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Research Article

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Cytotoxic, antibacterial and antioxidant activity of triterpenoids from *Kopsia singapurensis* Ridl.

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ABSTRACT

*Kopsia singapurensis Ridl. is one of the species of the genus Kopsia, also called white kopsia or locally known as 'selada' which about 18 species were distributed in Malaysia. This species is endemic only to Peninsular Malaysia and Singapore. This plant is known to produce a large number of biologically active compounds. In the present study, six known compounds were obtained from the hexane crude extract from the leave and bark of Kopsia singapurensis and identified as: lupeol **1**, lupeol acetate **2**, β-amyrin **3** β-amyrin acetate **4,** β-amyrone **5** and stigmasterol **6**. The structures of these compounds were elucidated by combination of various spectroscopic methods such as MS, UV, IR, 1D and 2D NMR, involving also comparison with data from the literature. All the isolated compounds exhibited cytotoxic effects against MCF-7 cell line, compound **6** was the most active compared to the rest of the compounds in the cytotoxic activity with IC_{50}= 14.5µg/mL, while all tested compounds showed no significant on antibacterial (gram-positive bacteria, Bacillus cereus) and antioxidant activity using the DPPH method.*

Key words: *Kopsia singapurensis* Ridl, cytotoxicity assay, antibacterial activity, antioxidant activity

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INTRODUCTION

Plants of the genus *Kopsia* is classified as member of the tribe Vinceae in subfamily Rauvolfioideae. The genus *Kopsia* (belonging to Apocynaceae family) comprises 30 species, distributed mainly over Southeast Asia, China and India [1]. *Kopsia singapurensis* Ridl.; one of the species of the genus *Kopsia*, also called white *kopsia* or locally known as 'selada' which about 18 species were distributed in Malaysia. In Singapore, this tree can be found and protected in the Nee Soon Swamp Forest of the Central Catchment's Nature Reserve [2]. This species is endemic only to Peninsular Malaysia and Singapore and rich in indole alkaloids [1-3]. Previous studies [4-6] have been reported that the alkaloids obtained from the plants in the family of Apocynaceae have antioxidant and anticancer properties. Various medicinal uses of this genus have been reported; the roots of *K. larutensis* King & Gamble, *K. macrophylla* Hook f., *K. singapurensis* Ridl. and *K. pauciflora* Hook f. were used to treat poulticing ulcerated noses in tertiary syphilis [7]. However, the isolation of terpenoids was only reported from the species *K. fruticosa* [8] and *K. longiflora* [9]. There is no report on the isolation of terpenoids from this species in the literature to date.

The purpose of this study is to isolate and identify the chemical compounds from the leaves and bark of *K. singapurensis* and to determine the bioactivities of the isolated compounds. In this paper, we report the isolation and characterization of known compounds from *K. singapurensis* namely lupeol **1**, lupeol acetate **2**, β-amyrin, **3** β-amyrin acetate **4,** β-amyrone **5** and stigmasterol **6**. The isolated compounds were then tested for cytotoxic activity by

using MCF-7 cell line, antibacterial activity, *Bacillus cereus* was selected for this activity and antioxidant inhibitory activity (standard DPPH assay).

EXPERIMENTAL SECTION

General Methods

Nuclear Magnetic Resonance spectra (NMR) were recorded in deuterated chloroform ($CDCl_3$) on a BRUKER 400MHz, BRUKER 600MHz and JEOL 500MHz. Chemical shifts (δ) were expressed in ppm and the coupling constants (J) are given in Hz. Mass spectra (MS) were measured by using the GC-mass spectroscopy (GC-MS Agilent 5975 Series). The infrared spectra (IR) were recorded on a Nicolet 6700 FTIR spectrophotometer, with CH_2Cl_2 as dilution solvent of the sample. The ultra violet spectral was obtained in dichloromethane (CH_2Cl_2) on a Perkin Elmer UV- visible spectrophotometer and the wavelength of the spectrum was recorded in the range of 200 to 400nm. Solvent used for diluted the sample was CH_2Cl_2.

Column chromatography were prepared by using Silica Gel 60F, 70-230 mesh ASTM and, 230-400 mesh ASTM and Silica Gel 60 containing Gypsum F_{254} as stationary phase. Analytical thin layer chromatography (TLC) was performed on commercially precoated aluminium supported silica gel $60F_{254}$ TLC sheets.

Reagents

10.0% solution of sulphuric acid was used as a spraying reagent to detect the presence of terpenoids spotting on the TLC plates. A positive result was indicated by the formation of purple spot on the TLC plates once the plate was heated to 110ºC for 5 minutes.

Plant Material

The species selected for the current study is *Kopsia singapurensis* Ridl. (KL5334) collected from Kluang, Johor and identified by the phytochemical group, Chemistry Department, Faculty Science, University of Malaya. The Voucher specimens were deposited at the Chemistry Department, Faculty Science, University of Malaya, Kuala Lumpur, Malaysia and the Herbarium of the Forest Research Institute, Kepong, Malaysia.

Extraction and Isolation

The dried ground leaves (2.5kg) and barks (3.3kg) of *Kopsia singapurensis* were defatted separately with hexane by Soxhlet extractor for 17 hours. Then, the extracts were dried on the rotary evaporator. For preliminary fractionation, the both hexane crude extracts were subjected to column chromatography over silica gel and eluted with different combinations solvent systems of hexane, dichloromethane and methanol mixture by increasing its polarity.

Eluents were collected in fractions and were concentrated using rotary-evaporator. Then, each concentrated fraction was analyzed by using aluminium supported TLC plate with a suitable solvent system. After the development of the TLC, 10% sulphuric acid as spraying agents, the fractions having spots with same retention factor (R_f) value were grouped together. Each grouped fraction was treated separately by extensive column chromatography or preparative TLC for further purification process.

Preparation of the Bioactivity

Cell Culture and MTT Cytotoxicity Assay

A preliminary screening against cancer cells was performed using the Microculture Tetrazolium (MTT) method [10] against MCF-7 cell line. The MCF-7 used in this study was obtained from the American Type Cell Collection (ATCC). The cell was cultured using RPMI 1640 culturing media (PAA, Leverkusen, Germany). Besides, cell was trypsinized and counted using hemocytometer and plated in a microtiter plate of 96-wells. After an overnight incubation to allow cell attachment, the medium was changed and 0.2 mL of new supplemented medium was added into each well. Cells were then treated with different drug concentrations and incubated at 37 °C, 5% CO_2 for 24 hours. Each concentration of the samples was assayed in triplicate. The colorimetric assay was performed at an absorbance of 570 nm. Results were expressed as a percentage of control giving a certain percentage of cell viability after 24 hour exposure to the test agent. The potency of cell growth inhibition for test agent was expressed as an IC_{50} value [11].

Antibacterial Activity

A gram positive bacterium, *Bacillus cereus* was selected for the *in vitro* antibacterial activity. The strain was stored in the appropriate medium before use. Antibacterial activity was determined by the disc diffusion method [12] with slight modifications in terms of sample concentration, volume of sample loaded and use of paper discs. The bacteria were cultured at 37 °C for overnight in nutrient broth and potato dextrose broth, respectively. The concentrations of the cultures were adjusted turbidometrically at a wavelength of 600 nm which gave 10^5–10^6 colony forming units

(CFU) per mL. The compounds to be tested were dissolved in dimethyl sulphoxide (DMSO) at concentration of 1 mg/mL. About 10 µL of each sample solution was loaded on Whatman No. 1 filter paper disc (0.6 mm). The disc was placed on the surface of the agar plate (nutrient agar or potato dextrose agar) previously inoculated with bacteria. The agar plates were then inverted and incubated for 24 hours at 37 °C. The antimicrobial activity was recorded by measuring the zone of inhibition (IZ) in mm around each disc, against the test organisms. The experiments were repeated in triplicate and the results were expressed as average values. The antibiotic streptomycin sulfate (10 µg/disc) was used as positive control and DMSO as negative control in the assay [11].

Antioxidant Inhibitory Activity

In this activity, the standard 2, 2-diphenyl-1-picrylhydrazyl (DPPH) assay was used with slight modification. In this technique, 96 micro-well flat bottom plates were used instead of cuvettes to consume minimal amounts of sample, vehicle and DPPH solutions. Each compound was prepared at 1 mg/mL in DMSO. Each well was filled in with 100 µL of diluent (DMSO). The first row (row A) of the 96 wells was filled in with 100 µL of diluted sample at 1 mg/mL. Concentration in the first row was 500 µg/mL of sample in each well and was serially diluted (two fold dilution) in 96 micro-well plates to varying concentrations, topping from 500 µg/mL to the lowest 7.8 µg/mL. Then, 5 µL of the DPPH solution (2.5 mg/mL in DMSO) was added to each well. The DPPH solution should be kept in the dark at 4°C. This procedure should not be done under direct light. The plate was shaken to ensure thorough mixing before placed in the dark. After 30 minutes, the optical density of each well was read using ELISA Reader (EL340 Biokinetic Reader, Bio-Tek Instrumentation) at wavelength 517 nm. Percentage inhibition was calculated using the following formula:

% Inhibition = 1 − OD(DPPH + Sample)/OD(DPPH) × 100

The IC_{50} value was determined as the concentration of each sample required or able to scavenge 50% of the DPPH. All tests and analyses were run in triplicate and averaged. Antioxidant assay by using DPPH method was used to assess the capacity of the compounds to scavenge stable free radical DPPH at different concentration. The radical scavenging effect was examined and compared with natural antioxidants a-tocopherol (Sigma, USA), ascorbic acid (Sigma USA), and quercetin (Sigma, USA) used as positive controls (Table 1) [12].

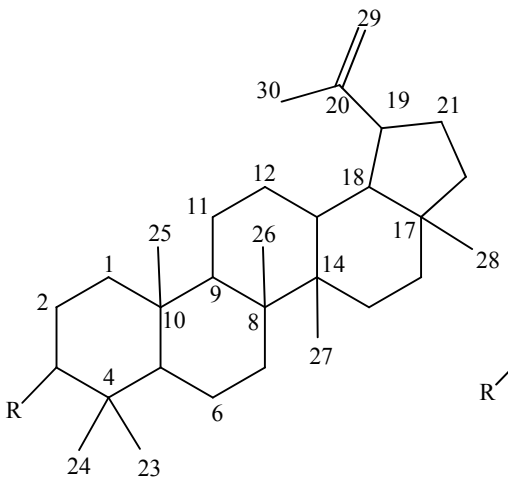
Table 1: The IC_{50} values of the standard against DPPH (free radical).

Standard	**IC_{50}(µg/mL)**
Ascorbic acid	20 ±1.2
α-tocopherol	60 ±2.9
Quercetin	40 ±5.8

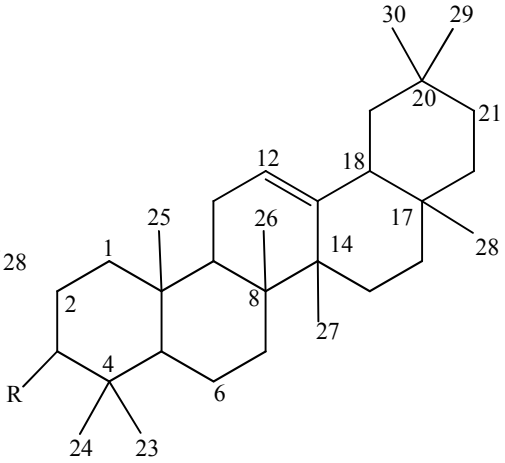
RESULTS AND DISCUSSION

Repeated chromatographic separation and purification of the hexane crude extracts of the leaf and bark of *Kopsia singapurensis* separately provided a total of six compounds (compound **1**, **2**, **3**, **4, 5** and **6**), the structure of the compounds were determined by ^{1}H and ^{13}C NMR spectral analysis as well as by comparison of their spectral data with previously reported values (Table 2 and 3). Compound **1** and **2** were belongs to lupane type triterpenoids while compound **3, 4** and **5** were belongs to oleanane type triterpenoids. Compound **6** was identified as stigmasterol from its physical constants and spectral data.

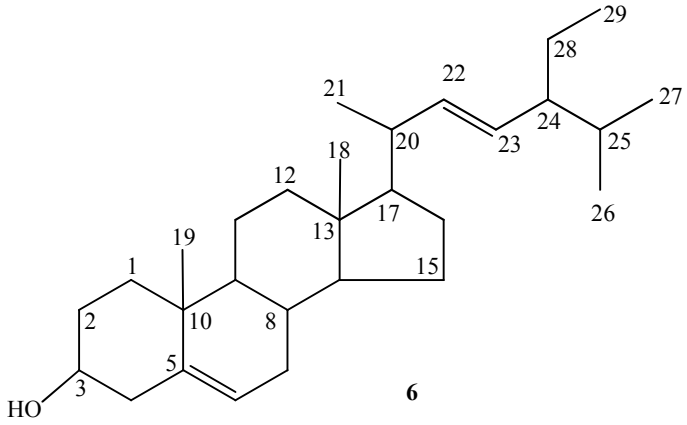
Compound **1** is a pentacyclic triterpenoid. It was obtained as white amorphous. In the GC-MS, the molecular formula $C_{30}H_{50}O$ was established from the molecular ion peak at m/z 426$[M]^{+}$. The IR spectrum of **1** displayed stretching band for O-H (3325cm^{-1}), olefin (1704cm^{-1}) and C-O (1204cm^{-1}) and the UV spectrum revealed the maxima absorption at 229.9 and 206.2nm. The ^{1}H NMR (500 MHz, CDCl3) spectrum showed seven tertiary methyl singlets (H-23, 24, 25, 26, 27, 28 and 30) and a proton of H-3 appeared as double doublet (*dd*) at δ 3.52 corresponding to hydroxyl methine proton. It also showed olefinic protons at δ 4.56 (H-29, *d*, *J* = 3.04Hz) and 4.68 (H-29, *d*, *J* = 2.32Hz). ^{13}C NMR (500 MHz, $CDCl_3$) spectrum of the compound showed 30 carbon signals for the triterpenoid of lupine skeleton which was represented by seven methyl groups. The carbon bonded to the hydroxyl group C-3 appeared at δ 79.1, while the two olefinic carbons appeared at δ 151.0 (C-20) and δ 109.3 (C-29). The spectra were compared with published data of Mouffok et al. works [13].



1 R: OH
2 R: COOH



3 R: OH
4 R: COOH
5 R: $OCOCH_3$



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Compound **2** was isolated as white amorphous. This compound was found to be a lupane type triterpenoid, which is having similar skeleton with compound **1**. The mass spectrum of compound **2** exhibited a molecular ion peak at m/z 468 $[M]^+$ corresponding to its molecular formula $C_{32}H_{52}O_2$. The IR spectrum displayed an ester carbonyl and a C-O stretching band at 1712cm^{-1} and 1264cm^{-1}. The UV spectrum showed maxima absorption at 277.6nm. The ^{1}H NMR spectrum (400 MHz, $CDCl_3$) showed the presence of eight tertiary methyl singlets at δ 0.84(H-23), δ 0.83 (H-24), δ 0.85 (H-25), δ 1.03 (H-26), δ 0.94 (H-27), δ 0.78 (H-28), δ 1.68 (H-30) and δ 2.05 (H-32). Two protons appeared at δ 4.57 (*d, 2.0Hz*) and 4.69 (*d, 2.0Hz*) as doublet, representing the exocyclic double bond protons H-29a and H-29b, respectively. The ^{13}C NMR (100 MHz, $CDCl_3$) spectrum of compound 2 has a total of 32 carbon atoms. Compound **2** has an ester carbonyl signal at δ 171.1 (C-31), C-3 at δ 81.2 and the alkene carbons at δ 151.20 and 109.6. The identity of 2 as lupeol acetate was confirmed by comparison published values of Ajithabai, Sreedevi, Jayakumar, Mangalam, Deepa & Sunitha, (2011) [14, 15].

Compound **3** was isolated as white amorphous. The UV spectrum revealed the maximum absorptions at 273.4 and 229.0nm indicating the presence of a triterpenoid with carbon C-12 unsaturated oleanane skeleton [16]. The IR spectrum showed the band at 3360cm^{-1}, 1790cm^{-1} and 1294cm^{-1} which were assigned to functional groups for O-H, C=C and C-O, respectively. The molecular formula of **3** was established as $C_{30}H_{50}O$ from its quasi-molecular ion peak at m/z 449 $[M+Na]^+$ in the GC-MS analysis. The ^{1}H NMR spectrum (400 MHz, $CDCl_3$) displayed the eight tertiary methyl singlets at δ 0.98(H-23), δ 0.92 (H-24), δ 0.77 (H-25), δ 1.96 (H-26), δ 1.12 (H-27), δ 0.81 (H-28), δ 0.85 (H-29) and δ 0.85 (H-30). A signal at δ 3.20 (*dd, J = 11.0, 5.0 Hz*) was assigned to H-3 whereas another downfield signal, δ 5.16 (*t, J = 3.6Hz*) was attributed to the olefinic proton (H-12). The ^{13}C NMR spectrum (100 MHz, $CDCl_3$) showed a total of 30 carbon atoms. Compound **3** has a hydroxyl carbon signal at δ 79.1. Besides, the signal at δ 121.8 and δ 145.3 were assignable to C-12 and C-13 (olefinic carbon). The above spectral features are in close agreement to those observed for β-amyrin in literature [17].

Table 2: ^{1}H NMR spectroscopic data for compound 1-6 [400,500,600 MHz,δH (J, Hz), $CDCl_3$]

Position	**1**	**2**	**3**	**4**	**5**	**6**
1	0.87 *(m)*	0.99 (m)	1.61 *(m)*	1.64 *(m)*	1.85 (m)	1.07 *(m)*
	1.66 (m)		0.98 (s)	1.04 (m)	1.36 (m)	1.83 (m)
2	1.54 (m)	1.62 (m)	1.59 (m);	1.88 (m)	2.33 (m)	1.52 (m)
	1.60 (m)		0.77 (m)	1.60 (m)	2.50 (m)	1.83 (m)
3	3.18 (*dd* , 11.5; 5.1Hz)	4.47 *(dd* , 10.7; 5.5Hz*)*	3.20 *(dd,* 11.0; 5.0Hz*)*	4.50 (*t* , 8.0Hz)		3.52 *(m)*
4						2.23 *(m)*
						2.28 (*dd* 4.6Hz; 1.7Hz)
5	0.68 *(m)*	0.79 *(m)*	0.73 *(m)*	0.84 *(m)*	1.28 (*m*)	
6	1.38 *(m)*	1.50 *(m)*	1.52 *(m)*	1.53 *(m)*	1.46 (*m*)	5.35 (*br, s*)
	1.52 *(m)*	1.38 *(m)*	1.38 *(m)*	1.40 *(m)*		
7	1.38 *(m)*	1.49 *(m)*	1.49 *(m)*	1.52 *(m)*	1.35 (*m*)	1.46 *(m)*
		1.38 *(m)*	1.31 *(m)*	1.33 *(m)*	1.50 (m)	1.99 *(m)*
8						1.52 *(m)*
9	1.29 *(m)*	1.28 *(m)*	1.54 *(m)*	1.58 *(m)*	0.91 (*m*)	0.92 *(m)*
10						
11	1.25 *(m)*	1.39 *(m)*	1.86 *(m)*	1.80 *(m)*	1.87 (*m*)	1.52 *(m)*
	1.38 *(m)*	1.21 *(m)*		1.63 *(m)*		
12	1.07 *(m)*	1.66 *(m)*	5.16 (*t,* 3.6Hz)	5.18 (*t* , 3.5Hz)	5.20 (*t* , 3.6Hz)	1.15 *(m)*
	1.68 *(m)*	1.05 *(m)*				2.02 *(m)*
13	1.68 *(m)*	1.65 *(m)*				
14						0.99 *(m)*
15	1.33 *(m)*	1.67 *(m)*	1.74 *(m)*	1.76 *(m)*	1.08 (*m*)	1.02 *(m)*
	1.68 *(m)*	1.00 *(m)*	0.95 *(m)*	0.85 *(m)*	1.74 (m)	1.58 *(m)*
16	1.47 *(m)*	1.47 *(m)*	1.97 *(m)*	1.98 *(m)*	0.78 (*m*)	1.25 *(m)*
		1.36 *(m)*		0.79 *(m)*		1.68 *(m)*
17						1.07 *(m)*
18	1.38 *(m)*	1.35 *(m)*	1.94 *(m)*	1.93 (*d* , 4.2Hz)	1.92 (*m*)	0.68 (*s*)
19	2.38 *(m)*	2.40 *(m)*	1.64 *(m)*	1.66 *(m)*	1.61 (*m*)	1.00 (*s*)
			1.01 *(m)*	1.00 *(m)*		
20						2.02 *(m)*
21	1.33 *(m)*	1.91 *(m)*	1.31 *(m)*	1.32 *(m)*	1.05 (*m*)	1.18 (*d,*
	1.91 *(m)*	1.33 *(m)*	1.09 *(m)*	1.08 *(m)*	1.31 (m)	5.7Hz)
23	0.96 (*s*)	0.84 *(s)*	0.98 *(s)*	0.88 *(s)*	1.08 (*s*)	5.00 (*dd* , 15.5Hz; 9.2Hz)
24	0.76 (s)	0.83 *(s)*	0.77 *(s)*	0.96 *(s)*	1.04 (*s*)	1.52 *(m)*
25	0.83 (s)	0.85 *(s)*	0.92 *(s)*	0.86 *(s)*	1.05 (*s*)	1.50 *(m)*
26	1.03 (*s*)	1.03 *(s)*	0.95 *(s)*	0,97 *(s)*	1.00 (*s*)	0.85 (*d* ,
27	0.94 (*s*)	0.94 *(s)*	1.12 *(s)*	1.13 *(s)*	1.13 (*s*)	0.79 (*d* ,
28	0.78 (*s*)	0.78 *(s)*	0.81 *(s)*	0.83 *(s)*	0.83 (*s*)	1.00 *(m)*
29	4.56 (*d,* 3.0Hz)	4.69 *(d,* 2.0Hz*)*	0.85 *(s)*	0.88 *(s)*	0.86 (*s*)	0.80 (*t,* 6.9Hz)
	4.68 (*d* , 2.3Hz)	4.57 *(d,* 2.0Hz*)*				
30	1.68 (*s*)	1.68 *(s)*	0.85 *(s)*	0.87 *(s)*	0.86 (*s*)	-
OAc	-	2.05 *(s)*	-	2.05 *(s)*		-

Compound **4** was obtained as colorless needles. This compound was found to be an oleanane type triterpenoid, which is having same skeleton with compound **3**. The IR spectrum displayed stretching band for C=O (1712cm^{-1}), and C-O (1264cm^{-1}). The UV spectrum showed maxima absorption at 277.8 and 229.7nm. The GC-MS of **4** gives an ion peak at m/z 468 $[M]^+$ corresponding to the molecular formula of compound of $C_{32}H_{52}O_2$. The ^{1}H NMR spectrum (400 MHz, $CDCl_3$) indicate the eight tertiary methyl singlets which is almost same with compound **3** except for the presence of acetoxyl group at δ 2.05 (H-32). A signal appear at downfield shift, δ 4.50 (t, J = 8.0 Hz),

compared with the corresponding proton in compound **3**. The ^{13}C NMR (100 MHz, $CDCl_3$) spectrum indicated a total of 32 carbons where the two olefinic carbon signals at δ 121.8 and δ 145.3, an acetoxyl group attached to C-3 (δ 81.0) with two carbon signals at δ 171.1 (C-31) and δ 21.4 (C-32). These spectra features are in close agreement to those observed for β-amyrin acetate by Segovia, et al. (2011) [18]. On this basis, the identity of compound **4** was confirmed as β-amyrin acetate.

Table 3: ^{13}C NMR spectroscopic data for compounds 1-6 [100, 125 MHz, δC, $CDCl_3$]

Position	**1**	**2**	**3**	**4**	**5**	**6**
1	38.8	38.4	38.7	38.3	39.4	37.3
2	27.5	23.7	27.3	23.5	24.3	31.7
3	79.1	81	79.1	81	218	71.9
4	38.9	37.8	39.9	39.8	47.6	42.4
5	55.3	55.4	55.3	55.2	55.4	140.8
6	18.4	18	18.5	18.3	19.7	121.8
7	34.3	34.2	32.7	32.6	32.2	31.9
8	40.8	40.9	38.9	37.7	39.9	31.9
9	50.5	50.3	47.7	47.6	46.9	50.2
10	37.2	37.1	37	36.8	36.8	36.6
11	21	20.9	23.6	23.6	23.7	21.2
12	25.2	25.1	121.8	121.6	121.6	39.8
13	38.1	38	145.3	145.2	145.4	42.3
14	42.9	42.8	41.8	41.7	41.9	56.8
15	27.5	27.4	26.2	26.1	26.2	24.4
16	35.7	35.6	27	26.9	27	29
17	43.1	43	32.6	32.5	32.6	56
18	48.4	48.3	47.2	47.2	47.4	12
19	48.1	48	46.9	46.8	46.8	19.5
20	151	151	31.2	31.1	31.2	40.6
21	29.9	29.8	34.8	34.7	34.8	21.2
22	40.1	40	37.2	37.1	37.2	138.3
23	28.1	28	28.2	28	26.5	129.3
24	15.5	16.5	15.7	15.6	21.6	51.3
25	16.2	16.2	15.6	16.7	15.3	32
26	16.1	16	16.9	16.8	16.8	21.2
27	14.6	14.5	26.1	26	26	19.1
28	18.1	18.2	28.5	28.4	28.5	25.4
29	109.3	109.4	33.5	33.4	33.4	12.1
30	19.4	19.3	23.8	23.7	23.8	-
31	-	171.1	-	171.1		-
32	-	21.3	-	21.4		-

Compound **5** was obtained as white amorphous. This compound was also found to be an oleanane type triterpene, which is having the same skeleton with compound **3** and **4**. The elucidation of compound **5** was then supported by the detail analysis of 1D and 2D NMR, also the GC-MS analysis which exhibited a molecular ion peak at m/z 424 $[M]^+$ in agreement with molecular formula $C_{30}H_{48}O$. The IR spectrum of **5** was almost similar to compound **3** but differ in the presence of carbonyl group at 1709cm^{-1}. The ^{1}H NMR spectrum (400 MHz, $CDCl_3$) indicate the eight tertiary methyl singlets which was almost same with compound **3** and **4.** A downfield signal at δ 5.20 (*t*, J = 3.6Hz) was probably positioned for an olefinic proton at C-12. The ^{13}C NMR (100 MHz, $CDCl_3$) spectrum indicated a total of 30 carbons The present of a carbonyl group of C-3 at δ 218.0 was also observed in agreement with the absorption band at 1709cm^{-1} in the IR spectrum and the two olefinic carbon signals at δ 121.6 (C-12) and δ 145.4 (C-13), Detailed analysis of the spectral data obtained led to the conclusion that compound **5** is β-amyrone after comparison with the literature [19].

Compound **6** was isolated as colourless needles. Its molecular formula was assigned as $C_{29}H_{48}O$ from GC-MS at m/z 413$[M+H]^+$. This compound was found to be a C-5 unsaturated modified tetracyclic triterpenoid, which showed

only 29 carbons instead of 30 carbons for tetracyclic triterpenoids. This was further supported by the UV spectrum which indicated maxima absorption at 276.8nm [16]. The IR spectrum displayed stretching band for O-H (3300 cm^{-1}), olefin (1711 cm^{-1}) and C-O (1263 cm^{-1}). The ^{1}H NMR spectrum of **5** showed characteristic signals for six methyls at δ 0.68 (Me-18, *s*), δ 1.00 (Me-19, *s*), δ 1.18 (Me-21, *d*, *J* = 5.7 Hz), δ 0.85 (Me-26, *d*, *J* = 4.0 Hz), δ 0.79 (Me-27, *d*, *J* = 6.8 Hz) and δ 0.80 (Me-29, *t*, *J* = 6.9 Hz). The proton of H-3 appeared as multiplet at δ 3.52 corresponding to hydroxyl methine proton. The typical signals for olefinic protons at δ 5.35 (H-6, *m*), δ 5.14 (H-22, *dd*, *J* = 15.5 and 8.6 Hz), δ 5.00 (H-23, *dd*, 15.5 Hz and 9.2 Hz). In the ^{13}C NMR spectrum, 29 carbon signals were observed. There are four olefinic carbon signals at δ 140.8 (C-5), δ 121.8 (C-6), δ 138.4 (C-22) and δ 129.3 (C-23). The compound was assigned as stigmasterol based on the physical and spectral data, also comparison with the literature values [15, 20].

All the compounds (**1 - 6**) were evaluated for the cytotoxicity assay agent MCF-7 cell line, antibacterial activity against *Bacillus cereus* and antioxidant activity using DPPH method.

In the cytotoxicity activity, a preliminary screening against cancer cells was performed using the MTT method [10] against MCF-7 cell line. Doxorubicin was used as a positive control in this study. The results showed that the compounds: lupeol **1**, lupeol acetate **2**, β-amyrin **3,** β-amyrin acetate **4,** β-amyrone **5** and stigmasterol **6** exhibited cytotoxic effect against cancer cell line with IC_{50} values ranging from 14.5 – 26.0µg/mL (Table 4). Compound **6**, stigmasterol was the most active compared to the rest of compounds in the cytotoxic activity with the IC_{50} value = 14.5µg/mL.

The preliminary screening results of antibacterial activity against *Bacillus cereus* was summarized in Table 4. Antibacterial activity was indicated by a clear zone of growth inhibition. All the compounds were evaluated for antibacterial activity using disc diffusion method and streptomycin sulfate was used as a positive control in the assay. The results indicated that all the tested isolated compounds were found to be inactive against the gram-positive bacteria, *Bacillus cereus* [21, 22].

Compounds **1 - 6** were tested for the antioxidant assay by using DPPH method. This assay was used to assess the capacity of the compounds to scavenge stable free radical DPPH at different concentration. From Table 4, triterpenoids showed weak antioxidant activity with IC_{50} >500 µg/mL, compare with the standard natural antioxidants a-tocopherol, ascorbic acid and quercetin.

Table 4: Cytotoxic effects against MCF-7 cell line, antibacterial and antioxidant activity on isolated compounds.

Compounds	Cytotoxic	Antibacterial activity	Antioxidant activity
	IC_{50} values (µg/mL)	Diameter of inhibition zone (mm)	IC_{50} values (µg/mL)
	MCF-7	*B.cereus*	DPPH
Lupeol **1**	17	NI	>500
Lupeol acetate **2**	26	NI	>500
β-amyrin **3**	15.5	NI	>500
β-amyrin acetate **4**	22.5	NI	>500
β-amyrone **5**	21.5	NI	>500
Stigmasterol **6**	14.5	NI	>500
*No inhibition (NI)			

CONCLUSION

The phytochemical study was aimed to investigate the chemical constituents of *Kopsia singapurensis* Ridl. (KL 5334) collected from Kluang (Johor) and to determine the bioactivitis of the isolated compounds. Structural elucidation was established by using various modern spectroscopic techniques such as ultraviolet (UV), infrared (IR), nuclear magnetic resonance (NMR) including 1D-NMR (^{1}H, ^{13}C, and DEPT), 2D-NMR (COSY, HSQC, and HMBC) and MS (GC-MS). The study on the leaves and bark of *K. singapurensis* yielded a total of 5 pentacyclic triterpenoid: lupeol **1**, lupeol acetate **2**, β-amyrin **3**, β-amyrin acetate **4,** β-amyrone **5** and a steroid namely stigmasterol **6** which were common occurrence in plant. The structures of the compounds were also elucidated by comparison with literature values. All the isolated compounds exhibited cytotoxic effects against MCF-7 cell line, compound **6** was the most active compared to the rest of compounds in the cytotoxic activity with IC_{50}= 14.5µg/mL, while all tested compounds showed no significant on antibacterial (gram-positive bacteria, Bacillus cereus) and

antioxidant activity using the DPPH method. Based on the previous reports on cytotoxic properties of *Kopsia singapurensis* with that of the present results, it clearly indicates that *Kopsia singapurensis* has potential cytotoxic properties and can be used as a source of antitumor agents.

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