extract is able to extract secondary metabolites better than other organic solvents, which is shown from the stain profile on the TLC plate, where methanol extract has the dominant spot stain compared to other extracts.

B. Extraction of *S. jamaicensis* leaves

Extraction is a technique of separating a compound from natural materials based on differences in solubility in certain solvents with the principle of "Like dissolves like" [50][51]. In the study, sample extraction was carried out using solid liquid (maceration) method. The maceration method is an effective method for extracting large quantities of samples at room temperature by adjusting the length of the immersion process in certain solvents [52][53]. This can be caused by the simple molecular structure of methanol so that it easily enters into sample fibers such as leaves. The 3840 g dried powder of *S. jamaicensis* leaf was macerated with 19 L of methanol for 3x24 hours. The maceration results were concentrated with a rotary vacuum evaporator and obtained 1305.8 g concentrated dark brown extract with a yield of 34%.

C. Fractionation and Isolation of Compound

100 g of crude methanol extract of *S. jamaicensis* leaves was fractionated by using the partitioning method with n-hexane: methanol (6: 1) eluent. Methanol fraction as much as 40 g then further separation using the KCV method with eluent ethyl acetate: acetone (100: 0 \( \rightarrow \) :0.100) using the KCV column with a column height of 6.7 cm and a diameter of 11.4 cm. From this fractionation process 5 fractions (M1-M5) were obtained.

The M3 fraction with a mass of 26 g has a better TLC profile when compared with other fractions so that further fractionation is continued to simplify the compounds to be isolated. The M3 fractionation process was carried out by using the KCV method with eluent ethyl acetate: acetone with a polarity enhancement system and M3A-M3H subfraction was obtained.

M3G subfraction when the evaporation process forms solids, so that the solid (1.6 g) is separated from the mixture and analyzed by using TLC to find out whether the solid is a pure compound or not. From the results of TLC, it shows a chromatogram profile that is almost pure but there are still stains below the line (baseline) so that it needs to be purified by recrystallization using a solvent that can separate impurities from the compounds to be isolated. The solvent used in recrystallization of compound is n-hexane: acetone, where compound dissolves completely in acetone and is insoluble in n-hexane. Compound 1 obtained from the recrystallization process was 448.4 mg, then purity was tested using TLC 3 eluent (Figure 2), 2D TLC (Figure 3) and melting point test. Testing 3 eluent systems is intended to see a single spot using three types of eluents. The expected profile is in the form of a single spot at the bottom, middle and top position on the TLC plate. The bottom spot position means that the single compound is seen from the top of the spot, there is no other spot, the middle position spot gives the meaning of a single compound because there is no other spot and the spot in the upper position means that the compound is single because it is not there is another spot below the spot. Further identification of the purity of compounds was carried out using the 2D TLC method. Isolates can be said to be pure if the spot shown is a single spot. Structure elucidation of compound 1 was carried out using IR instruments, 1H NMR, 13C NMR, DEPT 135, 2D NMR (HMQC and HMBC).

D. Eludiation of Structure

Compound is a white powder with a mass of 448.4 mg, with melting point of 130-131 °C. This compound is dissolved in acetone and methanol solvents. Compound 1 was analyzed using a UV spectrophotometer, IR spectrometer, 1D NMR (1H and 13C NMR), DEPT 135 and 2D NMR (HMQC and HMBC).

From UV spectrum analysis shows the intense absorption band at \( \lambda = 230 \) nm and there is no shift in wavelength when given shear reagent. This shows that compound 1 does not have a conjugate system in its structure. Daichiyanus (2004) states that a batochromic shift (shift towards a larger wavelength) will occur when a conjugate system has or is bound to a functional group [54]. From the results of the UV analysis also provides information that compound 1 is not a compound of the flavonoid group, where this compound group when added to the shear reactor NaOH 1M solution, AlCl\(_3\) solution and AlCl\(_3\) + HCl solution will provide a batochromic shift due to the substitution of OH [55]. The results of the identification of structures using IR
Based on the results of the structure identification and literature study from previous studies showed that compound 1 is 6β-hydroxyipolamiide, with the molecular formula C_{17}H_{26}O_{12}. This compound is a compound that has been isolated from the genus Stachyta raphe from S. mutabilis species [59]. This compound is the compound that was first isolated from the species S. jamacaeans. The structure of compound (6β-hydroxyipolamiide) is as Figure 6.

**Figure 6.** 6β-hydroxyipolamiide.

indicate a strong absorption band which indicates the presence of hydroxyl groups (-OH) at the frequency of 3394 cm⁻¹. Carbonyl groups (C=O) shown in wave numbers (vmaks) 1697, 1301 cm⁻¹. The presence of CH numbers (νmax) 1697, 1301 cm⁻¹. The presence of hydroxyl groups (-OH) at the frequency of indicate a strong absorption band which indicates the spectroscopy: (2H, m) are typical proton signals from glycosides. From the methyl group (CH_3) 0.965 (3H, s) proton signal from the methylene (CH_2) 2.020 (2H, m) is a signal from the methyl group (CH3), 4.342 (1H, d); 2.913 (1H, t); 3.113 (1H, t); 3.043 (1H, t), 3.021 (1H, m) and 3.653 (2H, m) are typical proton signals from glycosides. From the analysis of 13C NMR (DEPT-6D, 600 MHz) compound shows the presence of 17 carbon signals. Chemical shift (δc) at: 37.234 ppm is a carbon signal from methylene (CH2), 23.761 ppm is a signal of methyl (CH3), 166.342 ppm shows signals of carbonyl (C = O) at 8c 98.379; 73.446; 76.459; 70.279; 77.317 and 61.49 show a typical chemical shift from glycosides. The structure of compound was strengthened by analysis using DEPT 135 which showed that the isolated compound had two methylene groups (CH2) shown at δc 37.243 and 61.490 ppm. Based on the literature study, compound has a similarity in the amount of carbon and the value of 1H NMR and 13C NMR chemical shifts with 6β-hydroxyipolamiide compound shown in Table 1.

Furthermore, a correlation of 1H,13C was confirmed using 2D HMOC (Heteronuclear Multiple Quantum Coherence) and HBMC (Heteronuclear Multiple Bond Connectivity) analysis [56]. From the HMOC data it can provide information about protons that are bound to carbon with a distance of one bond, so that from HMOC data it can be seen that carbon binds protons and carbon that do not bind protons [57]. Based on the HMOC spectrum it is known that there are 13 proton signals that correlated with carbon, namely 2 methyl (CH_3) signals, 2 methylene (CH_2) signals and 9 methine (CH) signals (Figure 4). The HBMC analysis shows that there is a long distance relationship between neighboring 1H-13C, so it can provide more precise information on the position of hydrogen and carbon atoms [58] (Figure 5 and 6).

**REFERENCES**

[1] Lembaga Ilmu Pengetahuan Indonesia, “Status kekinian keane-karagaman hayati Indonesia,” Indonesia, 2014.
[2] S. Facciola, *Cornucopia II: Kampong Publication California,* 1998.
[3] G. Arumugam, P. Manjula, and N. Paari, “A review: Anti diabetic medicinal plants used for diabetes mellitus,” *J. Acute Dis.*, pp. 196–200, 2013.
[4] A. Inran et al., “Thymoquinone: A novel strategy to combat cancer: A review,” *Biomed. Pharmacother.*, vol. 106, pp. 390–402, 2018.
[5] Yuliana and S. Fatmawati, “Senyawa Metabolit Sekunder dan Aspek Farmakologi Aloscarnus Macrorrhizos,” *Akta Kim. Indones.*, vol. 3, pp. 141–158, 2018.
[6] M. Mamun-or-Rashid, H. Shamim, K. H. Naim, S. Biphal, Asharaufuzzaman, and S. Monokesh, “A review on medicinal plants with antidiabetic activity,” *J. Pharmacogn. Phytochem.*, vol. 3, pp. 149–159, 2014.
[7] M. Liew and Y. K. Young, “Stachyta raphe Jacq (L.) Vahl: from traditional usage to pharmacological evidence,” *Evidence-Based Complement. Altern. Med.*, pp. 1–7, 2016.
[8] M. Kaaikamam and J. K. Akina, *Hawaiian herbs of medicinal value*. University Press of The pacific Honolulu-Hawaii, 2000.
[9] N. Liang and D. Kitts, “Antioxidant property of coffee components: Assessment of methods that define mechanisms of action,” *Molecules*, vol. 19, pp. 1918–19208, 2014.
[10] M. Rahmatullah, S. A. R. Jahan, H. M. S. Hossan, and T. Rahman, “Folk Medicinal Uses of Verbeneaceae Family Plants in Bangladesh,” *African J. Tradit. Complement Altern. Med.*, pp. 53–65, 2011.
[11] O. Adedeji, “Palyonomology of the Genus Stachyta raphe Vahl. (Verbeneaceae),” *Not. Sci. Biol.*, vol. 2, pp. 27–33, 2010.
[12] M. Sulaiman, Z. A. Zakaria, H. S. Chong, S. K. Lai, D. A. Israif, and T. M. Shah, “Antinociceptive and Anti-Inflammatory Effects of Stachyta raphe Jacq (L.) Vahl (Verbeneaceae) in Experimental Animal Models,” *Med. Princ. Pract.*, vol. 18, pp. 272–279, 2009.
[13] N. A. da Silva, J. K. da Silva, E. H. Andrade, L. M. Carreira, P. J. Sousa, and J. G. Maia, “Essential oil composition and antioxidant capacity of Lippia schomburgkiana,” *Nat. Prod. Commun.*, vol. 4, pp. 1281–1286, 2009.
[14] V. Machumi et al., “Antimicrobial and antiparasitic abietane diterpenoids from the roots of Clerodendrum eriophyllum,” *Nat. Prod. Commun.*, vol. 5, pp. 853–858, 2010.
[15] K. Rufino, A. Bimie, and B. Tengnas, “Edible Wild Plants of Tanzania,” 2002.
[16] O. Akobundun and C. N. Agyakwa, “A hand book on west African weeds,” Nigeria, 1998.
[17] S. Liang and M. G. Gilbert, “Verbeneaceae,” *Flora of China*, vol. 17, pp. 1–49, 1994.
[18] F. Nascimento, L. Juliana, C. F. Paulo, and L. S. Giuliana, “Antibacterial activity of plant extracts and phytochemicals on antibiotic-resistant bacteria,” *Brazilian J. Microbiol.*, vol. 31, pp. 247–256, 2000.
[19] R. Dewoto, “Pengembangan Obat Tradisional Indonesia Menjadi Fitofarmaka,” *Maj. Redokt. Indones.*, vol. 57, no. 7, 2007.
[20] N. Malviya, S. Jain, and S. Malviya, “Antidiabetic potential of medicinal plants,” *Acta Pol. Pharm. Drug Res.*, vol. 67, pp. 113–118, 2010.
[21] K. Upadhyay, “Antidiabetic potential of plant natural products: A review,” *Int. J. Green Pharm.*, vol. 10, pp. 96–113, 2016.
[22] L. Rıos and M. C. Recio, “Medicinal plants and antimicrobial activity,” *J. Ethnopharmacol.*, vol. 100, pp. 80–84, 2005.
[23] M. Cowan, “Plant products as antimicrobial agents,” *Clin.
Microbiol. Rev., vol. 12, pp. 1564–1567, 1999.

[24] R. Sivananjan, K. Ramakrishnan, and G. Bhuvaneswari, “Pharmacognostic studies on root of Stachytarpha cayennensis,” Int. Lett. Nat. Sci., vol. 8, pp. 100–105, 2014.

[25] O. Silambujanaki, V. Chitra, D. Soni, D. Raju, and M. Sankari, “Hypoglycemic activity of Stachytarpha indica on streptozotocin induced wistar strain rat,” Int. J. PharmTech Res., vol. 1, pp. 1564–1567, 2009.

[26] R. Sahoo, R. R. Dash, and S. Bhatnagar, “Phytochemical screening and bioevaluation of medicinal plant Stachytarpha indica (L.) Vahl.,” Pharmacol. Toxicol. Res., vol. 1, pp. 1–5, 2014.

[27] G. Christian, U. P. Monday, and U. E. Charles, “Antibacterial potential of the methanol stem bark extract of Stachytarpha indica,” Asian J. Med. Sci., vol. 4, pp. 5–10, 2013.

[28] S. Olalode, O. O. Ogunmola, S. E. Kuyooro, and O. O. Aibola, “Stachytarpha jamaicensis leaf extract: Chemical composition, antioxidant, anti-arthritis, anti-inflammatory and bactericidal potentials,” J. Sci. Innov. Res., vol. 6, pp. 119–125, 2017.

[29] S. Schapoval, de V. M. R. Winter, and C. G. Chaves, “Antiinflammatory and antiocceptive activities of extracts and isolated compounds from Stachytarpha cayennensis,” J. Ethnopharmacology, vol. 60, pp. 53–59, 1998.

[30] C. Penido et al., “Anti-inflammatory and anti-ulcerogenic properties of Stachytarpha cayennensis (L. Rich) Vahl.,” J. Ethnopharmacol., vol. 104, pp. 225–233, 2006.

[31] A. C. Adebajo et al., “Hypoglycemic constituents of Stachytarpha cayennensis leaf,” Planta Med., vol. 73, pp. 241–250, 2007.

[32] R. A. Pandian, A. Srinivasan, and I. C. Pelapula, “Evaluation of wound healing activity of hydroalcoholic extract of leaves of Stachytarpha cayennensis in streptozotocin induced diabetic rats,” Der Pharm. Lett., vol. 5, pp. 193–200, 2003.

[33] R. M. Hassans, Y. N. Ezzatie, and N. Samsulrizal, “Hypoglycemic and Antioxidant activities of Stachytarpha cayennensis leaf,” Brazilian J. Pharmacogn., vol. 39, pp. 407–410, 2009.

[34] C. Oyoke, P. A. Akah, C. O. Okoli, A. C. Ezike, and F. N. R. M. Hassan, Y. N. Eizzatie, and N. Samsulrizal, “Hypoglycemic activity of Stachytarpha indica on streptozotocin induced wistar strain rat,” Int. J. PharmTech Res., vol. 1, pp. 1564–1567, 2009.

[35] K. Ramakrishnan and R. Sivananjani, “Pharmacognostical and phytochemical studies on stem of Stachytarpha cayennensis (L.) Vahl.,” Int. Res. J. Pharm., vol. 4, pp. 44–47, 2013.

[36] M. Idu, E. K. I. Omogbai, G. E. Ughiemien, F. Amaechina, O. Timothy, and S. E. Omonho, “Preliminary phytochemistry, antimicrobial properties and acute toxicity of Stachytarpha cayennensis (L.) Vahl. Leaves,” Trends Med. Res., vol. 2, pp. 193–198, 2007.

[37] D. E. Okwu and O. N. Ohenhen, “Isolation, characterization and antibacterial activity of lanostane triterpenoid from the leaves of Stachytarpha cayennensis Linn Vahl.,” Der Chem. Sin., vol. 1, pp. 6–14, 2009.

[38] D. E. Okwu and O. N. Ohenhen, “Isolation and characterization of Steroidal Glycosides from the leaves of Stachytarpha cayennensis Linn Vahl.,” Der Chem. Sin., vol. 1, pp. 6–14, 2010.

[39] S. Raj, M. Berthonnier, M. A. Babu, S. Karthkeyan, A. Sivakumar, and K. M. Gothandam, “Antioxidant, Antibacterial and Anti-Proliferative Activity and Phytochemical Analysis of Selected Medical Plants from Dasapushpam of Kerala,” Int. J. Pharm. Sci., vol. 23, pp. 172–179, 2013.

[40] F. S. Habene, M. B. Fumkina: Penetentia cara modern menganulisis tumbuhan. Bandung: ITB Press, 1987.

[41] N. Steiger and W. Liebenberg, “Recrystallization of Active Pharmaceutical Ingredients,” 2012.

[42] A. Pandey and S. Tripathi, “Concept of standardization, extraction and pre phytochemical screening strategies for herbal drug,” J. Pharmacogn. Phytochem., vol. 2, pp. 115–119, 2014.

[43] F. S. Sibul, D. Z. Orcic, E. Svircev, and N. M. Mimica-Dukic; “Optimization of extraction conditions for secondary biomolecules from various plant species,” Hem. Ind., vol. 70, pp. 473–483, 2016.

[44] Dachirianus, “Analisis Struktur Senyawa Organik Secara Spektroskopii,” Padang, 2004.

[45] Suyatno, Penetranan Molekul Senyawa Organik dengan Metode Spektroskopi. Surabaya: Unesa University Press, 2016.

[46] E. Fukushima, “Advanced NMR approaches for a detailed structure analysis of natural products,” Biosci. Biotechnol. Biochem., vol. 70, pp. 1803–1812, 2006.

[47] M. Kock, B. Reif, W. Fenical, and C. Griesinger, “Differentiation of HMBC two- and three-bond correlations: a method to simplify the structure determination of natural products,” Tetrahedron Lett., vol. 37, pp. 363–366, 1996.

[48] C. D. Luca, M. Guiso, and C. Martinott, “6f-Hydroxipolamiide, an iridoid glucoside from Stachytarpha mutabilis,” Phytochemistry, vol. 22, pp. 1185–1187, 1983.