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I am very pleased receiving the gally proof of our article. However, the gally proof contains some mistakes. I wonder if you could kindly rectify these as mentioned in the table below:

Thank you for your cooperation

Best regards,

Prof. Dr. Maulidiyah

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Dear Author
Many thanks for your email.
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Sincerely
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--
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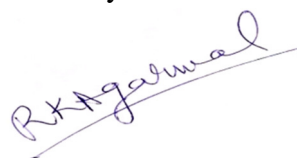
Dear Dr. Maulidiyah Maulidiyah,

We are pleased to inform you that your research article No. 22414/2019 entitled, *“Isolation the Natural Toxic Compounds derivated Steroidal from Bark Stem of Melochia Umbellata (Houtt) Stapf var. degrabrata K.”* has been accepted for publication in Asian Journal of Chemistry. The manuscript will appear in Volume 31 (2019) of Asian Journal of Chemistry. Here, we are enclosing an invoice bill No. 2753 dated 14th October 2019 of US \$ 400=00 (US Dollars Four Hundred Only) towards the Printing/Publication charges.

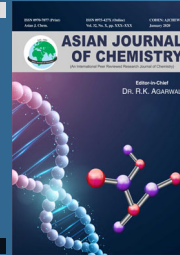
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Isolation of Natural Derivated Steroidal from Bark Stem of *Melochia umbellata* (Houtt) Stapf var. *degrabrata* K.

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Exploration of the natural product as pharmaceutical drug widely discover continuously for human life. The unique chemical compounds have been explored from bark stem of *Melochia Umbellata* (Houtt) Stapf var. *degrabrata* K. (*M. umbellata*) which is obtained from South Sulawesi, Indonesia. We discovered two group compounds isolated namely stigmasta-5,22-dien-3- β -ol (**1**) and stigmasta-5,22-dien-on (**2**), from *n*-hexane and chloroform extracts. The chemical structure of compounds **1** and **2** were identified based on IR spectroscopic, ¹H NMR and ¹³C NMR and compared spectra data with data of compounds by other researchers. Compounds **1** and **2** are evaluated of their toxicity activity against shrimp larvae (*Artemia salina*) shows that the compound **1** shows weak toxicity activities against *Artemia salina* with LC₅₀ value of 548.48 μ g/mL, meanwhile the compound **2** exhibits a high inhibitory by LC₅₀ value of 410.81 μ g/mL. Based on this study, we suggest that the compound **2** has good bioactivity compared with compound **1**.

Keywords: *M. umbellata*, Stem bark, Steroid compounds, Toxicity, *Artemia salina*.

INTRODUCTION

Metrosideros umbellata plant is widely found in shrubs similar to trees form and can be obtained at Indonesia's tropical forests consisting of 70 genera and 1,500 species [1]. These plants have been utilized by community in South Sulawesi area as traditional medicines, especially for treating diseases, liver, hypertension and hepatitis, cholesterol and diabetes [2-4]. The Muna tribe in Southeast Sulawesi uses *M. umbellata* leaf tissue as a remedy for hives [5]. These unique plants have many advantages based on the variation extract can improve a high bioactivity. Methanolic extract has been used for the isolation of biological active secondary metabolite from *Metrosideros umbellata* [6,7]. Methanolic extract can improve liver function of mice induced with carbon tetrachloride at concentrations of 10 and 15 % [8].

The high performance for extracting bark stem *M. umbellata* using MeOH due to high-ability diffusion in materials to extract polar and non-polar compounds, low cost and easy for getting

it [9]. The MeOH can be extracting many compounds from bark stem *M. umbellata* such as; alkaloids, flavonoids, triterpenoids, steroids, phenolics and saponins [10]. The preliminary test results of MeOH extract have an inhibitory effect on *Mycobacterium tuberculosis* growth at concentrations of 200 and 400 ppm [11-13]. It is associated with a previous study which was reported that the phytochemical test of MeOH extract of *M. umbellata* Stapf var. *degrabrata* showed positive results against alkaloids, flavonoids, steroids and saponins [14]. The chemical content of the hexane extract of bark stem *M. umbellata* is alkaloids and triterpenoids [15-17]. Ethyl acetate extract of bark stem *M. umbellata* var. *Visenia* contains steroid, alkaloids and terpenoids [18]. In addition, chloroform extract also contains steroid and alkaloid compounds [19,20].

In other hands, the antibacterial activity test results against hexane extract showed inhibition on *B. subtilis* and *S. aureus* bacteria with diameter inhibition zones of 12.0 and 10.4 mm [21]. The extract of hexane, CHCl₃, EtOAc and MeOH from *M. umbellata* are toxic to *Artemia Salina* with LC₅₀ 407.38,

460.79, 405.58 and 408.79 $\mu\text{g/mL}$. The EtOAc extract is most toxic compared to the other extracts [13]. Meanwhile, ethyl acetate extract of bark stem *M. umbellata* var. *Vicenia* is toxic against *A. salina* with a LC_{50} value of 101.66 $\mu\text{g/mL}$ and the extract is also very toxic to dengue virus with a value of IC_{50} 1.67 $\mu\text{g/mL}$, so there is a correlation relationship between toxicity to *A. salina* and toxicity to dengue virus [22]. The results of another research reported that the chloroform extract of bark stem *M. umbellata* var. *Vicenia* is toxic to *A. salina* with a value of LC_{50} 53.57 $\mu\text{g/mL}$ [18].

The secondary metabolite compound was isolated from *n*-hexane fraction such as oleanane derivative compound (3-acetyl-12-oleanane-28-ol) can inhibit of *B. subtilis* bacteria and the fungus *C. albicans* with a diameter of inhibition zones 15.8 and 15.2 mm and also toxic against *A. salina* with a value of LC_{50} 361.93 mg/mL [23]. The compound 5,22-stigmastadien-3 β -ol was found in the part root tissue of *M. umbellata* which has the potential as an antibacterial, stigmast-5,22-dien-3-O- β -D-glucopyranoside compound is antifungal. Then, compounds 9,10-epoxy melochinon was toxic against *A. salina* sand murine cell of leukemia P-388. The 6,6'-dimethoxy-4,4'-dihydroxy-3',2'-furano-isoflavone compound is not toxic against *A. salina* and murine cell of leukemia P-388 [24].

Methyl- β -(*p*-hydroxyphenyl)acrylate compound was isolated from the chloroform extract of root bark showed that the significant toxicity to murine cell of leukemia P-388 with the IC_{50} value of 5.351 $\mu\text{g/mL}$ [13]. Subsequently, two new compounds were isolated on the part tissue of stem *M. umbellata* are namely Waltherion C compound which was very toxic to *A. salina* and murine cell of leukemia P-388 and cleomiscosin compounds but it was not toxic to *A. salina* and P-388 [6]. Based on the literature, in this study we isolate and elucidation structure from steroid compounds *M. umbellata* and its bioactivity test against *A. salina* to produce the anticancer potential contributing for developing a natural product as a pharmaceutical drug.

EXPERIMENTAL

The *M. umbellata* type of *M. umbellata* (Houtt) Stapf var. *degrabrata* K. was taken from biodiversity area at Universitas Hasanuddin, Tamalanrea, Makassar, South Sulawesi, Indonesia. We then identified this species at Herbarium Bogor, Lembaga Ilmu Pengetahuan Indonesia (LIPI) Biology Research Center,

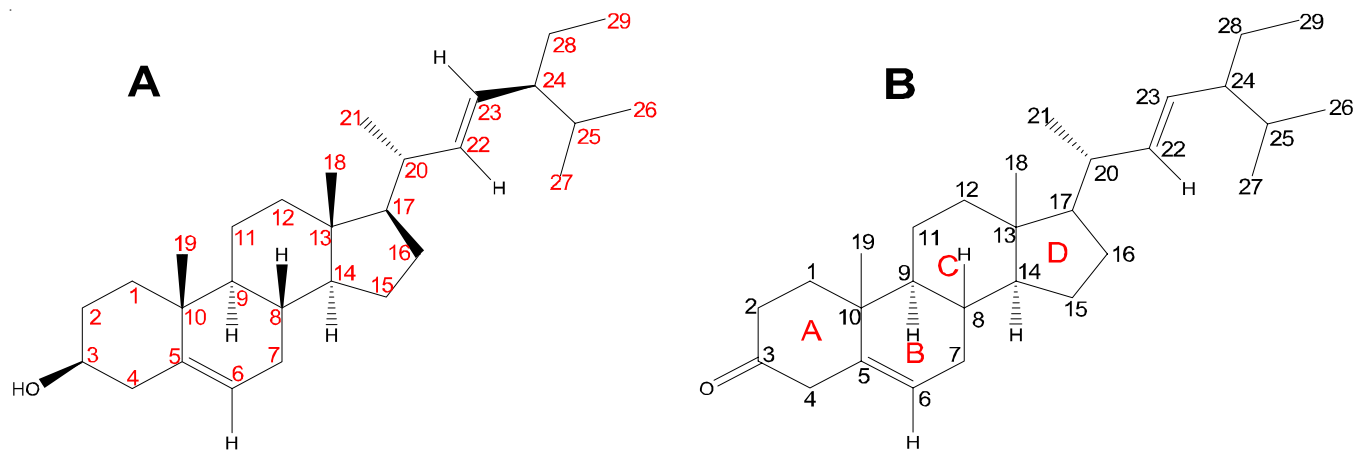
with species references: BO-1912171. The sample was smoothed using a blender and dried under sunlight to remove the water content in the sample and facilitate for the easy extraction process.

Determination of the melting point characteristic was obtained using a corrected micro melting point measurement tool (John Fisher). The IR spectrum was measured by a Perkin Elmer ONE spectrometer. The $^1\text{H NMR}$, $^{13}\text{C NMR}$ and HMBC spectra were measured using a JEOL JNM EX-400 FTNMR spectrometer that worked at 500 MHz ($^1\text{H NMR}$) and 125 MHz ($^{13}\text{C NMR}$). Chemical shifts are given at a scale of δ (ppm) with TMS as an internal standard. Chromatographic columns using Merck 60 silica gel (230-400 mesh) and TLC analysis on laminated plates of Merck gel Kiesel gel 60 F₂₅₄ 0.25 mm, The solvents used in this experiment are pro-analyst (Merck).

Extraction, separation and purification: *M. umbellata* (5.25 Kg) powder was extracted by maceration using MeOH solvent for 3 times (3 \times 24 h). Then, extract was evaporated with a rotary evaporator vacuum and we were obtained of thick extract (390 g) as brown colour. The MeOH extract (300 g) is partitioned by liquid-liquid extraction using a solvent with an increased level of polarity, starting from a solvent; C_6H_{14} , CHCl_3 and EtOAc. Each of the filtrate was separated using a rotary evaporator vacuum, to obtain C_6H_{14} extract of 36 g, CHCl_3 extract 24 g, EtOAc extract 16 g, respectively.

Subsequently, the C_6H_{14} fraction (30 g) was separated using a chromatographic technique such as: vacuum column chromatography (VCC) using C_6H_{14} -EtOAc solvent which increased its polarity to produce 32 initial fractions. Based on the thin layer chromatography (TLC) analysis fractions that have the same profile are combined to obtain 12 main fractions. Crude crystal F fraction was fractionated with preparative TLC and obtained FK 850 mg subfraction (yellowish white powder) and 290 mg FB fraction (blue paste form).

Approximately, 850 mg FK subfraction is fractionated with VCC, resulting in 12 subfractions; FK1 (55.3 mg), FK2 (75.2 mg), FK3 (45.5 mg), FK4 (56.0 mg), FK5 (60.6 mg), FK6 (45.2 mg), FK6 (45.2 mg), FK7 (55.4 mg), FK8 (72.8 mg), FK9 (64.6 mg), FK10 (66.3 mg), FK11 (60.1 mg) and FK12 (55.5 mg). The FK3, FK4, FK5 and FK6 subfractions each form a needle crystal. The crystal is purified by recrystallizing repeatedly using a MeOH solvent to produce fine white powder.



Structure from bark stem of *M. umbellata* (A) stigmast-5,22-dien-3- β -ol and (B) stigmast-5,22-dien-on

Based on the results of the TLC analysis, FK3 and FK4 compound isolates had the same stain profile and R_f value, so that they were combined and expressed as compound isolates **1**. The purity test of isolate compound **1** with TLC analysis of three eluent systems, each showing one stain with an R_f value different and the yield of the melting point of compound **1** is 115-117 °C. Compound **1** is positive as a steroid, which is characterized by the purple deposits after added LB (Liebermann-Burchard) reagent. Furthermore, compound **1** isolates were tested for their toxicity and analyzed by UV, FTIR, NMR spectrophotometers (^1H NMR, ^{13}C NMR, HMBC and COZY).

Then, CHCl_3 extract (15 g) was fractionated by VCC using C_6H_{14} , $\text{C}_6\text{H}_{14}:\text{EtOAc}$, EtOAc , CH_3COCH_3 and MeOH which increased polarity. and produced 48 initial fractions. Based on the TLC analysis, these fractions were combined and 12 sub-fractions were obtained, namely; subfraction of K1 (122.4 mg), K2 (90.26 mg), K3 (144.3 mg), K4 (155.4 mg), K5 (572.3 mg), K6 (440.5 mg), K7 (992.4 mg), K8 (614.1 mg), K9 (1432.3 mg), K10 (967.3 mg), K11 (559.5 mg) and K12 (536.2 mg). Subfraction K5 (572.3 mg) in the form of a blue paste form. Subsequently, K5 subfraction (570 mg) was separated by compressive column chromatography (CCC) with eluent $\text{EtOAc}-\text{C}_6\text{H}_{14}$, EtOAc , $(\text{CH}_3)_2\text{CO}$ and MeOH , with solvents that increased polarity and produced ten-ten subfractions namely; subfraction K5.1 (56 mg), K5.2 (45 mg), K5.3 (58 mg), K5.4 (70 mg), K5.5 (39 mg), K5.6 (31 mg), K5.7 (36 mg), K5.8 (55 mg), K5.9 (50 mg) and K5.10 (44 mg).

In subfraction K5.7 precipitate is formed in the form of a blue paste. Furthermore, the precipitate is crystallized and recrystallized using $(\text{CH}_3)_2\text{CO}-\text{C}_6\text{H}_{14}$, after filtering it is obtained the isolates that form deposits in the form of blue paste (18.2 mg). The purity test of isolates K5.7 (compound **2**) with TLC analysis of three eluent systems and showed one stain with different R_f values, the results of the analysis showed that compound **2** was pure. Test group against compound **2** using LB (Liebermann-Burchard) reagent, positive steroid which is indicated by the formation of blue deposits.

Toxicity test: Toxicity test of C_6H_{14} extract, compound **1** and compound **2** from the bark stem of *M. umbellata* using the brine shrimp lethality test (BSLT) method using shrimp larvae (*A. salina*) through the following stages:

Hatching of shrimp larvae (*A. salina*): Shrimp eggs (*A. salina*) are put in a beaker (1000 mL) which has 500 mL sea water (half of the total volume of the beaker). A chemical glass containing *A. salina* is covered with aluminum foil and placed under a 5 watt light source (28 °C). After 48 h, *A. salina* are collected using a pipette, then it is ready to be used for toxicity tests [25].

Preparation of test solutions (compound extracts and isolates): The C_6H_{14} extract, CHCl_3 extract, compound **1** and compound **2** were tested with variations in concentrations of 125, 250, 500 and 1000 ppm in seawater, if the extract and compound are insoluble, add 1.0 % dimethyl sulfoxide then stirred solution until homogeneous.

Meyer method toxicity test: In this method, each concentration were taken as much as 0.5 mL and included 15 *A. salina*. For each concentration three repetitions were carried out. As a control, it is done by without extracts or isolates of the compound into the test vial containing *A. salina*. Then, it was left

for 24 h and counted the total of dead and living *A. salina*. Mortality is calculated by comparing the total of dead larvae divided by the number of *A. salina*. The data obtained is used to calculate the LC_{50} value. Calculation of LC_{50} was carried out using a linear regression equation: $y = a + bx$ obtained from the relationship between log concentration and probit mortality graph. An extract is said to be active or toxic if the value of $\text{LC}_{50} \leq 1000 \mu\text{g/mL}$, for pure compounds is said to be active if it has an LC_{50} value $\leq 30 \mu\text{g/mL}$ [26].

RESULTS AND DISCUSSION

The white powder was isolated from bark stem *M. umbellata* and we provide code by compound **1**. It has a melting point of 115-117 °C and positive steroid with reagent Liebermann-Burchard (L-B) gave the colour blue after the addition of anhydrous acetate and H_2SO_4 solution. The data is supported by absorption bands at wavenumber 3428 and 1244 cm^{-1} originating from the hydroxyl group. The absorption band at 2935 and 2886 cm^{-1} for aliphatic C-H supported by absorption bands at 1462 and 1377 cm^{-1} showed the presence of CH_3 and CH_2 . The absorption band appeared due to the presence of double bond $\text{C}=\text{C}$ at 1645 cm^{-1} and $\text{C}-\text{O}$ at 1056 cm^{-1} .

Based on NMR data (Table-1), compound **1** has six degrees of saturation. The ^{13}C NMR spectrum of compound **1** has 29 carbon atoms consisting of six methyl, nine methylene, eleven methine and three quaternary carbon, so compound **1** is thought to have a basic steroid framework containing one hydroxyl group and two double bonds [27]. ^1H NMR spectrum of compound **1** shows 25 proton signals consisting of 6 singlet methyl groups namely at δ_{H} 0.69 ppm (3H, s, H-18) and 1.02 ppm (3H, s, H-19) both are signals singlet of methyl tertiary, three signals of secondary methyl at δ_{H} 0.92 ppm (3H, d, 6.3, H-21), 0.88 ppm (3H, d, 6.5, H-26) and 0.84 ppm (3H, d, 6.5, H-27) and one signal of methyl primary at δ_{H} 0.83 ppm (3H, t, 6.5, H-29), one signal proton methine which binds the group of oxy groups to area δ_{H} 3.52 ppm (H-3, 6H, septet, 6.5 Hz, H-3), and three olefinic substituted proton signals in the area of 5, H 5.35 ppm (1H brd = 5.2, H-6), 5.16 ppm (1H, dd, 8.5; 15 Hz, H-22), and 5.03 ppm (1H, dd, 8.5; 15 Hz, H-23).

The relationship of the long distance correlation between protons ^1H and carbon ^{13}C is shown by the HMBC spectrum. Long distance correlation of proton at δ_{H} 5.35 ppm (H-6) with methylene carbon at δ_{C} 42.5 ppm (C-4), with carbon methine at δ_{C} 32.08 ppm (C-8), with quaternary carbon at δ_{C} 36.68 ppm (C-10), with olefinic quaternary carbon at δ_{C} 140.29 ppm (C-5) and methylene carbon at δ_{C} 31.9 ppm (C-7), this confirms that the double bond is located at C-5 and C-6 on the second ring of the steroid skeleton. Long distance correlation of proton δ_{H} 5.16 ppm (H-22) with carbon methine at δ_{C} 40.7 ppm (C-20), with carbon methine olefinic at δ_{C} 129.4 ppm (C-23), with carbon methine at δ_{C} 51.42 ppm (C-24).

Long distance correlation between proton signal δ_{H} 5.03 ppm (C-23) with carbon signal methine at δ_{C} 40.7 ppm (C-20), with carbon signal methine double bond at δ_{C} 138.5 ppm (C-22), with the carbon signal methine at δ_{C} 51.42 ppm (C-24), these signals indicate that the double bond is on the C-22 and C-23. Some proton signals from the methyl group at δ_{H} 0.69 ppm (Me-18) with carbon at δ_{C} 39.95 ppm (C-12), δ_{C} 42.48

TABLE-1
 SPECTROMETRY DATA ANALYSIS OF NMR FROM COMPOUNDS 1 AND 2 IDENTIFIED AND COMPARED WITH REFERENCE [14]

Total C	H NMR, δ ppm (m, J [Hz])	C NMR δ ppm	C NMR literature	COSY H-H	HMBC H-C	Total C	H NMR, δ ppm (m, J [Hz])	C NMR δ ppm	C NMR lit.	COSY H-H	HMBC H-C
1	2.34 (1H, m); 1.84 (1H, m)	37.43	37.6	2	C2; C5; C10; C3;	1	2.34 (1H, m); 1.63 (1H, m)	38.8	38.19	H2	C3; C2; C9
2	1.83 (1H, m); 1.50 (1H, m)	31.83	32.1	1; 3	C10; C3; C4	2	2.50 (1H, m); 2.40 (1H, m)	32.2	31.95	H1	C3; C4
3	3.52 (1H, m, 6.5 Hz)	71.99	72.1	2; 4	-	3	-	199.91	212.11	-	-
4	2.23 (1H, m); 1.98 (1H, m)	42.5	42.4	3	C2; C3; C5; C6; C10.	4	3.07(1H, m); 2.42 (1H, m)	42.6	129.52	-	C3; C6; C5
5	-	140.29	141.1	-	-	5	-	138.3	139.55	-	-
6	5.35 (1H br d = 5.2)	121.9	121.8	7	C4; C7; C8; C10	6	5.72 (1H bs)	123.9	19.01	H7	C7; C10
7	1.99 (1H, m); 1.56 (1H, m)	31.9	31.8	8; 6	C5; C6; C9; C14	7	2.32 (1H, m); 1.84 (1H, m)	33.1	34.42	H6; H8	C5; C8; C9
8	1.45 (1H, m)	32.08	31.8	9;	C9	8	1.27 (1H, m)	34.2	30.06	H9; H7; H14	-
9	1.02 (1 H, m)	50.32	50.2	11	C1; C10; C19	9	1.17 (1 H, m)	46.0	51.26	H8; H11	-
10	-	36.68	36.6	-	-	10	-	39.2	39.33	-	-
11	1.53 (2H, m)	21.29	21.5	12	-	11	1.63 (2H, m); 1.38 (2 H, m)	21.1	21.71	H9; H12	C8; C10
12	1.96 (1H, m); 1.21 (1H, m)	39.95	39.9	11	C9; C14; C17	12	1.56 (1H, m); 1.31 (1H, m)	39.7	40.07	H11	C14; C17
13	-	42.48	42.4	-	-	13	-	42.5	41.27	-	-
14	1.01 (1H, m)	57.04	56.8	15	C7; C8; C18	14	1.04 (1H, m)	56.1	55.85	H15; H8	-
15	1.60 (1H, m); 1.35 (1H, m)	24.55	24.4	14; 16	C14; C17	15	1.86 (1H, m); 1.50 (1H, m)	24.4	24.66	H14	-
16	1.71 (1H, m); 1.28 (1H, m)	29.30	29.3	17	-	16	1.83 (1H, m); 1.52 (1H, m)	28.4	28.53	H17	-
17	1.15 (1H, m)/	56.11	56.2	20	C13; C14; C21; C22	17	1.24 (1H, m)	54.0	55.03	H16	C13; C20
18	0.69 (3H, s)	12.17	12.2	-	C12; C13; C14; C20	18	1.01 (3H, s)	19.0	21.14	-	C13; C17
19	1.02 (3H, s)	19.59	18.9	-	C1; C5; C9; C10	19	0.84 (3H, s)	12.3	21.14	-	C9; C10;
20	1.52 (1H, m)	40.7	40.6	22	-	20	2.08 (1H, m)	40.6	138.11	H22; H17	-
21	0.92 (3H, d, 6, 3)	21.40	21.7	-	C17; C20; C22	21	1.0 (3H, d, 6, 3)	20.2	117.01	-	C17; C22
22	5.16 (1H, dd, 8.5; 15)	138.5	138.7	23	C20;; C23; C24	22	5.12 (1H, dd, 8, 5; 15)	138.3	130.04	H20; H23	C20; C23; C24
23	5.03 (1H, dd, 15; 8.5)	129.4	129.6	-	C20; C22; C24	23	5.02 (1H, dd, 15; 9.5)	129.6	129.58	H24; H22	C20; C22
24	1.54 (1H, m)	51.42	46.1	25; 28	C25; C28	24	1.87 (1H, m)	56.2	48.04	H23; H25;	C25; C28
25	1.56 (1H, m)	32.13	29.6	26; 27	C24;	25	1.25 (1H, m)	35.9	29.40	H26;	C26; C27
26	0.88 (3H, d, 6, 5)	21.29	20.2	-	C24; C25; C27	26	0.72 (3H, d, 6, 5)	18.9	23.04	-	C24
27	0.84 (3H, d, 6, 5)	19.2	19.8	-	C24; C25; C26	27	0.91 (3H, d, 6, 5)	19.	21.40	-	C24; C25
28	1.5 (1H, m)	26.21	18.9	29	C24; 29	28	1.44 (1H, m)	26.3	28.53	H29	C29
29	0.83 (1 H, t, 6, 5)	12.46	12.2	28	C28	29	0.84 (1 H, t, 6, 5)	12.3	12.12	-	C28

ppm (C-13), δ_c 57.04 ppm (C- 14) and δ_c 40.7 ppm (C-20). proton signal at δ_H 1.02 ppm (Me-19) with carbon at δ_c 37.43 ppm (C-1), δ_c 140.29 ppm (C-5), δ_c 50.32 ppm (C-9) and δ_c 36.68 ppm (C-10). The proton signal at δ_H is 0.92 ppm (Me-21) with carbon at δ_c 56.11 ppm (C-17), δ_c 40.7 ppm (C-20) and δ_c 138.5 ppm (C-22). The proton signal at δ_H is 0.88 ppm (Me-26) with carbon δ_c 51.42 ppm (C-24), δ_c 32.13 ppm (C-25) and δ_c 19.2 ppm (C-27). The proton signal at δ_H is 0.84 ppm (Me-27) with carbon at δ_c 51.42 ppm (C-24), δ_c 32.13 ppm (C-25) and δ_c 21.29 ppm (C-26). The proton signal at δ_H

0.83 ppm (Me-29) with carbon at δ_c 26.21 ppm (C-28). In detail, the correlation of HMBC compound 1 can be seen in Table-1 and analysis of HMBC spectrum of compound 1 can be seen in Fig. 1.

Based on the analysis of 1D and 2D NMR spectral data described above, it can be concluded that compound 1 is 5.22-stigmastadien-3 β -ol (stigmasterol), with a molecular structure of C₂₉H₄₈O. NMR spectral data of compound 1 are similar with stigmasterol compounds that have been reported by Chaturvedula and Prakash [28] so that supporting compound 1 is stigma-

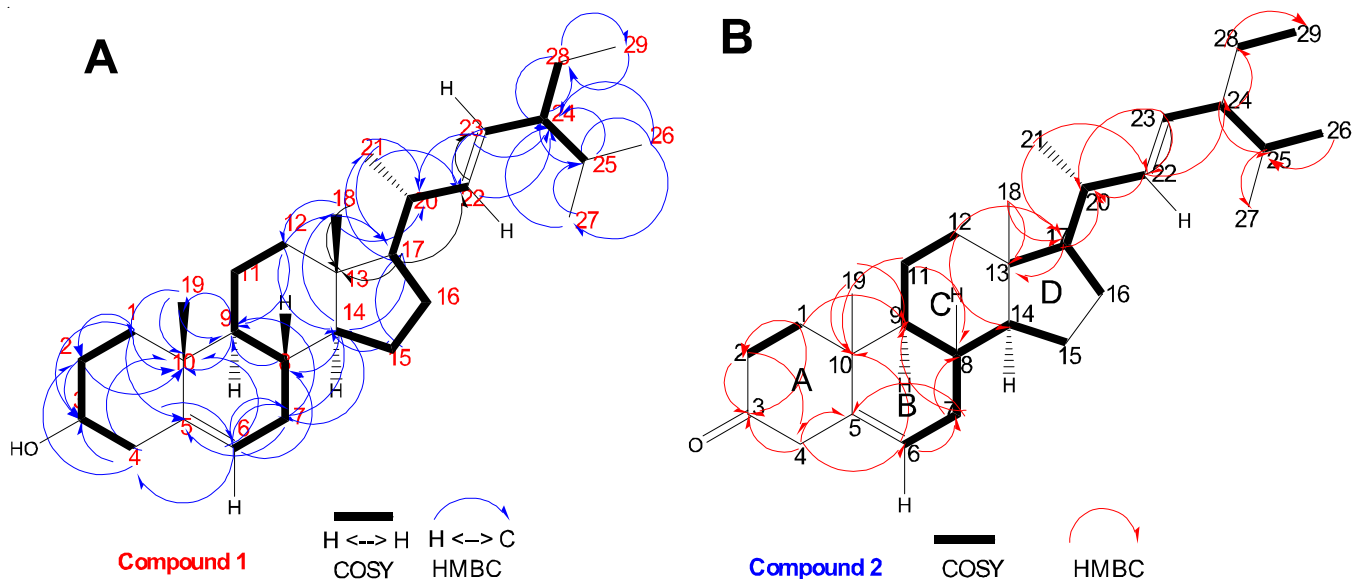


Fig. 1. Analysis of HMBC and COSY (A) compound **1** and (B) compound **2**

sterol. The comparative data for compound **1** are presented in Table-1.

We also obtain the metabolite secondary based on chloroform extract (15.30 mg) and labeled by compound **2**. It is paste form and blue colour and based on NMR data (Table-1) compound **2** also has six degrees of unsaturation. It is positive for steroids because of gives the colour blue after the addition of Lieberman-Bucher reagent (L-B). The test results are supported by IR spectrum compound **2**, where there is absorption at wavenumber 2953.02 and 2852.72 cm^{-1} for C-H aliphatic and strengthened by the presence of absorption bands at 1482.04 and 1377.17 cm^{-1} for CH_3 and CH_2 . The absorption band at 1735.93 cm^{-1} for C=O, the absorption band of double bond C=C at 1641.42 cm^{-1} and the absorption band for C-O at 1056.92 cm^{-1} .

Subsequently, the ^{13}C NMR spectral data and DEPT-135 (Table-1) shows that compound **2** has 29 carbon atoms consisting of six methyl, nine methylene, ten methine and four quaterners, which are thought to be composed of a steroid framework substituted by two methyl groups and one alkyl groups consist of ten carbon chains. δ_{C} 199.91 ppm shows the presence of 1 carbonyl group (C=O) on C-3 atoms, this group is also observed in the IR spectrum.

The ^1H NMR spectrum of compound **2** shows a pattern similar to that of compound **1** ^1H NMR spectrum **1**, which shows 25 proton signals consisting of: seven methylene proton signals, at δ_{H} : 2.34, 2.50, 3.07, 2.32, 1.63, 1.56, 1.86 and 1.83 ppm, five proton signals methine at δ_{H} : 1.45, 1.02, 1.01, 1.15 and 5.35 ppm, and one olefinic proton signal. Two methyl proton signals bound to quaternary carbon at δ_{H} 1.01 ppm (3H, s, H-18) and 0.84 ppm (s, H-19). The proton signals indicate a steroid skeleton substituted by two methyl groups.

Ten other signals that appear and are indicated as an alkene unit consisting of; seven proton signals from the methine group each in δ_{H} : 1.27 (H-8); 1.17 (H-9); 1.04 (H-14); 1.24 (H-17); 2.08 (H-20); 1.87 (H-24) and 1.25 (H-25) ppm, and three olefinic proton signals on δ_{H} : 5.72 (H-6); 5.12 (H-22) and 5.02 ppm (H-23). One methylene proton signal at δ_{H} 1.44 (H-28,

1H, m) and there are six proton signals from the methyl group at δ_{H} 1.01 (H-18, 3H, s), 0.84 (H-19, 3Hs), 1.0 ppm (H-21, 3H, d, 6.3), 0.72 ppm (H-26, 3H, d, 6.5), 0.91 ppm (H-27, 3H, d, 6.5) and 0.84 ppm (H-29, 1H, t, 6.5). ^1H NMR data of compound **2** can be seen in Table-1.

The long distance correlation relationship between ^1H protons and ^{13}C carbon is shown by the HMBC spectrum. The HMBC spectrum shows a long-distance correlation of protons in δ_{H} 5.72 ppm (H-6) with methylene carbon at δ_{C} 33.1 ppm (C-7), with quaternary carbon carbon δ_{C} 39.2 ppm (C-10), this make it clear that the double bond is located at C-5 and C-6 on ring B. The long-distance correlation of proton δ_{H} 5.12 ppm (H-22) with carbon methine δ_{C} 40.6 ppm (C-20), with methyl olefinic carbon at δ_{C} 129.6 ppm (C-23), with carbon methine δ_{C} 56.2 ppm (C-24). Long distance correlation between proton signal δ_{H} 5.02 ppm (C-23) with carbon signal methine δ_{C} 40.6 ppm (C-20), with olefinic carbon signal at δ_{C} 138.3 ppm (C-22), signals this indicates that the double bond is on the C-22 and C-23.

Some proton signals from the methyl group at δ_{H} 1.01 ppm (Me-18) correlate with carbon at δ_{C} 42.5 ppm (C-13) and δ_{C} 54.0 ppm (C-17). The proton signal at δ_{H} 0.84 ppm (Me-19) correlates with carbon at δ_{C} 46.0 ppm (C-9) and δ_{C} 39.2 ppm (C-10). The proton signal at δ_{H} 1.0 ppm (Me-21) correlates with carbon at δ_{C} 54.0 ppm (C-17) and δ_{C} 138.3 ppm (C-22). The proton signal at 72H 0.72 ppm (Me-26) correlates with carbon δ_{C} 56.2 ppm (C-24). The proton signal at δ_{H} 0.91 ppm (Me-27) correlates with carbon at δ_{C} 56.2 ppm (C-24) and δ_{C} 35.9 ppm (C-25). The proton signal at δ_{H} 0.84 ppm (Me-29) correlates with carbon at δ_{C} 26.3 ppm (C-28). In detail the correlation of HMBC compound **2** can be seen in Table-1 and an analysis of the relationship of Cozy correlation and HMBC compound **2** is shown in Fig. 1.

We have calculated LC_{50} value (Table-2) exhibits that the C_6H_{14} extract of compounds **1** and **2** from bark stem of *M. umbellata* against inhibitory *A. salina* in a row: 407.38; 584.48; and 410.81 $\mu\text{g}/\text{mL}$. These results indicate that C_6H_{14} extract and CHCl_3 extract of compound **1** (stigmasta-5,22-dien-3- β -

TABLE-2
TOXICITY TESTS OF EXTRACTS AND COMPOUNDS
FROM BARK STEM OF *M. umbellata* AGAINST *A. salina*

Extract	LC ₅₀ (µg/mL)	Compound	LC ₅₀ (µg/mL)
<i>n</i> -hexane	407.38	1	584.48
Chloroform	460.79	2	410.81

ol) and compound **2** (stigmasta-5,22-dien-3-one) have successive LC₅₀ values 584.48 and 410.81 µg/mL, respectively. These results indicate that both of these compounds are less toxic to the *A. salina* because the LC₅₀ value of the compound is greater than 100 µg/mL.

Pure compounds are said to be toxic (high toxic) to *A. salina* if the LC₅₀ value is less than 100 µg/mL [25,29]. However compound **2** (stigmast-5,22-dien-3-one) is more toxic to *A. salina* than compound **1** (stigmast-5,22-dien-3-β-ol). The difference in toxicity shown by these three compounds to *A. salina* is probably caused by differences in the polarity of the compound caused by differences in the number of functional groups in the compound.

Conclusion

We have discovered the two secondary metabolite compound from bark stem *Melochia umbellata* (Houtt) Stapf var. *degrabrata* K. which are extracted using variation solvent namely C₆H₁₄ and CHCl₃ extracts exhibit that the steroidal structure identified is stigmasta-5,22-dien-3-β-ol (**1**) and stigmasta-5,22-dien-on (**2**). Toxicity test has been explored for inhibiting *A. salina* shows that the LC₅₀ values of 548.48 and 410.81 µg/mL, respectively.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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