



# Active Cytotoxic Compounds and Essential Oil from *Bougainvillea Alba*

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## Abstract

Chemical analysis of methanol extract of flowers and leaves of *Bougainvillea alba* led to isolation of four compounds identified as 3', 4', 6 - trimethoxy, 5 - hydroxyl 7-prenyl 3-O- (feruloyl (1→2) - rhamnopyranosyl) flavone [1], 3-O β-D-glucopyranosyl oleanolic acid 28-O-β-D-glucopyranosyl (1→3'') β-D-glucopyranosyl (1→3''') α-L -xylopyrenoside [2], Lepidolide [3], E feruloyl 3-O- Coumaroyl α-L Rhamnopyranoside [4]. In addition, two oil fractions, the major identified components of oil are Elemene<β->, Lanceol <cis->, Nerolidol <cis->, Methyl palmitate and Abietatriene. Cytotoxic activity of methanol extract of both leaves and flowers of *Bougainvillea alba*, isolated compounds and oil fractions were determined using the SRB Assay on human tumor cell line (Hepatocyte generation 2, HepG2). The isolated compounds showed cytotoxic activity at LC<sub>50</sub> 15.2, 17.2, 15.6 and 2.82 µg/ml for compound 1, 2, 3 and 4 respectively, while the two oil fraction showed activity at 2.67 µg/ml.

**Keywords:** *Bougainvillea alba*, essential oil, isolation, nyctaginaceae, SRB assay

## 1. Introduction

Cancer is considered one of the most common causes of mortality worldwide. The most common methods for treatment of cancer are chemotherapy, radiotherapy and surgery, most of the above methods exhibit severe toxicity and/or resulting undesirable side effects. There is a long history of medicinal use of plants; it is a most promising site for discovery of novel biologically-active substances [1].

Nyctaginaceae is a family of around 33 genera and 290 species of flowering plants, widely distributed in tropical and subtropical regions. Saponins [2], flavonoids [3, 4] and phenolic acid [5] were isolated from the family.

*Bougainvillea* is a genus of flowering plants native to South America from Brazil to Peru and Argentina. It is a popular ornamental plants, rapid growing and flower all year in warm climate. Flavonoids were found as active principle isolated from the plant leaves where it showed a strong activity on xanthone oxidase inhibition [6]. Glycosides of oleanolic acid and Quercetin were isolated from the plant [7].

This work investigate the cytotoxic effect of methanol extracts of the leaves and flowers of *Bougainvillea alba*, four isolated compounds and two essential oil fractions using the SRB Assay on human tumor cell line HepG2.

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## 2. Material and Methods

### 2.1 Plant Material

Flowers and leaves of *Bougainvillea glabra* 'Alba' (Bunga Kertas) were collected from of Banha Agriculture university gardens, The plant was kindly identified by Mrs. Terasa Labib, General Manager and head of plant Taxonomy in El-Orman Botanical Garden, Giza, Egypt. Voucher specimens (BA-I) were deposited at laboratory of Medicinal Chemistry, Theodor Bilharz Research Institute. The flowers and the leaves of the plant were isolated, dried separately in shade, then finely powder with an electric mill.

### 2.2 Equipment

$^1\text{H-NMR}$  ( $\delta$  [ppm],  $J$ [Hz]) and  $^{13}\text{C-NMR}$  spectra were recorded in  $\text{CD}_3\text{OD}$ , operating at 300 MHz for proton and 75 MHz for carbon 13 spectrometer. Chemical shifts ( $\delta$ ) are reported in parts per million, using TMS as internal standard. Mass spectra were recorded on a finnigan TSQ 700 GC/MS equipped with a finnigan electrospray source (ESI-MS). Paper chromatography sheet [Whatman 1], using 15 % Acetic acid as solvent system, the chromatograms were visualized under UV light (at 254 and 366 nm). Column chromatography was performed using a glass column 120 x 7 cm and using polyamide as a stationary phase.

GC/MS analysis of essential oil was performed with a finnigan mat 7000 gas chromatograph coupled to mass detector. The capillary column used was DB5, 30m x 0.25mm, 0.5  $\mu\text{m}$  film thickness.

Operating conditions: Injector and ion source temperatures 220°C/M interface temperature program 3 min. isothermal at 50°C, then programmed from 59-250°C at 30°C/min Carrier gas: 0.88ml He/min. Ion source 70 e V.

### 2.3 Extraction and Isolation

The flower dry powder (1 Kg) were soaked in 85 % methanol (7 L x 3), reflux for 6 weeks to give 120 g crude methanol extract. The crude methanol extract was successively extracted with petroleum ether, chloroform, and ethyl acetate to give petroleum Ether extract (1.16 gm), chloroform extract (2.03 gm) and ethyl acetate extract (1.43 gm.).

The remaining methanol extract was desalted by dissolving the extract with water then precipitated with methanol. The filtrate evaporated till dry to give 90 gm which was subjected to polyamide column chromatography using elution system water, water: Methanol with different gradients, finally pure methanol [8].

Fractions (500 ml each) were collected; tested using paper chromatography (PC) developed with solvent system 15 % Acetic acid and/or TLC plates. Similar fractions were collected together to give 8 groups [A-H]. Group A was free from compound except traces of sugar. Group B subjected to more purification with organic solvent to give compound 1. Both group C and D separately were purified on silica gel sub column with elution system Chloroform:methanol:water 15:6:1 to give compound 2 and 3. Group E purified on SephadexLH20 sub column with elusion system Methanol: water, 8:2 to give compound 4. Group F give traces of unidentified compounds. Group G & H afforded oil collection which analyzed by GC/MS system.

### 2.4 Identification of Essential Oil

Qualitative identification was based upon matching the resulting mass spectra with those given by data system using the computer mass spectra identification program Amdis 2.0 (NIST, Gaithersburg, USA), retention time as well as comparison with published data<sup>R</sup>. The peak area measured for the total ion current was followed by quantitative determination of the different constituents (Table 2, 3 ) [9].

### 2.5 Measurement of Potential Cytotoxicity (SRB Assay)

The sulforhodamine B (SRB) assay which was developed in 1990 remains one of the widely used methods for in vitro cytotoxicity screening [10]. The assay relies on the ability of SRB to bind to protein components of cells that have been fixed to tissue culture plates by trichloroacetic acid. SRB is a bright-pink amino oxanthene dye with two sulfonic groups that bind to basic amino acid residues under mild acidic conditions, and dissociate under basic conditions. The SRB method has proven to be sensitive, practical and suited to large scale screening applications as well as research.

## 2.6 Human Tumor Cell Lines

They were obtained frozen in liquid nitrogen (-180 °C) from the American Type Culture Collection. The tumor cell lines were maintained in the National Cancer Institute, Cairo, Egypt, by serial sub-culturing.

## 2.7 Chemicals

Dimethylsulphoxide (DMSO), RPMI-1640 medium, Sodium bicarbonate, Trypan blue, Fetal Bovine Serum (FBS), Penicillin/Streptomycin, Acetic acid, Sulphorhodamine-B (SRB), Trichloroacetic acid (TCA), 100 % isopropanol and 70 % ethanol. All chemicals were purchased from Sigma Chemicals Co (St. Louis, Mo U.S.A.).

## 2.8 Buffers

Tris base 10 mM (PH 10.5): It was used for SRB dye solubility. 121.1 gm of tris base was dissolved in 1000 ml of distilled water and PH was adjusted by HCl acid (2 M).

### 2.8.1 Sulphorhodamine-B (SRB) Assay of Cytotoxic Activity

This method was carried out according to that of Skehan et al. [11]. Cells were used when 90 % confluence was reached in T25 flasks. Adherent, cell lines were harvested with 0.025% trypsin. Viability was determined by trypan blue exclusion using the inverted microscope (Olympus 1x70, Tokyo, Japan). Cells were seeded in 96-well microtiter plates at a concentration of  $5 \times 10^4$ - $10^5$  cell/well in a fresh medium and left to attach to the plates for 24 hrs. After 24 hrs, cells were incubated with the appropriate concentration ranges of drugs, completed to total of 200  $\mu$ l volume/well using fresh medium and incubation was continued for 24, 48 and 72 hrs. Control cells were treated with vehicle alone. For each drug concentration, 4 wells were used. Following 24, 48 and 72 hrs treatment, the cells were fixed with 50  $\mu$ l cold 50 % trichloroacetic acid for 1 hr at 4 °C. Wells were washed 5 times with distilled water and stained for 30 min at room temperature with 50  $\mu$ l 0.4 % SRB dissolved in 1 % acetic acid. The wells were then washed 4 times with 1 % acetic acid. The plates were air dried and the dye was solubilized with 100  $\mu$ l/well of 10 mM tris base (ph 10.5) for 5 min on a shaker (Orbital shaker OS 20,

Boeco, Germany) at 1600rpm. The optical density (O.D.) of each well was measured spectrophotometrically at 564nm with an ELIZA microplate reader (Meter tech.  $\Sigma$  960, U.S.A.). The mean background absorbance was automatically subtracted and mean values of each drug concentration was calculated. The percentage of cell survival was calculated as follows:

$$\text{Survival fraction} = \text{O.D. (treated cells)} / \text{O.D. (control cells)}$$

The IC50 values (the concentrations of thymoquinone required to produce 50 % inhibition of cell growth). The experiment was repeated 3 times for each cell line.

## 3. Results

### 3.1 3',4',6 - trimethoxy, 5 - hydroxyl 7-prenyl 3-O- (feruloyl (1 $\rightarrow$ 2) - rhamnopyranosyl) flavone (1)

<sup>1</sup>H NMR :  $\delta$  0.81, 1.08 3.1, 3.12, 3.34, , 3.32, 3.7, 5.02, 5.68, 7.47, 6.76, 7.4, 6.22 6.25, 7.03, 6.58, 7.56, 6.78. <sup>13</sup>C NMR spectra: (Table 1). Positive ESI-MS: m/z 734.89 [M]<sup>+</sup>, m/z 557.06 [M-177]<sup>+</sup>, m/z 411 [M-323]<sup>+</sup>.

### 3.2 3-O $\beta$ -D-glucopyranosyl oleanolic acid 28-O- $\beta$ -D-glucopyranosyl (1 $\rightarrow$ 3'') $\beta$ -D-glucopyranosyl (1 $\rightarrow$ 3''') $\alpha$ -L - xylopyrenoside (2)

<sup>1</sup>H NMR :  $\delta$  0.63, 0.70, 0.8, 0.91, 1.02, 1.18 ,1.29, 3.10, 4.51, , 4.99, 5.11, 5.17, 5.19, <sup>13</sup>C NMR spectra: (Table 1). negative ESI-MS : m/z 1060 [M-2H]<sup>-</sup>, 934.5 [M- (2H + 132)], m/z 771.2 [M- (2H + 294)]. Positive ESI-MS : m/z 776 [M-294]<sup>+</sup>, m/z 614 [ M- 2 x 162 + 132]<sup>+</sup>, m/z 452 [aglycone]<sup>+</sup>.

### 3.3 Lepidolide (3)

<sup>1</sup>H NMR spectrum: at  $\delta$  5.25, 5.36, 5.65, 5.96. <sup>13</sup>C NMR spectra: (Table 1). Negative ESI-MS: m/z 495 [M-H]<sup>-</sup>, m/z 355(M-H-side chain)

### 3.4 E feruloyl 3-O- Coumaroyl $\alpha$ -L Rhamnopyranoside (4)

<sup>1</sup>H NMR spectrum: 1.23 (3H, Br,s, 3H-6'),3.07 (3H, s), 5.2 (1H, Br s, H-1'),6.0 & 6.06 each (d, J=17.7, H- $\alpha$ ), 7.58

**Table 1:** <sup>13</sup>C NMR of compound 1, 2 and 3

Carbon No	Compound 1	Carbon No	Compound 2	Compound 3
2	156.3	1	39.5	23.1
3	132.8	2	23.8	38.9
4	175.0	3	85.0	176.1
5	160.1	4	40.1	122.6
6	132.0	5	55.5	143.6
7	167.5	6	17.18	145.6
		7	33.2	108.6
8	93.0	8	40.0	48.3
9	156.7	9	47.6	38.9
10	101.3	10	39.5	40.7
1'	125.7	11	24.0	30.4
2'	116.2	12	122.2	30.2
3'	145.3	13	143.2	46.1
4'	142.1	14	40.5	50.5
5'	115.8	15	30.8	32.0
6'	124.9	16	23.9	28.8
OMe	56.9,56.0,51.6	17	46.0	49.9
Rhminose		18	40.0	15.0
1''	101.1	19	43.0	28.8
3''	73.9	20	44.1	33.5
4''	71.1	21	31.5	20.0
5''	73.0	22	34.0	145.5
6''	14.4	23	30.2	122.7
Feruloyl		24	18.5	136.9
A	115.6	25	15.7	125.0
B	144.7	26	16.9	162.3
1'''	130.6	27	28.0	20.9
2'''	115.3	28	175.7	188.3
3'''	109.0	29	28.5	10.0
4'''	146.2	30	25.5	16.7
5'''	149.6	Glucose		
6'''	120.0	1'	94.62	
OMe	55.1	2'	76.5	
C=O	168.0	3'	88.7	
Prenyl		4'	72.8	
1''''	23.3	5'	78.2	
2''''	120.0	6'	61.1	
3''''	131.0	Glucose		
4''''	22.6	1''	105.80	
5''''	25.3	2''	77.1	

(Continue)

3''	88.4
4''	73.1
5''	77.0
6''	60.9
Glucose	
1''	105.17
2''	76.5
3''	74.8
4''	70.0
5''	77.2
6''	61.1
Xylose	
1'''	104.59
2'''	73.5
3'''	72.7
4'''	73.7
5'''	70.0

**Table 2:** Percentage composition of flower heads essential oil (fraction 1)

No	Identified compound	M <sup>+</sup>	Rt	Base peak	Area %
1	Methyl tiglate	114	228	55	1.58
2	Ethyl Hexanoate	145	418	43	1.64
3	Ethyl heptanoate	159	626	41	1.38
4	Elemene <β->	189	1375	67	25.26
5	Jasmone <cis->	164	1384	79	1.19
6	Cinnamic acid <trans->	147	1489	103	2.09
7	Nerolidol <cis->	189	1724	41	7.12
8	Cedrol	207	1876	95	0.72
9	Lanceol <cis->	187	2248	93	9.16
10	Bergamotol acetate<[Z]-α-trans>	202	2327	43	2.83
11	Nonodecane <N->	152	2539	57	1.90
12	Heneicosane <N->	152	2931	57	15.63
Identified compounds					70.5
Unidentified compounds					29.5

& 7.62 each (d, J=17.7, H-β). 6.75 (1H, d, J=8.0 Hz), 7.1 (1H, dd, J= 8.0, 1.5 Hz), 7.3 (1H, m) Negative ESI-MS : m/z 487 [M+H]<sup>+</sup>, m/z 457 [M+H - OMe]<sup>+</sup>, m/z 363 [M+H- C<sub>7</sub>H<sub>7</sub>O<sub>2</sub>]<sup>+</sup>.

#### 4. Discussion

Methanol extract of both flowers and leaves of *Bougainvillea alba* was tested on PC chromatogram and

TLC plates. They showed great similarity in number and kind of compounds but flowers showed more number of spots than that of leaves, the methanol extract of the flowers showed higher cytotoxic effect against human tumor cell line (Hepatocyte generation 2). The flowers methanol extract was subjected to chromatographic fractionation to yield four compounds and two oil fractions. The oil fraction showed significant cytotoxic effect, they were analyzed by GC/MS spectrophotometer.

**Table 3:** Percentage composition of flower heads essential oil (fraction 2)

No	Identified compound	M <sup>+</sup>	Rt	Base peak	Area %
1	Ethyl Hexanoate	159	626	41	1.37
2	Hexyl tiglate	185	1216	101	0.06
3	Caryophyllene oxide	187	1837	41	3.17
4	Viridifloral	205	1859	205	0.38
5	Pipitzol<O-Methyl,α>	262	2397	262	5.10
6	Methyl palmitate	270	2592	74	38.66
7	Abietatriene	270	2841	173	38.33
8	Dehydro abietal	284	3226	253	1.08
	Identified compounds				88.15
	Unidentified compounds				11.85

Fraction 1 (23 peak), Fraction 2 (11 peak)

**Table 4:** LC<sub>50</sub> of the extracts, oil and isolated compounds

Plant extract /compound	LC <sub>50</sub>
MeOH extract of the flowers of <i>Bougainvillea alba</i>	8.7 µg/ml
MeOH extract of the leaves of <i>Bougainvillea alba</i>	14.9 µg/ml
Compound 1	15.2 µg/mL
Compound 2	17.2 µg/ml
Compound 3	15.6 µg/ml
Compound 4	2.82 µg/ml
Oil 1	2.67 µg/ml
Oil 2	2.67 µg/ml
Doxorobsin*	0.6

\*Positive control

The isolated compounds identified using different spectroscopic analysis.

Compound 1, brown powder showed a flavonoid nature, it give yellow color spot change to dark brown after spraying with H<sub>2</sub>SO<sub>4</sub> on TLC chromatogram. It gave R<sub>f</sub> = 0.8 on TLC plates With solvent system [BuOH : MeOH: H<sub>2</sub>O, 2:1:1, v/v/v].

<sup>1</sup>H NMR spectrum, showed three one-proton signals at δ 7.47 (1H, d, J=2.0Hz, H-2'), δ 6.76 (1H, d, J=8.4 Hz, H-5') and δ 7.45 (1H, dd,8.4,2.0 Hz, H-6'). The size of coupling constants is characteristic of *meta* and *ortho* coupling 3', 4'-oxigenated flavonoids [11]. <sup>1</sup>H NMR spectrum, also, showed singlet signal at δ 6.22 (1H, s, H-8) at ring A. <sup>13</sup>C NMR spectra (Table 1), showed presence of 39 signals, from which 23 were attributed

to the aglycone, 10 to the E-feruloyl unit, 6 to the α-L-rhamnopyranosyl sugar unit. The anomeric carbon of rhamnose sugar unite showed peak at δ101.1 confirmed by the anomeric proton at δ 5.68 (1H, br, s Hz, H-1"). In aromatic zone of <sup>1</sup>H NMR spectrum there is peaks at δ 6.25 (1H, d, J=15.5, H α), δ 7.03 (1H, d, J= 15.5) Hz, H-β), δ 6.58 (1H, d, J=8.5 Hz, H-5"), δ 7.56 (1H, d, J= 2.5 Hz,H-2"), δ 6.78 (1H, dd, J= 8.5, 2.0 Hz, H-6") suggesting presence of ferulic acid unit which is confirmed by <sup>13</sup>C NMR spectrum and literature data [4,12]. Compound 1 showed presence of four singlet methoxyl groups at 3.34, 3.32, 3.1 and 3.7 confirmed by <sup>13</sup>C NMR spectrum signals at 56.9, 56.0, 51.6 and 55.1. It contain chelated 5-OH group appear downfield at δ12.6. Both <sup>1</sup>H & <sup>13</sup>C spectrum showed presence of prenyl unit, it showed signals at δ 3.12, 5.02 (olefinic proton, d), 0.81 (Me, s) and 1.08 (Me,s) corresponding with their carbons at 23.3, 120.0, 131.0, 22.6 and 25.3 [13].

Positive ESI-MS spectrum give molecular ion at m/z 734.89 [M]<sup>+</sup> corresponding to molecular formula (C<sub>39</sub>H<sub>42</sub>O<sub>14</sub>), m/z 557.06 [M-177]<sup>+</sup> meaning the loss of terminal feruloyl unite, m/z 411 [M-323]<sup>+</sup> (C<sub>23</sub>H<sub>23</sub>O<sub>7</sub>) indicating loss of terminal feruloyl unite in addition to rhaminose sugar unit. The point of attachment of side chain confirmed by <sup>13</sup>C NMR spectra.

From the above data compound 1 was identified as 3', 4', 6 - trimethoxy, 5 - hydroxyl 7-prenyl 3-O- (feruloyl (1→2) - rhamnpyranosyl) flavone.

Compound 2 brown powder showed positive libermann-burchard test and positive molish test which indicate its triterpenoid glycosidic nature. It showed

violet spot on TLC after spraying with  $H_2SO_4$ . Its  $R_f$  value 0.47 with solvent system [ $CHCl_3$ : EtOAc: MeOH: H<sub>2</sub>O 28:32:35:5 v/v]. The  $^1H$ NMR spectrum showed the presence of seven  $sp^3$  methyl protons at  $\delta$  0.63, 0.70, 0.8, 0.91, 1.02, 1.18 and 1.29. There was a signal at 3.10 (1H, dd,  $J=11.45, 7.0$ Hz, H-3), it contain four anomeric protons at 5.17 (1H, d,  $J=8.4$ Hz), 5.11 (1H, d,  $J=6.6$ Hz), 4.99 (1H, Br S) and 4.51 (1H, Br S). A doublet olefinic proton appeared at 5.19 (1H, d, 12-H). Acid hydrolyses afforded oleanolic acid as an aglycone and glucose and xylose as sugar moieties.  $^{13}C$  NMR spectrum (Table 1) supported the above data, it showed 53 peaks, 30 for the aglycone and 23 corresponding to four sugars unites, three hexose and one pentose sugar.  $^{13}C$  NMR spectrum showed presence of four anomeric carbons at 105.80, 105.17, 104.59 and 94.62. The resonance of C-3 at  $\delta_c$  85.0 and C-28 at  $\delta_c$  175.7 together with  $^1H$  NMR signals at  $\delta$  6.21 indicate the bidesmosidic nature of a compound with 3- $\alpha$  hydroxyl group.  $^{13}C$  NMR spectroscopy was used for the determination of the interglycosidic linkage. negative ESI-MS showed Molecular ion at  $m/z$  1060 [ $M-2H$ ]<sup>-</sup>, 934.5 [ $M-(2H+132)$ ],  $m/z$  771.2 [ $M-(2H+294)$ ]. Positive ESI-MS spectra showed fragment ions at  $m/z$  776 [ $M-294$ ]<sup>+</sup> loss of glucose and xylose molecules,  $m/z$  614 [ $M-2 \times 162+132$ ]<sup>+</sup> loss of two molecules of glucose and one mole of xylose,  $m/z$  452 [ $M$ ]<sup>+</sup> loss of sugar side chain. Both type of fragmentation indicate the presence of terminal Xylose connected with two glucose units at C-28,

From the above data compound 2 identified as 3-O  $\beta$ -D-glucopyranosyl oleanolic acid 28-O- $\beta$ -D-glucopyranosyl (1 $\rightarrow$ 3'')  $\beta$ -D-glucopyranosyl (1 $\rightarrow$ 3''')  $\alpha$ -L-xylopyrenoside.

Compound 3, brown powder substance, soluble in acetone water [1:1, v/v], m.p. 268°C, it has  $R_f$  value 0.9 in solvent system [BuOH: MeOH: H<sub>2</sub>O, 4:2:2 v/v], it give violet color after spraying with  $H_2SO_4$ . Four olefinic protons were revealed in  $^1H$  NMR spectrum at  $\delta$  5.25, 5.36, 5.65 and  $\delta$  5.96 which coupled with four double bonds in  $^{13}C$  NMR data at 108.6 (CH), 122.6 (C), 120.0 (CH), 126.5 (C), 136.9 (CH), 143.7 (C) (Fig. 20).

$^{13}C$  NMR signals (Table 1) showed the presence of two carboxylic acid groups at C-3 (176.1), C-26 (162.3) and carbonyl group at C-28 (188.3). Negative ESI-MS showed molecular ion at  $m/z$  495 [ $M-H$ ]<sup>-</sup>,  $m/z$  355( $M-H$ -side chain) [ $C_8H_{11}O_2$ ]. All spectroscopic data

are matched with Lepidolide a seco-ring Cucurbitane triterpenoid which previously isolated from *Russula lepida* [4, 13, 14].

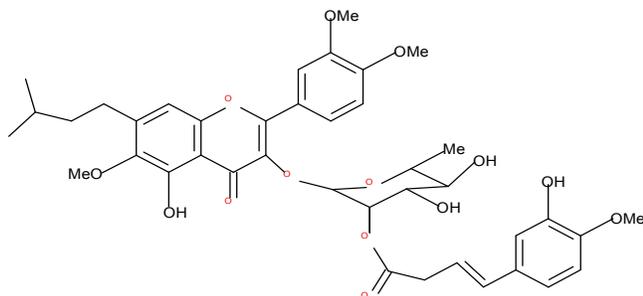
Compound 4, off white powder has a phenolic character, it give blue color under UV changed to brown color after spraying with  $H_2SO_4$ , m.p.187°C, its  $R_f$  value 0.33 on TLC with solvent system [BuOH:MeOH:H<sub>2</sub>O, 4:2:2 v/v].

$^1H$  NMR spectrum revealed presence of two moles of phenolic acid, it showed two signals at 6.0 & 6.06 each (d,  $J=17.7$ , H- $\alpha$ ) and two signals at 7.58 & 7.62 each (d,  $J=17.7$ , H- $\beta$ ). It also showed two signals at 6.75 (1H, d,  $J=8.0$  Hz) and at 7.1 (1H, dd,  $J=8.0, 1.5$  Hz), which means presence of di-substituted aromatic ring. Negative ESI-MS fragmentation showed a molecular ion peak at  $m/z$  487 [ $M+H$ ]<sup>+</sup>[ $C_{25}H_{26}O_{10}$ ],  $m/z$  457 [ $M+H-OMe$ ]<sup>+</sup>,  $m/z$  363 [ $M+H-C_7H_7O_2$ ]<sup>+</sup> (Fig. 24). The signal at 7.3 (1H, m) represent mono-substituted aromatic ring. It showed one methoxy group appeared as strong singlet at 3.07 (3H, s).  $^1H$  NMR spectrum also showed broad singlet at 5.2 (1H, Br s, H-1'), the anomeric proton of rhaminose sugar unit, its methyl group showed signal at 1.23 (3H, Br,s, 3H-6').

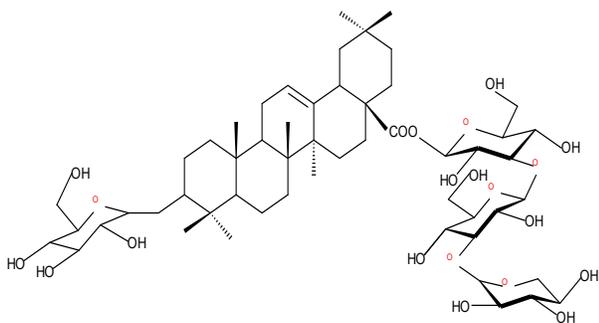
From the above data compound (4) was identified as: E feruloyl 3-O- Coumaroyl  $\alpha$ -L Rhamnopyranoside.

Two essential oil fractions isolated during chromatographic fractionation of methanol extract were analyzed using GC/MS analysis, fraction 1 showed 23 peaks. Analysis revealed the presence of Oxygen containing compounds (75 %), and hydrocarbon (25 %), represented as (41 %) monoterpenes, (8 %) diterpenes and (51 %) sesquiterpenes. The major identified components are Elemene< $\beta$ -> (25.26), Lanceol <*cis*-> (9.16 %), and Nerolidol <*cis*-> (7.12 %) (Table 2). Analysis of fraction (2) showed 11 peaks, Its analysis revealed the presence of Oxygen containing compounds (50 %), and hydrocarbon (50 %), represented as (25 %) monoterpenes, (25 %) diterpenes and (50 %) sesquiterpenes. The major identified components are Methyl palmitate (38.66), Abietatriene (38.33 %), and Pipitzol <O-Methyl, $\alpha$ > (5.10 %) (Table3).

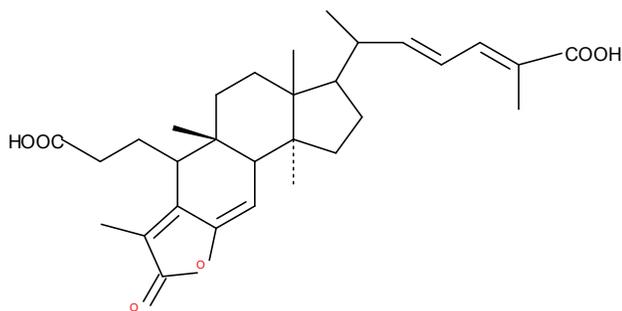
Both methanol extract of flowers and leaves of *Bougainvillea alba* and the isolated components were tested for their toxicity using HEPG2 cell line test. The four isolated compounds showed activity at  $LC_{50}$  15.2, 17.2, 15.6 and 2.82  $\mu$ g/ml for compounds 1,2 3 and 4 (Fig. 1–4). The last compound showed significant



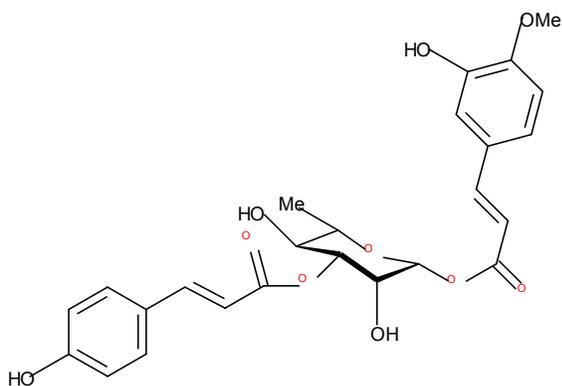
**Fig. 1.** Compound 1.



**Fig. 2.** Compound 2.



**Fig. 3.** Compound 3.



**Fig. 4.** Compound 4.

cytotoxic activity which may be due to its phenolic nature and the two phenolic acid which connected to a sugar moiety. The two oil fractions showed high cytotoxic effect at 2.67  $\mu\text{g/ml}$ .

## 5. Conclusion

Four compound and two essential oil fractions were isolated from *Bougainvillea alba*, they were tested for Cytotoxic activity using the SRB Assay on human tumor cell line. The oil fractions and compound 4 showed significant activity compared to original methanol extract and other three compounds.

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