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Research Article

α–AMYRIN AND β-SITOSTEROL FROM BARK EXTRACT OF *RHIZOPHORA MUCRONATA* LAMK. AND THEIR CYTOTOXIC ACTIVITIES AGAINST HeLa CELL LINE

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ABSTRACT

The aims of this study are to isolate and identify active compounds of mangrove plants *Rhizophora mucronata* Lamk bark extract obtained from the mangrove forest of Kendari Bay, Southeast Sulawesi Province Indonesia and study their cytotoxic activities against cervical cancer (HeLa) cell line. The isolation and purification of the compounds were carried out under several chromatography methods, including thin layer chromatography, vacuum liquid chromatography and radial chromatography with silica gel as adsorbent and various solvents mixture as an eluent. Elucidation of the structure of the isolated compounds was done based on FTIR, ^{1}H-NMR, ^{13}C-NMR (1D and 2D) spectroscopic data and confirmed with an existed data from literatures. From this research, it was obtained two pure compounds, α-amyrin (1) and β-sitosterol (2). The isolation of this α-amyrin was firstly reported so far from the bark of this plant. Cytotoxic activities, marks by an IC_{50} value, of both α-amyrin and β-sitosterol are 804.762 ± 0.22 µg/mL and 353.871 ± 0.53 µg/mL respectively and shows that cytotoxic of β-sitosterol is stronger than α-amyrin.

Keywords: *Rhizophora mucronata*, bark extract, α-amyrin, β-sitosterol, cytotoxic

INTRODUCTION

Several kinds of literature have reported the traditional usage of mangroves by some community to treat various diseases. Indian peoples, for examples, used mangroves to treat diseases like, flatulence, epilepsy, smallpox, diabetic, asthma, rheumatism, stomach pains, fevers, malaria, cholera, hepatitis, cancer, ulcer, wounds and AIDS[1,2,3] Furthermore, people in Bangladesh used mangrove plants as an antinociceptive, anti-inflammatory, and antipyretic[4], while in Myanmar peoples used this plant to treat inflammatory diseases and diarrhea[5]. Mangrove bark extract had also been used by local Thai people to treat diarrhea, nausea, vomiting, and to stop bleeding in new fresh wounds[6,7]. Leaf extract of *R. mucronata* also has been traditionally used to treat diarrhea[8] and as a blood sugar-lowering drug[9]. The root extract of *R. mucronata* have the ability as an antioxidant and can repair the damage of the experimental rat liver due to CCl_4 hepatotoxins[10]. This research is focused on the isolation and determination of some chemically active compound, with further examination of the biological activity of bark extract of *Rhizophora mucronata*. Lamk. We previously have reported toxicity and anti-oxidant activity of the bark extracts[11]. In this study, we reported the isolation and cytotoxic assessment of α-amyrin and β-sitosterol compounds from an n-hexane extract of the bark of *R. mucronata* against HeLa cell line.

MATERIALS AND METHODS

The isolation, identification, and purification of the active constituents of the samples were carried out at Halu Oleo University by use several chromatographic techniques, such as vacuum liquids chromatography methods (VLC) and radial chromatography (RC). VLC and RC methods were filled with Merck Si-gel 60 G PF_{254} and monitored by TLC analysis on pre-coated Si-gel plates with Merck Kieselgel 60 F_{254}, 0.25 mm. The IR spectra were measured at the Organic Chemistry Laboratory of Faculty of Mathematics and Natural Sciences Hasanuddin University. ^{1}H and ^{13}C NMR (1D and 2D) spectra were recorded with an Agilent 500 MHz spectrometer which operated at 500 MHz for ^{1}H and 125 Mhz for ^{13}C. This work was conducted at the Natural Product Organic Chemistry Laboratory, ITB Bandung. The cytotoxic assays were conducted at the Agency for Research and Development of Medicinal Plants and Traditional Medicine (B2P2TO2T) Laboratory, Tawangmangu, Solo Indonesia.

Plant Material

Plant samples of *R. mucronata* Lamk were obtained from the mangrove forest of Kendari Bay, Southeast Sulawesi Province. These samples have been identified by the Research Center for Biology of Indonesian Institute of Sciences (LIPI) and taken as a collection under voucher number of FR.7.5.1.PU.01-02.

Isolation and Purification of Compounds

A total of 3 kg dry powder of *R. mucronata* bark were macerated using n-hexane for 3 days and the solvent replacement was carried out every 24 hours. The filtrate was collected, combined and concentrated under vacuum to obtained 6 g of thick n-hexane extracts. These n-hexane extracts were then separated under vacuum liquids chromatography with the Si-Gel as a stationary phase and eluent mixture of n-hexane:ethyl acetate as a mobile phase with a gradient increased of polarity from 100% n-hexane,

n-hexane:ethyl acetate 9:1 (*v/v*) to ethyl acetate 100%, to obtained 4 fractions, namely F1, F2, F3, and F4. Approximately 1.6 gram of combined fraction of F1 and F2 were separated further with electronic radial chromatography using a mixture of n-hexane:ethyl acetate 9.5:0.5 (*v/v*) as an eluent and Si-Gel PF_{254} containing gypsum as a stationary phase to obtained 4 combined fractions of $F1_1$, $F1_2$, $F1_3$, $F1_4$. The crystals of FI_3 and $F1_4$ were combined and recrystallized using methanol to give 36.3 mg of white crystals of Compound 1. Crystals of F3 and F4 were combined and purified using electronic radial chromatography and recrystallized with methanol to resulted in 42.5 mg of white crystals of Compound 2.

Determination of Molecular Structure of Pure Compound

The molecular structure of the isolated compounds was characterized using an IR, ^{1}H-NMR, ^{13}C-NMR (1D and 2D) spectroscopy.

Cytotoxic assay

The quantitative analysis procedure was adopted from[12] with minor modifications. Cervical cancer (HeLa) cell line the B2P2TO2T collection was cultured on RPMI 1640 (Gibco) 5% at 37ºC for 3 days in CO_2 incubator then the medium was descended then cells were washed with PBS (Sigma-Aldrich). Confluent cell cultures were harvested with trypsin, calculated by hemocytometer then distributed into 96-well microplate wells with a total of 8000 cells / well. Cells were incubated for 24 hours in a CO_2 incubator. The test solution was stocked in DMSO solvent then diluted using culture media according to the specified concentration series. Cells were washed with PBS then the test solution was put into the well (triple). Cells were incubated again for 24 hours in the CO_2 incubator. After incubation, the test solution was discarded and the MTT reagent added to 100 µl / well. Stopper reagents were added after 3 hours of incubation with MTT. Furthermore, cells are incubated overnight at room temperature and protected from light. At the end of the incubation, the plate was shaken with a horizontal shaker for 10 minutes then read of absorbance (OD) with an ELISA reader at a wavelength of 595 nm and the viability of cells was calculated using the following equation:

$$\text{Cells viability (\%)} = \frac{\text{OD of treated cells}}{\text{OD of control cells}}\, x\ 100\%$$

The IC_{50} values were obtained from the curves of toxicity, i.e. plots of percentage of the viability of cells vs. concentration of sample test by simple linear regression[13]

RESULTS AND DISCUSSION

Compound 1 Form: white crystal melting point 187-190 ºC.
IR spectrum (KBr) ῡ (cm^{-1}): 3394.42: OH, 1037.99: CO, 2945.3: C-H_{str} of CH_3, 2866.8: C-H_{str} of CH_2, 1661.07: C=C three substitution, 1462.04: C-H_{bend} of CH_2, 1382: C-H_{bend} of CH_3, 651.07: C=C bond.
^{1}H-NMR spectrum (500MHz) δH ppm, (m,ΣH): 5.12 (t,1H), 3.22 (dd,1H), 1.31 (d,1H), 2.03 (t, 2H), 1.91 (t,2H), 1.83 (t,2H) 1.68 (t,2H), 1.61 (t,2H), 1.57 (m,2H), 1 , 54 (t,1H), 1.41 (t,2H) 1.39 (m,2H) 1.36 (t,1H), 1.31 (d,1H) 1.07 (s,3H), 1.02 (s,3H), 0.99 (s,3H), 0.96 (s,3H), 0.91 (s,3H), 0.87 (m,1H), 0.80 (s,3H), 0.79 (s,3H), 0.79 (s,3H), and 0.74 (t,1H).
^{13}C-NMR spectrum (135 MHz): δC (ppm): 139.72, 124.55, 79.19, 59.19, 55.32, 47.85, 42.22, 41.68, 40.15, 39.81, 39.76, 38.93, 38.73, 37.04, 33.90, 33.08, 31.41, 28.91, 28.28, 28.25, 27.42, 26.76, 23.52, 23.42, 21.57, 18.53, 17.63, 17.01, 15.84, 15.79.
DEPT-135 spectrum: δC (ppm), (type): 139.72 (C=C), 124.55 (=CH), 79.19 (CH), 59.19 (CH), 55.32 (CH), 47.85 (CH), 42.22 (Cq), 41.68 (CH2), 40.15 (Cq), 39.81 (CH), 39.76 (CH), 38.93 (CH_2), 38.72 (Cq), 37.04 (Cq), 33.70 (Cq), 33.08 (CH_2), 31.41 (CH_2), 28.91 (CH_3), 28.28 (CH_3), 28.25 (CH_2), 27.42 (CH_2), 26.76 (CH_2), 23.52 (CH_2), 23.42 (CH_3), 21.57 (CH_3), 18.53 (CH_2), 17.63 (CH_3), 17.01 (CH_3), 15.84 (CH_3), 15.79 (CH_3)

Compound 2. Form: white crystal melting point 137-140 ºC
IR spectrum (KBr) ῡ (cm^{-1}): 3425.56: OH, 2958.8, 2935.66: C-$H_{str}$of CH_3, 2866.8: C-H_{str} of CH_2, 1463. 978: C-H_{bend} of CH_2, 1377.17: C-H_{bend} of CH_3, 1645. 28: C=C bond.
^{1}H-NMR spectrum δH (ppm) (m, ΣH): 5.34 (t,1H), 3.52 (M,1H), 2.26 (d,2H), 2.20 (t,2H), 1.99 (m,2H), 1.84 (m,2H), 1.68 (m,1H), 1.58 (m,2H), 1.49 (m,2H), 1.45 (m,1H) 1.38 (m,1H), 1.36 (m,1H), 1.32 (m,2H), 1.25 (m,2H), 1.16 (m,2H), 1.11 (m,1H), 1.01 (s,3H), 1.00 (m,1H), 0.94 (d,3H), 0.93 (m,1H), 0.87 (m,1H), 0.84 (m,2H), 0.82 (s,3H), 0.68 (d,3H).
^{13}C-NMR spectrum δC (ppm): 140.87, 121.85 71.93, 56.89, 56.18, 50.25, 45.96, 42.45, 42.41, 39.91, 37.39, 36.64, 36.29, 34.07, 32.05, 32.03, 31.77, 29.27, 28.39, 26.18, 24.45, 23.19, 21.22, 19.97, 19.54, 19.17, 18.92, 12.13, and 12.00
DEPT-135 δC (ppm) (type): 140.87 (Cq), 121.85 (CH), 71.93 (CH), 56.89 (CH), 56.18 (CH), 50.25 (CH), 45.96 (CH), 42.45 (Cq), 42.41 (CH_2), 39.91 (CH_2), 37.39 (CH_2), 36.64 (C), 36.29 (CH), 34.07 (CH_2), 32.05 (CH_2), 32.03 (CH), 31.77 (CH_2), 29.27 (CH), 28.39 (CH_2), 26.18 (CH_2), 24.45 (CH_2), 23.19 (CH_2), 21.22 (CH_2), 19.97 (CH_3), 19.54 (CH_3), 19.17 (CH_3), 18.92 (CH_3), 12.13 (CH_3), and 12.00 (CH_3)

Compound 1

Compound 1 was in the form of white crystals with a melting point of 187-190 ºC and gave a positive test of terpenoid with the Liebermann-Burchard reagent. The IR spectrum of compound 1 showed the characteristic absorption at wavenumber 3394.42 cm^{-1} for OH which was strengthened by the absorption at 1037.99 cm^{-1} by the C-O group. The absence of absorption in the area of 1700 cm^{-1} was typical for C=O and indicated that the molecule did not contain a carboxylic group. The absorption band at 2945.3 cm^{-1} is the CH stretching of the CH_3 and 2866.8 cm^{-1} groups, the CH stretching of CH_2 which is amplified by absorption in the absorption band at 1462.04 cm^{-1} is the CH bending of the CH_2 group and the absorption at 1382 cm^{-1} which is the CH bending of the CH_3 group. The absorption band at 1651.07 cm^{-1} is the absorption of the C-C stretching of the C=C bond. Such absorption frequency is identical to the absorption frequency of α-amyrin as stated by[14,15]. The ^{13}C-NMR spectrum of compound 1 shows 30 carbon signals, and the DEPT-135 spectrum shows compounds consisting eight CH_3, nine CH_2, one C=C, seven CH groups (including carbon which binds to OH groups), and four quarterner carbon atoms. Signals at δ 139.72 and δ 124.55 ppm are C-12 and C-13 atomic signals forming double bonds[13] and signals at δ 79.19 ppm are signals from carbon atoms that bind to hydroxyl groups and neighbor to quarterner carbon. The signals that appear at δ 28.91, δ 28.28 δ 23.42 δ 21.57 δ 17, 63, δ 17.01 δ 15, 84 and δ 15.79 ppm are methyl carbon signals. Signals at δ 41.68, δ 38.93, δ 33.08, δ 31.41, δ 28.25, δ 27.42, δ 26.76, δ 23.52 and δ 18.53 ppm are methylene carbon signals, and signal at δ 79.19, δ 59.19, δ 55.32, δ 47.85, δ 39.81, δ 39.76 ppm is the methine carbon signals and signal at 42.22, δ 38, 73, δ 33.9, δ 37.04 ppm are signals of quarterner carbon. Chemical shifts and types of carbon above are identical to the carbon chemical shift of α-amyrin compounds[15]. The ^{1}H-NMR spectrum shows a typical signal for the hydroxyl proton H-3 of a terpenoid nucleus that appears in the area around δ 3.24 ppm and the signal at 12 5.12 ppm is the proton signal bound to the substituted C-olefin (H-5). Proton signals at 36 1.36 ppm (1H, H-19) and δ 0.87 ppm (1H, H-20) indicate the position of the methyl group (C-29) bound to the C-19 atom (which distinguishes it from the β-isomer of amyrin). The position of a methyl group at C-19

is supported by HMBC data, that is a correlation of protons at δ 0.79 (H-29) to C-18 atoms (δ 59.19) and to C-20 atoms (δ 39.76). The proton at C-20 was shown by HSQC data, namely proton at δ 0.87 (H-20) with carbon δ 39.76 (C-20). The six singlet signal of protons containing three proton (s,3H) at δ 1.07, 1.01, 0.99, 0.96, 0.95, 0.8 ppm and two doublet signals (d,3H) at δ 0.91 and 0.79 ppm confirm the structure of the α-amyrin molecule which has eight methyl groups. From the description above, as well as identifying proton chemical and carbon compounds of isolate 1 with the α-amyrin compound proposed by[15], as shown in table 1, it can be concluded that compound 1 is α-amyrin with the formula $C_{30}H_{50}O$. According to[16] α-amyrin compounds have the ability to stimulate the increasing of Human Keratinocytes (HaCaT) proliferation in the speed up to 18% so that it can be an option for use in the cosmetics industry as a wound healing agent and accelerate the skin regeneration.

Compound 2

Compound 2 is white crystal, positive for a steroid test with the Liebermann-Burchard reagent. Melting point 137-140 ºC. The IR spectrum of compound 2 shows the characteristic absorption at wavenumber 3425.56 cm^{-1} for OH which was strengthened by the presence of absorption at 1056.99 cm^{-1} by the C-O group. The absence of absorption in the area of about 1700 cm^{-1} which is typical for C=O indicating that the molecule does not contain a carboxylic group. The absorption band at 2958.8 cm^{-1} is C-H stretching of the alkane where the absorption bands at 2935.66 and 2866.8 cm^{-1} are C-H stretching of the CH_3 and CH_2 groups which are amplified by the absorption band at 1463.978 cm^{-1} which are makeup C-H bending of CH_2 group and the absorption at 1377.17 cm^{-1} is C-H bending of the CH_3 group. The absorption band at 1645.28 cm^{-1} is the absorption of the C-C stretching from the C=C bond. This absorption frequency is identical to the absorption frequency of β-sitosterol[17]. The ^{13}C-NMR spectrum of compound 2 shows that the compound has 29 carbon atoms, and the DEPT-135 data it is known that the 29 carbon atoms consist of six CH_3, eleven CH_2, eight CH, two quarterner carbon and two carbon to form one double bond. Signals at δ 140.87 and δ 121.85 ppm are C-5 and C-6 atomic signals that form double bonds[5]. The signal at δ 71.93 ppm is a signal from the carbon atom that binds to the hydroxyl group. Signals that appear at δ 19.97 δ 19.54 δ 19.17 δ 18.92, 12.13 and δ 12.00 ppm are signals of the methyl group. Signal at δ 42.41, δ 39,91 δ 37,39 δ 34,07 δ 32,05 δ 31,77 δ 28,39 δ 26,18 δ 24,45 δ 23,19 and δ21,22 ppm is signal of methylene carbon, and the signals at δ 121.85 δ 71.93 δ 56.89 δ 56.18 δ 50.25 δ 45.96 δ 36.29 δ 32.03 and δ 29.27 ppm are signals of methine carbon and signals in δ 42.45 δ 36.64 ppm are signal of quarterner carbons. The ^{1}H-NMR spectrum shows the signal of methyl protons at δ 1.01 (3H, H-19), 0.94 (3H, H-26) 0.85 (3H, H-29) 0.84 (3H, H- 21), 0.82 (3H, H-18), and 0.68 (3H, H-27). The signal at δ 2.26 (2H, H-4) shows 2 H atoms in C-4. The signal at δ 3.53 is the signal of proton hydroxyl group (H-3) and the signal at 34 5.34 ppm is the proton that is bound to olefin carbon (H-6). The chemical shift of the proton above is identical to the chemical shift of the hydrogen and carbon of β-sitosterol[17,18]. The molecular structure of compound 2 similar to the molecular structure of β-sitosterol is supported by HSQC and HMBC spectrum data. Table 2. shows the chemical shifts of proton and carbon of compound 2 with the β-sitosterol which has the formula $C_{29}H_{50}O$. The molecular structure of isolate compound 1 (α-amyrin) and 2 (β-sitosterol) are presented in Figure 1.

Completion of the linear regression equation in the Graph in Figure 2, yielding IC_{50} values of 804.762 ± 0.22 μg/mL for α-amyrin and 353.871 ± 0.53 μg/mL for β-sitosterol.

Table 1: The NMR spectrum of compound 1 and α-amyrin[15] in $CDCl_3$

No	^{1}H-MRδ(∑H,m,J in Hz)		^{13}C-NMR		DEPT-135	HMBC
	Compound 1	α-amyrin[15]	1	[15]		
1	1.68(2H,m)	-	38.93	38.7	CH_2	C-2, C-10, C-5
2	2.03(2H,m)	-	28.25	28.7	CH_2	-
3	3.22(1H,dd,J=4.8,11.2)	3.16 (1H,dd,J=5.1,11.2)	79.19	79.6	CH	C-1,C-2,
4	-	-	38.73	38.7	Cq	-
5	0.74 (1H,d,J=11.8)	0.67(1H,d,J=11,6)	55.32	55.1	CH	C-6, C-10
6	1.57(2H,m)	-	18.53	18.4	CH_2	-
7	1.36(1H,m)	-	33.08	32.2	CH_2	-
8	-	-	40.15	40.7	Cq	-
9	1.54(1H,m)	-	47.85	47.7	CH	C-10
10	-	-	37.04	36.6	Cq	-
11	1.91(2H,m)	-	23.52	23.3	CH_2	C-12, C-13
12	5.12 (1H,t,J=3.8)	5.06 (1H,t,J=3.2)	124.55	124.4	=CH	C-9, C-11, C-14, C-18
13	-	-	139.72	139.5	C=C	-
14	-	-	42.22	42.0	Cq	-
15	1.61 (2H,t,J=4,2)	1.94 (2H,td,J=4.5,13.5)	27.42	27.2	CH_2	-
16	1.83 (2H,t,J=4.9)	1.76 (2H,td,J=5.0;13.5)	26.76	26.6	CH_2	C-14
17	-	-	33.9	33.7	Cq	-
18	1.31(1H,s)	-	59.19	59.0	CH	C-12, C-20
19	1.36(1H,m)	-	39.81	39.6	CH	C-20
20	0.87(1H,m)	-	39.76	39.6	CH	-
21	1,39(2H,m)	-	31.41	31.2	CH_2	-
22	1,41(2H,t,J=10.1)	1.85(2H,dt,J=3.0;7.0)	41.68	41.5	CH_2	C-17, C-20
23	0.99(3H.s)	0.93(3H,s)	28.28	28.1	CH_3	-
24	0.95(3H,s)	0.74(3H,s)	15.79	15.6	CH_3	C-5
25	0.96(3H,s)	0.73(3H,s)	15.84	15.6	CH_3	-
26	1.01(3H,s)	0.89(3H,s)	17.01	16.8	CH_3	C-14, C-8, C-9
27	1.07(3H,s)	1.01(3H,s)	23.42	23.2	CH_3	C-8, C-14, C-13
28	0.8(3H,s)	0.94(3H,s)	28.91	28.1	CH_3	C-17,C-22, C-18
29	0.79 (3H, d,J=3.45)	0.85 (3H,d,J= 6.0)	17.63	17.4	CH_3	C-20, C-18
30	0.91(3H,d,J=5.8)	0.73(3H,d,J=7.0)	21.57	21.4	CH_3	C-20, C-21

Table 2: The NMR spectrum of compound 2 and β-sitosterol[18] in $CDCl_3$

No	^{1}H-NMR δ(∑H,m,J in Hz)		^{13}C-NMR		DEPT-135	HMBC
	Compound 2	β-sitosterol[18]	2	[18]		
1	1.07(2H,m)	-	37.38	37.5	CH_2	C-3, C-5, C-9
2	1.95(1H,m)	-	31.77	31.9	CH_2	C-3, C-1
3	3.52(1H,dd,J=6.4;4.7)	3.53 (1H,tdd,J=4.2,3.8)	71.93	72.0	CH	C-1, C-4
4	2.28(2H,m)	-	42.41	42.5	CH_2	C-3, C-5, C-10
5	-	-	140.87	140.9	Cq	
6	5.34 (1H,t,J-4.5)	5.36(1H,t,J=6.4)	121.85	121.9	=C-H	C-4, C-8, C-10
7	1.84(2H,m)	-	32.05	32.1	CH_2	C-5, C-8
8	1.44(1H,m)	-	32.03	32.1	CH	C-6, C-7,C-14
9	0.93(1H,s)	-	50.25	50.3	CH	
10	-	-	36.64	36.7	Cq	
11	1.49(2H,m)	-	21.22	21.3	CH_2	C-11, C-12
12	1.98(2H,m)	-	39.91	39.9	CH_2	C-19
13	-	-	42.45	42.6	Cq	-
14	0.99(1H,s)	-	56.89	56.9	CH	C-9, C-15, C-17
15	1.57(2H,m)	-	24.45	26.3	CH_2	C-14, C-17
16	0.90(2H,m)	-	28.39	28.5	CH_2	C-15, C-17
17	1.13(1H,m)	-	56.18	56.3	CH	C-19, C-21
18	0.80(3H,s)	0.68 (3H,s)	18.92	19.0	CH_3	C-1, C-10, C-11
19	0.84(3H,s)	1.01(3H,s)	11.99	12.0	CH_3	C-17
20	1.35(1H,m)	-	36.29	36.3	CH	C-11, C-17
21	1.00(3H,d,J=7.4)	0.93(3H,d,J=6.5 Hz)	19.17	19.2	CH_3	-
22	1.01(2H,m)	-	34.07	34.2	CH_2	C-20, C-23
23	1.15(2H,m)	-	26.18	26.3	CH_2	C-24, C-25
24	0.66 (1H,m)	-	45.96	46.1	CH	C-25, C-28
25	1.66(1H,m)	-	29.27	29.4	CH	C-24, 26, C-27
26	0.83(3H,d,J=5.6)	0.83(3H,d,J=6.4)	19.97	20.1	CH_3	-
27	0.81 (3H,d,J=5.6)	0.81(3H.d,J=6.4)	19.54	19.6	CH_3	C-26
28	1.25(2H,m)	-	23.19	23.3	CH_2	C-29
29	0.85(3H,t,J=7.9)	0.84(3H,t,J=7.2)	12.13	12.2	CH_3	C-24

Table 3: The IC_{50} of isolate compounds

Compounds	1 (α-amyrin)	2 (β-sitosterol)
IC_{50} (µg/mL)	804.762 ± 0.22	353.871 ± 0.53

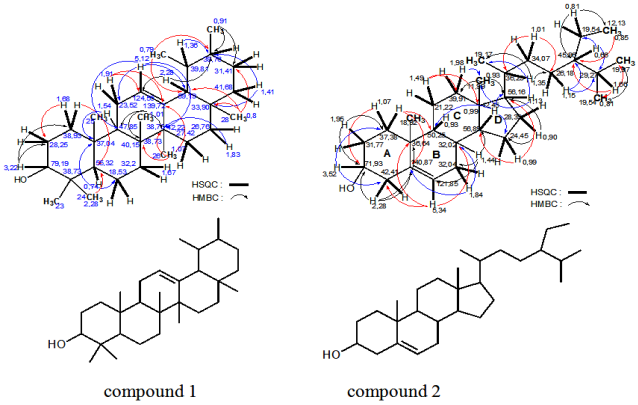

Figure 1: The molecular structure of isolate compound 1 (α-amyrin and 2 (β-sitosterol)

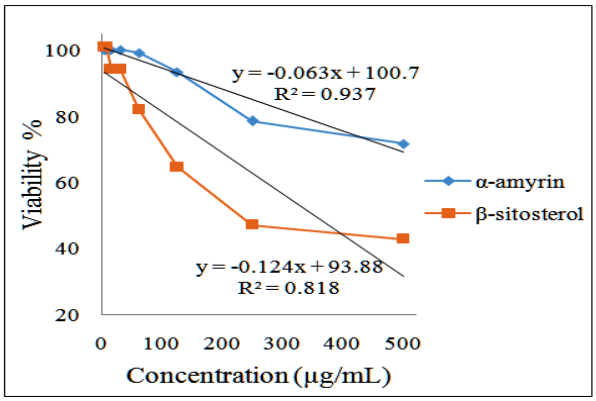

Figure 2: Graph of the relationship between the concentration of the isolate compounds vs viability % of HeLa cell line

CONCLUSION

Two compounds have been isolated and purified in this investigation which was α-amyrin (1) and β-sitosterol (2). The α-amyrin compound from the bark of this plant became firstly reported due to the fact that no past literature precedent has been found so far. However, these α-amyrin and β-sitosterol have been isolated from the root of *R. mucronata* from Andaman and Nicobar Island of India[19]. The result of the cytotoxic test showed that α-amyrin and β-sitosterol compounds have an IC_{50} value of 804.762 ± 0.22 µg/mL and 353.871 ± 0.53 µg/mL respectively which according to U.S. National Cancer Institute[20] both compounds were designated as weak category. Hence, cytotoxic effects of a compound mainly depend on several factors, such as: chemical structure, concentration and also on the type of cancer[21]

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