

6 β -hydroxyipolamiide of *Stachytarpheta jamaicensis* Leaves

Yuliana, F. Auwalayah, and S. Fatmawati

Abstract—*Stachytarpheta jamaicensis* is one species of Verbenaceae family that is used as a traditional medicine. 6 β -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—6 β -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 treatment of diseases has been accepted on a global scale [7][8], and [9].

Various family plants exist in Indonesia, one of which is from the Verbenaceae family, which has approximately 35 genera with around 1,200 species. The Verbenaceae family is known to have important bioactivity. There were 13 species of 8 genera that were reported as traditional medicine in several countries to cure several diseases [10][11]. Among them are ethanol extract *Stachytarpheta jamaicensis* that is commonly known as Brazilian tea, verbena cimarrona, roter comb or blue porter weed is known as horse whip which has anti-inflammatory activity [12], *Lippia schomburgkiana* Schauer leaves as antioxidants and have cytotoxic effects [13] and roots of *Clerodendrum eriophyllum* as antimalarials [14]. The large number of species in Indonesia attracts researchers to find out the content of active compounds and bioactivity in plants in Indonesia for use in medicine.

S. jamaicensis is a species that is widespread in Indonesia. *S. jamaicensis* can be found in forests, roadside, coastal environments, gardens and grasslands [15]. The plant's height is around 60-120 cm and breeds through seeds. The stem of the plant has a soft woody. *S. jamaicensis* has dark green leaves and blue flowers. The leaves are round and tapered to the tip, smooth on both surfaces and have short stalks [16][17], and [18]. (Figure 1).

The diversity of plant species in Indonesia is a source of natural ingredients. The use of traditional medicine has been going on for thousands of years, before synthetic drugs were discovered and marketed [19][20],



Figure 1. (a) Whole plant, (b) flower, (c) leaves and flower.

and [21]. In order for traditional medicine to work effectively, safely and efficiently, it needs to be supported by scientific evidences that can be obtained from systematic research [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 antidiabetic [25], antioxidants [26] and antibacterial [27][28], *Stachytarpheta cayennensis* has compound that active as anti-inflammatory [29][30], antidiabetic [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], antidiarrheal [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 (1,3,16 β -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) Cholestan-1 β -yl-6-O- (3,4,5-trimethoxybenzoyl) β -D (2) and 16- β (β -D - Glucopyranosyl 3,8,22-trihydroxy-cholest-

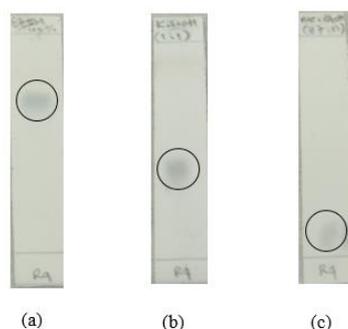


Figure 2. TLC Chromatogram using three eluents systems (a) petroleum ether (100%), (b) *n*-hexane:DCM (95:5), (c) *n*-hexane:chloroform (95:5).

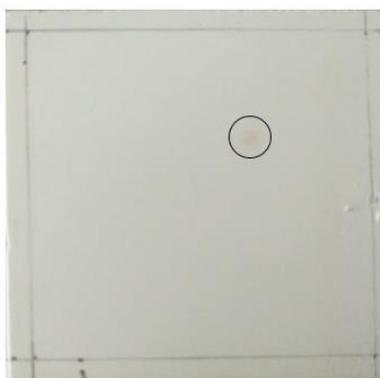


Figure 3. 2D TLC chromatogram with *n*-hexane:DCM (9: 1) and *n*-hexane:chloroform (5: 5) eluents.

5,14,16,23tetraiene β -yl, 6-O-(3,4,5-trimethoxybenzoyl) β -D glucopyranoside (**3**) [47][48]. The study aimed to isolate compounds of *S. jamaicensis* leaves.

The material use *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. 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_2SO_4 2N and heated in the oven. Extracts from solvents that have a better profile at TLC will be used in the sample extraction and fractionation process [49].

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 $\lambda = 254$ and 366 nm then the TLC plate was sprayed with 1.5% cerium sulphate solution in H_2SO_4 2N and heated in an oven.

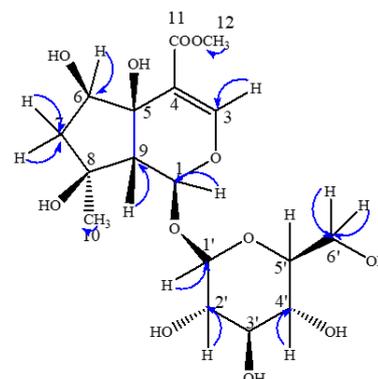


Figure 4. HMQC of 6 β -hydroxyipolamiide.

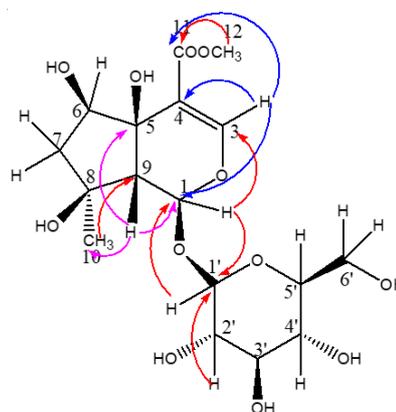


Figure 5. HMBC of 6 β -hydroxyipolamiide.

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 :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 nd 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, ^{13}C 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 eluen *n*-hexana:

TABLE 1.
 DATA 1H NMR, 13C NMR AND HMBC OF COMPOUND

No. C	¹ H (δ _H ppm)	¹³ C (δ _C ppm)	DEPT 135	HMBC (H → C)
1	5,621 (1H, s)	93,23	CH	3, 1'
3	7,313 (1H, s)	151,115	CH	1, 4, 11
4	-	114,664	C	-
5	-	77,842	C	-
6	3,043 (1H, t)	70,529	CH	-
7	2,020 (2H, m)	37,234	CH ₂	-
8	-	79,71	C	-
9	2,263 (1H, s)	61,175	CH	1, 10, 5
10	0,965 (3H, s)	23,761	CH ₃	9
11	-	166,342	C	-
12	3,595 (3H, s)	51,307	CH ₃	11
1'	4,362 (1H, d)	98,379	CH	1
2'	2,913 (1H, t)	73,446	CH	1'
3'	3,113 (1H, t)	76,459	CH	-
4'	3,043 (1H, t)	70,157	CH	-
5'	3,021 (1H, m)	77,317	CH	-
6'	3,653 (2H, m)	61,49	CH ₂	-

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 H₂SO₄ 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 dissolve 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 → :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. Elutidation 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. Dachriyanus (2004) states that a bathochromic 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, AlCl₃ solution and AlCl₃ + HCl solution will provide a bathochromic shift due to the substitution of OH [55]. The results of the identification of structures using IR

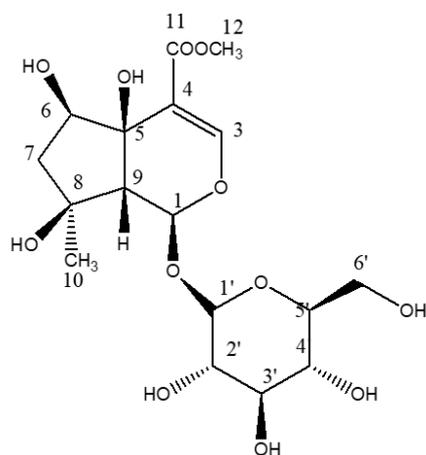


Figure 6. 6 β -hydroxyipolamiide.

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 sp³ groups is shown at wave number 2904 cm^{-1} .

Compound was also identified using NMR spectroscopy: ¹H (400 MHz) and ¹³C NMR (400 MHz), DEPT 135 (Distortionless Enhancement of Polarization Transfer) and 2D experiments (HMOC and HMBC). From the results of the ¹H NMR spectrum using DMSO-D₆ solvents, at a frequency of 400 MHz, there were 26 types of protons. Table 1 show that chemical shift (δ H) provides information about the type of proton of the structure analyzed. At δ H; 2,020 (2H, m) is a signal from the methylene (CH₂) and 0,965 (3H, s) proton 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 (DMSO-D₆, 400 MHz) compound shows the presence of 17 carbon signals. Chemical shift (δ C) 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) and δ C 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 δ C 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 6 β -hydroxyipolamiide compound shown in Table 1.

Furthermore, a correlation of ¹H-¹³C was confirmed using 2D HMOC (Heteronuclear Multiple Quantum Coherence) and HMBC (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 (CH₃) signals, 2 methylene (CH₂) signals and 9 methine (CH) signals (Figure 4). The HMBC 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).

Based on the results of the structure identification and literature study from previous studies showed that compound 1 is 6 β -hydroxyipolamiide, with the molecular formula C₁₇H₂₆O₁₂. This compound is a compound that has been isolated from the genus *Stachytarpheta* from *S. mutabilis* species [59]. This compound is the compound that was first isolated from the species *S. jamacaensis*. The structure of compound (6 β -hydroxyipolamiide) is as Figure 6.

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