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## Potential of stigmasterol from EtOAc extract *Melochia umbellata* (Houtt) Stapf var. *Visenia* as Dengue Antivirus

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**Abstract.** Stigmasterol has been isolated from the stem bark extract of *Melochia umbellata* (Houtt) Stapf var. *Visenia*. The methods used include multilevel maceration, fractionation, and crystallization. Elucidation structure of compound based on spectroscopy data IR, <sup>1</sup>H and <sup>13</sup>C NMR. Stigmasterol obtained has the potential as a dengue antiviral agent with IC<sub>50</sub> 9.11 µg / mL.

### 1. Introduction

Steroid compounds are abundant secondary metabolites and have been isolated from plants and marine biota. One of the most important steroid compounds in the body's metabolism is stigmasterol. Stigmasterol is an important role in the synthesis of the hormones progesterone, androgens, estrogens, and corticoids [1]. In addition, many studies that report on the bioactivity of stigmasterol include antidiabetic and can inhibit the  $\alpha$ -amylase enzyme [2,3], inhibits tumor growth ie the Human Umbilical Vein Endothelial cell (HUVSC) [4], potential as a skin anticancer (DMBA) [5], and antibacterial *S. aureus* [6]. Therefore stigmasterol can be used as a source of medicinal raw materials for various diseases.

Dengue Hemorrhagic fever (DHF) is an endemic disease in tropical countries caused by the dengue virus. This disease is transmitted by female *Aedes* mosquitoes [7]. Dengue is included in the Flavivirus family which is a type of pathogenic virus that causes many deaths in humans [8]. In 2019 WHO data reports that Asian countries are the countries that have the largest DHF cases, including the Philippines 72,076 cases, Vietnam 59,959 cases, Malaysia 46,607 cases, Singapore 3,233 cases [9]. In Indonesia, cases of DHF are increasing every year, currently, around 80% of districts/cities have been infected with the dengue virus [10]. Based on the Ministry of Health 2018 data, it is reported that South Sulawesi has 2,114 DHF cases [11]. Efforts that can be made to minimize these numbers are by utilizing stigmasterol which has been isolated from plants endemic to South Sulawesi. One of the endemic plants of South Sulawesi is *Paliassa* which belongs to the genus *Melochia*. Stigmasterol glycosides have been isolated from *Melochia umbellata* (Houtt) Stapf var. *Degrabrata* K. and appointed as antifungal *A. niger* [7], and *A. flavus* [8]. In addition, the stigmasterol derivative (22S, 23S) -22,23-dihydroxystigmast-4-en-3-one (22S, 23S) -3 $\beta$ -bromo-5 $\alpha$ , 22,23-trihydroxy stigmastan-6-one [9] 7-keto-stigmasterol [10] it is also reported to have the potential for antiviral herpes (HSV-1)



and (EHV-1), so it is possible that stigmasterol isolated from *Melochia umbellata* (Houtt) var staff.

*Visenia* has the potential to be antiviral dengue.

## 2. Materials and Methods

### 2.1 General Experiment Procedure

The melting points were taken using electro-thermal apparatus, the IR spectrum was measured using an IR spectrometer with variants of FTIR 8501 Shimadzu, JEOL 1H-NMR JMN A 5000 which worked at 500.0 MHz and 13C-NMR at 125.65 MHz, using residual and deuterated solvent peaks as reference standards. VLC was carried out using Merck Si-gel 60 GF254, and TLC analysis on precoated Si-gel plates (Merck Kieselgel 60 F254, 0.25 mm), centrifuge 4°C, T25 flask, CO<sub>2</sub> incubator, cone tube, incubator without CO<sub>2</sub>, ELISA reader (Benchmark), freezer, microwell plate, hemocytometer, inverted microscope. All the solutions were distilled before used.

### 2.2 Plant Material

*M. umbellata* (Houtt) Stapf var. *Visenia* is obtained from the village of Baring, Pangkep district, South Sulawesi. Plant specimens were identified by the Indonesian Flora Diversity of the Sereale Family Harmony (KKS) of Makassar.

### 2.3 Extraction and Isolation

Dry powder of *M. umbellata* stem bark 5 kg was extracted with n-hexane. The extract obtained was then evaporated to produce concentrated n-hexane extract. The residue was re-extracted using CHCl<sub>3</sub> solvent, followed by using EtOAc. All extracts were evaporated to produce concentrated n-hexane, CHCl<sub>3</sub>, and EtOAc extracts 4.51 g, 46.99 g, and 8.51 g, respectively.

Ethyl acetate extract (8.51 g) was fractionated early through vacuum column chromatography with eluent chloroform: n-hexane with enhanced polarity obtained 5 fractions (F1-F5). Furthermore F1 (1.86 g) was further fractionated through vacuum column chromatography with ethyl acetate eluent: n-hexane with gradient polarity and obtained 6 main fractions (A-F). B fraction (0.45 g) was fractionated again using flash column chromatography using eluents, n-hexane, ethyl acetate/n-hexane, ethyl acetate, acetone, and methanol, obtained 14 fractions (B1-B14). In the B8 fraction there were crystalline seeds, so crystallization and recrystallization were carried out and pure compounds (stigmasterol) were obtained as much as 14.4 mg and melting points 134 - 136 °C.

#### 2.3.1 Stigmasterol

Needle crystals are white; IR  $\nu_{max}$  (KBr): 3427, 2958, 2953, 2866, 1462, 1377, 1645, 1058 and 960 cm<sup>-1</sup>; 1H NMR (acetone-d<sub>6</sub>, 500 MHz), 13C NMR (acetone-d<sub>6</sub>, 125 MHz), HMBC see Table 1.

### 2.4 Bioactivity Test

2.4.1 Dengue Antivirus Test. The compound cytotoxicity test on dengue virus was carried out by several stages of testing, namely cell line vero culture, cell line C6/36 culture, making C6/36 cell line virus stock, making viral stock in cell line vero, FFURA, and ELISA reader. All activity test procedures refer to references [11] that have been modified by [12].

### 3. Discussion

White needle crystalline compounds weighing 14.4 mg, with a melting point of 134-136 °C have been isolated from the stem bark extract of *Melochia umbellata* (Houtt) Stapf var. *Visenia*. These compounds are included in the steroid class namely stigmasterol.

Stigmasterol has the molecular formula C<sub>29</sub>H<sub>48</sub>O. IR spectrum data shows the presence of an OH group (3427 cm<sup>-1</sup>) supported by the absorption of the C-O group (1058 cm<sup>-1</sup>). Absorption of 2958, 2953, and 2866 cm<sup>-1</sup> showed that the aliphatic C-H group was supported by the absorption of CH<sub>2</sub> (1462 cm<sup>-1</sup>) and CH<sub>3</sub> (1377 cm<sup>-1</sup>) groups. There were also absorption at 1645 cm<sup>-1</sup> and 960 cm<sup>-1</sup> respectively indicating the presence of C = C (olefins) and trans olefins. Determination of the structure of the compounds resulting from isolation was continued by analysis of <sup>1</sup>H-NMR, <sup>13</sup>C-NMR, HMBC and HSQC.

Based on <sup>13</sup>C-NMR data obtained 29 carbon atoms including one oxycarbon at δ 71.9 ppm and six methyl at δ: (12.4; 19.5; 21.3; 21.2; 19.1; and 12,19 ppm). Nine methylene at δ: (37.3; 31.8; 42.3; 32.04; 21.2; 39.9; 24.4; 28.4 and 26.1 ppm), ten metin at δ: (121.8; 32.06; 50.28; 56.9; 56.07; 40.6; 138.4; 129.3; 51.3 and 29.2 ppm) and the three-carbon quaternary at 140: 140, 8; 36.6 and 42.4 ppm. Olefinic resonance at δ 121.8; 138.4; 129.3; and 140.8 ppm each shows C-6, C-22, C-23 and C-5 signals. This indicates that there are two double bonds, namely one double bond in nineteen carbon signals that forms the steroid skeleton on C-5 and C-6 atoms and one double bond on the outside of the steroid skeleton, namely at C-22 and C-23 atoms.

<sup>1</sup>H NMR spectroscopic data shows CH<sub>2</sub> proton signals (δ: 1.09, 1.84, 2.27, 1.99, 1.49, 2.02, 1.55, 1.85, and 1.18 ppm) (2H, m), and CH (δ: 3.49, 1.45, 0.92, 1.00, 1.13, 2.02, and 1.68 ppm) (1H, m). At 77 0.77 ppm (Me-18) and 1.00 ppm (Me-19) (3H, s) shows two methyl bound to tertiary carbon. At δ 1.02 ppm (Me-21) (3H, m), δ 0.84 ppm (Me-26) and δ 0.80 ppm (Me-27) respectively (3H, d, J = 8, 15 & 8.25 Hz) shows three methyl bound to secondary carbon, and one methyl at δ 0.82 ppm (Me-29) (3H, d, J = 4.40 Hz). Three protons substituted by olefinic were at 5.35 ppm (H-6), δ 5.14 ppm (H-22) and 5.01 ppm (H-23) and one proton at δ 3.49 ppm (H-3). The proton signals indicate a steroid skeleton substituted by six methyl and one hydroxyl one.

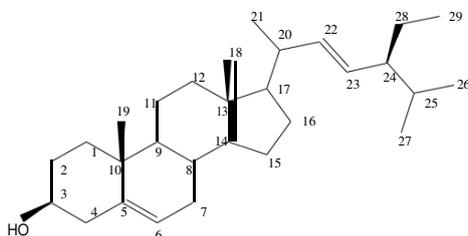
Correlation of bonds in the structure is proven by the long distance correlation between protons (<sup>1</sup>H) and carbon (<sup>13</sup>C) from the HMBC spectrum which can be seen in Table 1. Long distance correlation of protons at δ 5.35 ppm (H-6) with methylene carbon at δ 42.3 ppm (C-4), δ 32.04 ppm (C-7) and quaternary carbon at δ 36.6 ppm (C-10) reinforces that C-5 and C-6 as olefinic bonds in the second ring of the isolation structure. Furthermore, the long distance correlation of protons at 5.14 ppm (H-22) with methine carbon at δ 51.3 ppm (C-24) and olefinic metin carbon at δ 129.3 ppm (C-23) and the correlation proton long distance at δ 5.01 ppm (H-23) with methine carbon at δ 51.3 ppm (C-24) and olefinic metin carbon at δ 138.4 ppm (C-22) supporting that C-22 and C-23 is olefinic which is positioned between C-20 and C-24 on the structure of the isolated compound. NMR spectroscopic data of isolated compounds obtained were significant with absorption peaks indicated by stigmasterol compounds reported by [13].

**Table 1.** <sup>1</sup>H-NMR spectroscopic data, <sup>13</sup>C-NMR, and HMBC & comparisons with references

C	<sup>1</sup> H-NMR H* d ppm (H, multiplicity, J)	<sup>1</sup> H-NMR H** d ppm (H, multiplicity, J)	<sup>13</sup> C-NMR d ppm	<sup>13</sup> C-NMR d ppm	HMBC (H → C)
1	1,85 (1H, m) & 1,09 (1H, m)	1,86(1H, m) & 1,09 (1H,m)	37,3	37,4	C-3, C-19
2	1,84 (1H, m) & 1,51 (1H, m)	1,83(1H, m) & 1,51 (1H,m)	31,8	31,9	C-10
3	3,49 (1H, dd, 10,45 & 4,75)	3,52(1H,m)	71,9	72,0	-
4	2,27 (2H, m)	2,29(1H, m) & 2,23 (1H,m)	42,4	42,5	C-2, C-10, C-3, C-6, C-5

5	-	-	140,8	140,9	-
6	5,35 (1H, dd, 1,5 & 3,65)	5,35 (1H, br d, 3,9)	121,8	121,9	C-10, C-4, C-7
7	1,99 (1H, m) & 1,48 (1H, m)	1,97 (1H, m) & 1,50 (1H, m)	32,0	32,1	C-9, C-6, C-5, C-3, C-8
8	1,45 (1H, m)	1,45 (1H, m)	32,0	32,1	C-9, C-10, C-14, C-7
9	0,92 (1H, m)	0,92 (1H, m)	50,2	50,3	C-8, C-12, C-11, C-19
10	-	-	36,6	36,7	-
11	1,46 (2H, m)	1,47 (2H, m)	21,2	21,3	C-12, C-19, C-14
12	1,18 (1H, m) & 2,02 (1H, m)	2,02 (1H, m) & 1,19 (1H, m)	39,9	40,0	C-13
13	-	-	42,4	42,4	-
14	1,00 (1H, m)	1,00 (1H, m)	56,9	57,1	-
15	1,55 (1H, m) & 1,05 (1H, m)	1,55 (1H, m) & 1,07 (1H, m)	24,4	24,6	-
16	1,85 (1H, m) & 1,27 (1H, m)	1,71 (1H, m) & 1,28 (1H, m)	28,4	29,1	-
17	1,13 (1H, m)	1,16 (1H, m)	56,1	56,1	C-12
18	0,77 (3H, s)	0,70 (3H, s)	12,4	12,2	C-17, C-12, C-14, C-20, C13
19	1,00 (3H, s)	1,01 (3H, s)	19,5	19,6	C-1, C-5
20	2,02 (1H, m)	2,06 (1H, m)	40,6	40,7	-
21	1,02 (3H, m)	1,03 (3H, d, 6,3)	21,3	21,4	C-20
22	5,14 (1H, dd, 8,65 & 15,1)	5,15 (1H, dd, 8,5 & 9,1)	138,4	138,5	C-23, C-24
23	5,01 (1H, dd, 6,6 & 17,3)	5,03 (1H, dd, 9,1 & 8,5)	129,3	129,5	C-22, C-24
24	1,52 (1H, m)	1,52 (1H, m)	51,3	51,4	-
25	1,68 (1H, m)	1,54 (1H, m)	29,2	32,1	C-27, C-26
26	0,84 (3H, d, 8,15)	0,85 (3H, d, 7,2)	21,2	21,3	C-24, C-25, C-27
27	0,80 (3H, d, 8,25)	0,80 (3H, d, 7,5)	19,1	19,2	C-28, C-24
28	1,18 (2H, m)	1,42 (1H, m) & 1,18 (1H, m)	26,1	25,6	-
29	0,82 (3H, d, 4,4)	0,82 (3H, t, 6,5)	12,1	12,4	C-25

Based on analysis of FT-IR,  $^1\text{H-NMR}$ ,  $^{13}\text{C-NMR}$ , HMBC, and HSQC spectroscopic data. Stigmasterol compounds that have been reported have similarities with isolation compounds as data in Table 1, so it can be concluded that the isolated compounds are stigmasterol (Figure 1). This is also supported by the results of phytochemical tests that showed positive steroid compounds.



**Figure 1: Stigmasterol**

**Dengue Antivirus Activity.** Dengue antiviral testing of stigmaterol compounds from isolation of EtOAc extract of bark *Melochia umbellata* (Houtt) Stapf var. *Visenia* has been carried out through the FFURA method and measured using ELISA reader so that the IC<sub>50</sub> value is obtained at 9.1070 µg/mL. Measurement of IC<sub>50</sub> obtained <10 µg / mL which is classified as active in inhibiting the dengue virus (DENV-2) [14]. These results are positively correlated with the research conducted by [15] where stigmaterol compounds are very effective as antilarvasida *Aedes aegypti* in ex vivo, also positively correlated with research conducted by [12] which showed that EtOAc extract of stem bark *Melochia umbellata* (Houtt) Stapf var. *visenia* active in inhibiting the dengue virus (DENV-2) with an IC<sub>50</sub> value of 2.81 µg/mL.

### Conclusion

Stigmaterol has been isolated from the EtOAc extract of stem bark *Melochia umbellata* (Houtt) Stapf var. *Visenia* and active in inhibiting the dengue virus (DENV-2) with IC<sub>50</sub> values of 9.11 µg/mL.

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