Cytotoxicity and Antioxidant Activity of New Biologically Active Constituents from *Salvia Lanigra* and *Salvia Splendens*

Usama Y. Shaheen[1]*, Mohammed H. Hussain[1] and Hassan A. Ammar[2]

[1] Department of Pharmacognosy, Faculty of Pharmacy, Al-Azhar University, Cairo, Egypt. [2] Department of Pharmacognosy, Faculty of Pharmacy, Ahram Canadian University in Egypt, 6th October, Egypt.

ABSTRACT

Chromatographic fractionation of the acetone extracts of each of *Salvia lanigra* and *Salvia splendens* and of the n-butanol extract of *Salvia lanigra*, resulted in the isolation and identification of two known diterpenes; horminone (1) and 7-*O*-ethylhorminone (2), three new diterpenes; salviatane B (3), salvianol A (4) and salviaclerodan A (5), two known triterpenioc acids; ursolic acid (6) and oleanolic acid (7), a known sterol; β-sitosterol (8), two known flavones; salvigenin (9) and apigenin (10) and one new caffiec acid dimmer; 3,3′-dehydrodicaffeic acid (11). The cytotoxicity and the antioxidant activity of the different extracts (MeOH, acetone and n-butanol) of both *Salvia lanigra* and *Salvia splendens* and most of the isolated pure compounds (1-5, 9 and 10) were determined.

Key words: Salvia, Diterpenes, Phenolics, Cytotoxicity and Antioxidants Activity.

INTRODUCTION

Salvia is an important genus consisting of ca 900 species in the family Lamiaceae (formerly Labiatae) and some species of *Salvia* have been cultivated worldwide for use in folk medicines and for culinary purposes.[1] The genus has attracted great interest so much so that it has been the subject of numerous chemical studies. It is a rich source of diterpenoids, tanshinones and polyphenols.[2-4] These compounds constitute the major secondary metabolites and show interesting spectra of biological activities as antioxidant, antitumor, cytoprotective, antibacterial and as components of herbal teas which has been used extensively for the treatment of coronary heart disease, cerebrovascular disease, hepatitis, hepatocirrhosis, chronic renal failure, dysmenorrheal, flatulence, dyspepsia, gastritis, sore throat, tuberculosis, psoriasis, eczema and neuroasthenic insomnia.[1,5] In the flora of Egypt, the genus *Salvia* is represented by three major species; *S. lanigra* Poir, *S. splendens* Sello, and *S. farinacia* Benth. This study was undertaken to perform the isolation, structure elucidation of the interesting constituents of *S. lanigra* Poir and *S. splendens* Sello. Also to perform the screening of cytotoxic activity against certain human cell lines and the antioxidant properties using inhibition of DPPH• method of different extracts (MeOH, Acetone and *n*-butanol) as well as some isolated pure compounds (**1-5, 9** and **10**).

Address for correspondence:
E-mail: usamayosef2003@yahoo.com

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MATERIAL AND METHODS

General Experimental Procedures: UV spectra were determined with a Hitachi 340 spectrophotometer; IR spectra were carried out on a Nicollet 205 FT IR spectrometer connected to a Hewlett-Packard Color Pro. Plotter. The ^{1}H and ^{13}C NMR measurements were obtained with a Bruker NM spectrometer operating at 300, 400 and 500 MHz (for ^{1}H) and 100 and 75 MHz (for ^{13}C) in Acetone-d_6, DMSO-d_6 or $CDCl_3$ solution, and chemical shifts were expressed in δ (ppm) with reference to TMS, and coupling constant (*J*) in Hertz. ^{13}C multiplicities were determined by the DEPT pulse sequence (135°). HMQC NMR experiments were carried out using a Bruker AMX-500 high field spectrometer equipped with an IBM Aspect-2000 processor and with software VNMR version 4.1 or NUTS program for NMR. HRFAB mass spectra were performed on a VGZAB-HF reversed geometry (BE configuration, where B is a magnetic sector and E is an electrostatic analyzer) mass spectrometer (MS) (VG Analytical, Inc.). MALDI-TOFMS was conducted using perceptive Biosystems, Voyager DE-STR mass spectrometer. ESIMS (positive and

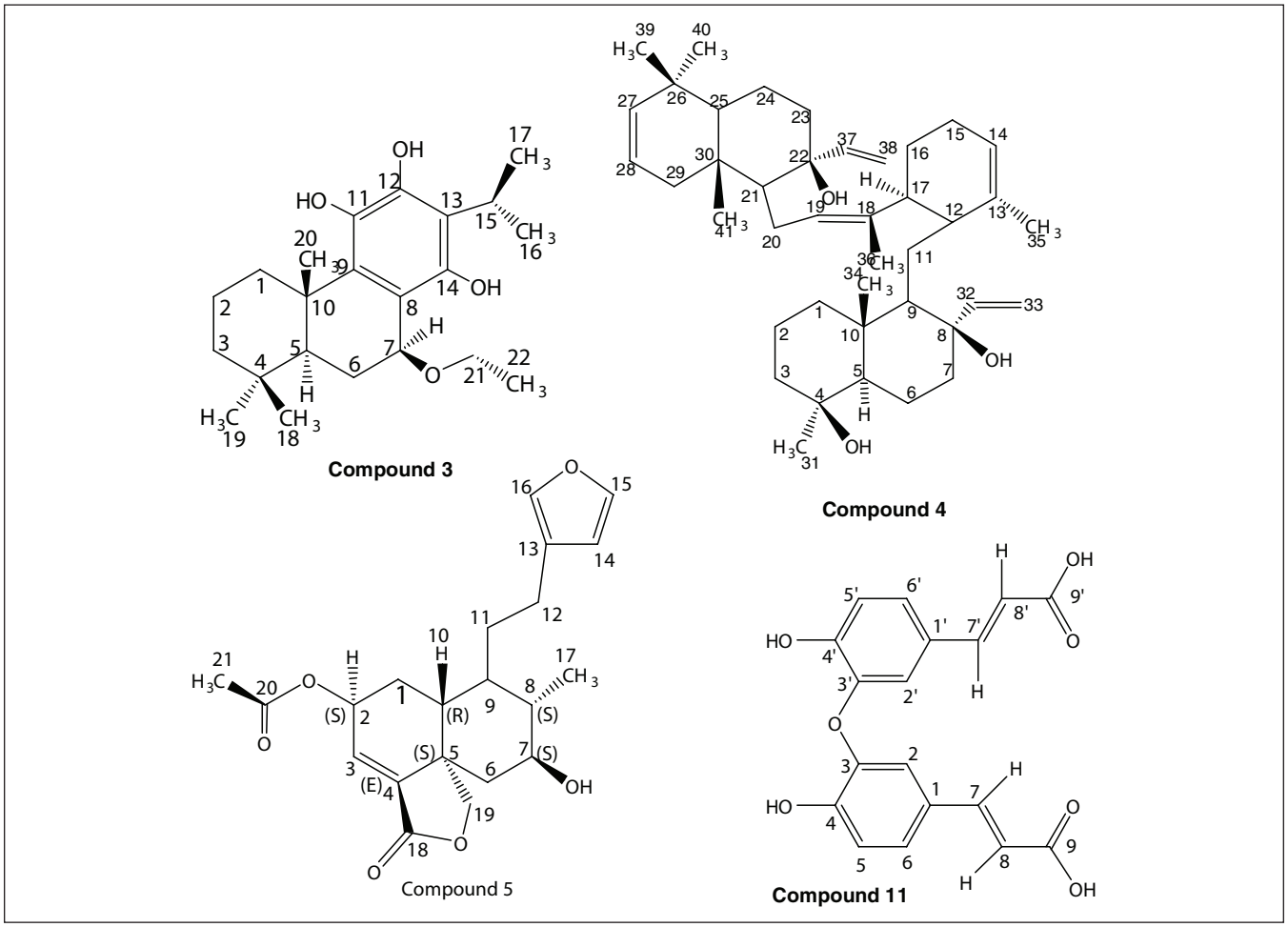

Figure 1: Structures of compounds 3-5 and 11.

negative ion acquisition mode) was carried out on a TSQ700 triple quadrupole instrument (Finnigan, SanJose, CA, USA) mass spectrometer. EIMS was carried on Scan EIMS-TIC, VG-ZAB-HF,X-mass (158.64, 800.00) mass spectrometer (VG Analytical, Inc.). Si gel (Si gel 60, Merck), were used for open column chromatography. Flash column liquid chromatography was performed using J.T. Baker glassware with 40 μm Si gel (Baker) and Sepralyte C_{18} (40 μm) as the stationary phase. TLC was carried out on precoated silica gel 60 F_{254} (Merck) plates. Developed chromatograms were visualized by spraying with 1% vanillin-H_2SO_4, followed by heating at 100 for 5 min and diazotized sulfanilic acid (Pauly's reagent) for phenols.

The following material and reagents were used for cell culture and cytotoxic assays. Human liver hepatocellular carcinoma (SNU-398 and Hep G2), Liver hepatoma (PLC/PRF/5), Kidney hypernephroma (SW 156), Kidney carcinoma (A-498), Urinary bladder carcinoma (HT-1376), Urinary bladder transitional cell carcinoma (UM-UC-3), Stomach gastric carcinoma (Hs 746T and Hs 740.T), ovary adenocarcinoma (NIH:OVCAR-3 and SK-OV-3), Lymph node Hodgkin's disease; Hodgkin's lymphoma (Hs 388.T and Hs 751.T) and Uterus Uterine sarcoma (MES-SA and MES-SA/MX2) cell lines were purchased from the American Type Culture Collection (ATTC). Dulbecco's Modified Eagle Medium (DMEM) was from (Gibco, Grand Island NY, USA). Eagle Minimum Essential Medium (EMEM) and Roswell Park Memorial Institute (RPMI) 1640 medium were from (Nissui Pharm. Co., Ltd., Tokyo, Japan). Flat-bottom plates, 96 well were from (Iwaki Glass Co., Ltd., Fumabashi-Chiba-Ken, Japan). 3-[4,5-dimethylthiazole-2-yl]-2,5-diphenyl tetrazolium bromide (MTT), for colorimetric assay was from Sigma (St. Louis, Mo., USA). 10% Fetal Bovine serum (FBS) was from (Gibco Br L, Rockville, MD, USA). All other chemicals used were of analytical reagent grade. The following material and reagents were used for antioxidant activity. 2, 2- Diphenyl -1-picrylhydrazyl, 95 % was from (Aldrich, D 211400 -1G, USA). Automatic pipette was from (Gilson, France). 96-well plates were from (USA). Incubator (30 ± 2 °C) was from (New Brunswick Scientific, Innova 5000 Gyratory tier shaker, USA). Multi well scanning spectrophotometry was from (Dynex MR 5000, Chantilly, VA, USA). D,

L-α-tocopherol was from (Nacalai, Tesuque, Tokyo, Japan). Butylated hydroxytoluene (BHT) was from (Sigma, chemical company, St. Louis, Mo, USA).

DPPH stock solution; dissolve 125 mg of DPPH in 100 ml of reagent alcohol, Sonicate for 10 minutes. The stock solution may be stored for 2 weeks in the refrigerator (–40°C) under Argon. DPPH working solution; dilute the stock solution 1: 10 (V/V) in reagent alcohol. The concentration of DPPH in the working solution is 316 μM.

Sample stock solution; dissolve a known amount of sample (extracts, compounds and standards) in 10 ml of reagent alcohol. The concentration of the stock solution is given by such that the working solutions prepared from it will provide an even distribution of data points around the 50% inhibition point (EC_{50}). For many samples, several trials may have to be run to determine the stock solution concentration that provides an appropriate range of data points. Stock solutions; mixed tocopherols (1.25 mg /ml), BHT 1.25 mg /ml, crude methanolic, acetone and *n*-BuOH extracts of *Salvia lanigra* and *Salvia splendens* (5 mg/ml), and isolated compounds (1 mg/ml).

Plant Material: The plant materials used in this work consisted of the leaves and stems of *Salvia lanigra* Poir, collected in April, 2002, from Borg Al-Arab Desert, Alexandria, Egypt. The leaves and stems of *Salvia splendens* Sello collected in April, 2002 from Zoo garden Giza, Egypt. The two plants were kindly identified by Late Dr. Nabil El-Hadidy, Professor of Plant Taxonomy, Faculty of Science, Cairo University, and Engineer Badia Hassan Aly Dewan, Consultant of Egyptian Flora, Agricultural Museum, Dokki, Giza.

***Extraction and Isolation*; *Salvia lanigra* Poir:** Three kg of air-dried powdered (leaves and stems) of *Salvia lanigra* were subjected to exhaustive extraction with acetone (3 x 12 L). The combined acetone extract was concentrated under *vacuo* at 40°C to dryness (140 g). The marc was then partitioned several times with *n*-butanol (3 x 3 L), the concentrated *n*-butanol extract was concentrated under *vacuo* at 40°C to dryness (14 g). The acetone extract was applied to silica gel column using n-hexane: EtOAc (100:00-70:30), to yield six fractions (A-F). Fr. A (4.7g) was further subjected to silica gel flash column chromatography using n-hexane: EtOAc (100:00-90:10) to give five fractions (A1-A5). Four fractions; Fr. A1 (195mg), Fr. A2 (180mg), Fr. A3 (560mg) and Fr. A4 (1.95g), were further subjected separately to a series of column chromatographic techniques including silica gel column (n-hexane: EtOAc- 95:5-85:15), silica gel flash column (Petroleum ether: EtOAc- 90:10-80:20), Sepralyte C_{18} flash column (H_2O: MeOH- 65:35-100:00) and final purification by Sephadex LH-20 column (MeOH) to afford compounds **1** (93mg), **2** (19 mg), **3** (23 mg) and **4** (35 mg), respectively. Fr. C (10.95 g) was chromatographed over silica gel flash column using n-hexane: EtOAc (90:10-80:20) to give three fractions of C1, C2 and C3. Fr. C1 (225mg) and Fr. C2 (6.8g) were further subjected separately to silica gel column using n-hexane: EtOAc (9550-85:15), Sepralyte C_{18} flash column (H_2O: MeOH- 50:50-90:10) and Sephadex LH-20 column (MeOH) to afford compounds **7** (3.7 g) and **9** (23 mg), respectively. The n-butanol fraction was subjected to silica gel column chromatography using $CHCl_3$: MeOH (100:00-70:30) to give six fractions (A-F). Fraction C (220 mg) and fraction E (354 mg) were further subjected separately to silica gel column using $CHCl_3$: MeOH (95:5-80:20), silica gel flash column using CH_2Cl_2: MeOH (90:10-75:25), Sepralyte C_{18} flash column (H_2O: MeOH-40:60-20:80) and Sephadex LH-20 column using MeOH and $CHCl_3$: MeOH (15:85) to afford compounds **10** (23 mg) and **11** (18 mg), respectively.

***Salvia splendens* Sello:** Two kg of air dried powdered (leaves and stems) of *Salvia splendens* was subjected to exhaustive extraction with acetone (3 x 8 L). The combined acetone extract was concentrated under *vacuo* at 40°C to dryness (76 g). The acetone extract was chromatographed over silica gel column using n-hexane: EtOAc (100:00-70:30), to give seven fractions (**A-G**). Fr. D (1.5 g) was subjected to silica gel flash column (Petroleum ether: EtOAc- 90:10-75:25) and Sepralyte C_{18} flash column (H_2O: MeOH- 60:40-85:15) to give two fractions of D1 and D2. Fr. D2 (455 mg) was further subjected to silica gel column using Petroleum ether: EtOAc (85:15-80:20) to afford compound **8** (205 mg). Fr. E (1.25 g) was subjected to silica gel column (Petroleum ether: EtOAc- 90:10-75:25), silica gel flash column using n-hexane: EtOAc (90:10-70:30) and Sepralyte C_{18} flash column (H_2O: MeOH- 70:30-90:10) to give two fractions of E1 and E2. Fr. E1 (50 mg) was further subjected to Sephadex LH-20 column (MeOH) to afford compound **5** (205mg), while Fr. E2 (50 mg) was further subjected to Sephadex LH-20 column (MeOH) to afford compound **6** (120 mg).

Cytotoxicity assay:[6,7] Cell lines SNU-398, Hep G2, PLC/PRF/5, A-498, HT-1376, UM-UC-3, Hs 746T, Hs 740.T, Hs 388.T, Hs 751.T, MES-SA and MES-SA/MX2 were cultured in Dulbecco's Modified Medium (DMEM) containing 10% heat-inactivated Fetal Bovine Serum (FBS). The SW 156, SK-OV-3, and NIH:OVCAR-3 cells were cultured in Eagle Minimum Essential Medium (EMEM) containing Earle's salts heated and sublimated with amino acids and 10% heat inactivated Fetal Bovine Serum (FBS). The SNU-398, A-498, UM-UC-3, MES-SA and MES-SA/MX2 cell lines were maintained in Roswell Park Memorial Institute (RPMI) 1640 Medium containing 10% heat inactivated Fetal Bovine serum (FBS). All cell lines were

cultivated in an incubator at 37°C in humidified air containing 5% CO_2. For routine cytotoxicity assays, all cell lines were adapted to one single medium RBMI 1640 medium sublimated with 10% FBS, 2 mM L-glutamine, penicillin (100 unites/1 ml), and streptomycin (100 µg/1 ml). For the micro assay, the growth medium was supplemented with 10 mM HPES (1-[2-hydroxy ethyl] piprazine-4-ethane sulfonic acid) buffer pH 7.3 and incubated at 37°C in CO_2 incubator. Cellular viability in the presence and absence of experimental reagents was determined using the standard MTT (3-[4, 5-dimethyl thiazole-2-yl]-2, 5-diphenyl tetrazolium bromide) colorimetric assay. The assay is based on reduction of MTT by the mitochondrial dehydrogenase of viable cells to give a blue formazan product that can be measured spectrophotometrically. In brief, exponentially growing cells were harvested and 200 µL cells suspension were seeded in 96-well microplates and preincubated for 24 h at 37°C under 5% CO_2 to allow cell attachment. After attachment 10 µL of an EtOH: H_2O (1:1 solution) containing varying concentrations of test samples; different extracts (MeOH, Acetone and *n*-BuOH) and isolated compounds (**1- 6, 11-19**) were added in wells in duplicate, and 10 µL EtOH: H_2O (1:1) was added into wells as a control. Sample containing microplates were further incubated for 6 days. Cell survival was evaluated by adding 10 µL of 5 mg/ml MTT in 0.1 mM, pH 7.4 phosphate buffered saline to each well, and re-incubating plates in 5% CO_2 /air for 4 hrs at 37°C. Plates were then centrifuged at 1500 × *g* for 5 minutes to precipitate cells and MTT formazan. An aliquot of 100 µL of the supernatant was removed, and DMSO (100 µL) was added to dissolve precipitated, reduced MTT. The plate was mixed on a microshaker for 10 minutes, and the absorbance was determined at 550 nm with a multiwell scanning spectrophotometer (Dynex MR 5000) Chaltilly VA, USA). The ED_{50} value, which reduces the viable cell number, was defined as the concentration of test samples resulting in a 50% reduction of absorbance compared to untreated controls. The 50% effective dose (ED_{50}) obtained by measuring growth inhibition with MTT, are shown in (Tables 4 and 5).

Statistical Analysis:[8] All cytotoxic data were expressed as mean (± SE. Student's *t*-test was applied for detecting the significance of difference between each sample; $P < 0.05$ was taken as the level of significance.

Antioxidant activity assay:[9] DPPH radical scavenging assay: Radical scavenging activity of plant extracts against stable DPPH (2,2-diphenyl-2-picrylhydrazyl hydrate) was determined spectrophotometrically. When DPPH reacts with an antioxidant compound, which can donate hydrogen, it is reduced. The changes in colour (from deep—violet to light—yellow) were measured at 515 nm on a UV/visible light spectrophotometer. The reduction in absorbance at 517 nm can be measured to determine the antioxidant potential measured by comparing IC_{50} to known antioxidant such as tocopherols, Butylated hydroxytoluene (BHT).

In a 96-well plate, add 10 µL of sample working solution to 190 µL of DPPH working solution. Prepare a blank by adding 10 µL of ethanol to 190 µL of DPPH working solution. The final volume in each is therefore equal to 200 µL. The concentration of DPPH in final solution is approximately 300 µM. Incubate solutions to (30 ± 2°C) for 30 minutes. After incubation, measure the absorbance of each solution at 517 nm. Plot % inhibition vs. the concentration (mg/ml) of test sample in the final solution. From the equation of the line, calculate the test sample required to reduce the absorbance at 517 nm by 50% (IC_{50}).

Calculations:

$$\text{DPPH Scavenging Effect (\%)} = \left[\frac{(A_{\text{control}} - A_{\text{sample}})}{A_{\text{control}}}\right] \times 100$$

1. Plot % inhibition vs. the concentration (mg/ml) of test sample in the final solution.
2. From the equation of the line, calculate the test sample required to reduce the absorbance at 517 nm by 50% (IC_{50}).

The results of different extracts, isolated compounds as well as mixed tocopherols and BHT were shown in (Table 6).

RESULTS AND DISCUSSION

The air-dried leaves and stems of *Salvia lanigra* and *Salvia splendens* were subjected separately to exhaustive extraction with acetone. The acetone extracts were partitioned several times with *n*-butanol. The acetone extracts of *Salvia lanigra* and *Salvia splendens* and the *n*-butanol extract of *Salvia lanigra* were repeatedly chromatographed over normal-phase silica gel, silica gel for flash and reversed-phase silica gel to give five diterpenes (**1**-**5**), two triterpenioc acids (**6** and **7**), one sterol (**8**), two flavones (**9** and **10**) and one phenolic acid (**11**). The known compounds were identified as horminone (**1**)[10-12] and 7-*O*-ethylhorminone (**2**),[10-13] ursolic acid (**6**) and oleanolic acid (**7**),[14] β-sitosterol (**8**),[15,16] salvigenin (**9**) and apigenin (**10**) by comparing their spectral data (mass spectrometry, ^{1}H- and ^{13}C NMR) with those reported previously.[17-19]

Compound 3: was obtained as a yellowish amorphous solid. Its molecular formula $C_{22}H_{34}O_4$ was determined from an $(M + H)^+$ ion at m/z 363 in the positive ion ESI-MS. Other fragment ion at 317 $(M + H - CH_3CH_2O)^+$ indicated the presence of an ethyl moiety. Analysis of the IR spectrum of (**3**) suggested that it contained a hydroxyl (3420 cm^{-1}), and aromatic (1502, 1580, 1615 cm^{-1}) groups. The UV

spectrum of (**3**) showed absorption bands at λ_{max} 245 and 280 nm supporting the presence of an aromatic ring. ^{1}H and ^{13}C chemical shifts for (**3**) suggested an aromatic C-ring, substituted with an isopropyl group at C-13 and hydroxyl groups at C-11, C-12 and C-14 in the abietane diterpene skeleton.[10-12] In the ^{1}H NMR spectrum, signals for three tertiary methyl singlets were observed at δ 0.92 (3H), 0.93 (3H), 1.22 (3H) which were attributed to the geminal dimethyl groups (C-18 (*a*), C-19 (*β*)) and C-20, respectively, two secondary methyl signals at δ 1.23 and 1.18 (each 3H, d, J = 6.8 Hz, Me-16, 17).

The latter two signals, together with a benzylic methine signal at δ 3.18 (1H, heptet, J = 7.0 Hz, H-15), showed the presence of an isopropyl group, characteristic for all abietane diterpenes.[10-12] ^{1}H and ^{13}C NMR spectral data of (**3**) shows an ethyl moiety at δ 3.65 (q, J = 7.0 Hz, H_2-21) and δ 1.12 (t, J = 7.0 Hz, Me-22). The H-5 proton appears as a doublet of doublet, indicating the existence of C-6 methylene protons at δ 2.08 (m, H-6α), and δ 1.99 (dd, J = 10.0, 3.3 Hz, H-6β), which in turn coupled with another methine proton at δ 4.40 (dd, J = 3.3, 1.4 Hz) attributed to H-7, establishing the closure of the ethoxyl group at C-7. The coupling patterns of a double doublet with J value of 1.8 and 8.5 Hz of H-7 permitted the assignment of an axially oriented ethoxyl group of C-7.[2] ^{13}C NMR spectrum of (**3**) showed well-resolved resonances for all 22 carbon atoms. The multiplicity of each carbon atom was determined using ^{13}C-NMR DEPT experiment which revealed the presence of six methyls (including three tertiary at δ (20.13 (C-20), 22.22 (C-18), 33.49 (C-19) and two secondary methyls at δ 16.1 (C-16), 18.82 (C-17)), and one primary methyl at δ 20.46 (C-22), five methylenes at (δ 36.58 (C-1), 19.58 (C-2), 41.98 (C-3), 23.84 (C-6) and 65.60 (C-21)), three methines (including one oxygenated at δ 69.92 (C-7)) and eight quaternary carbons including six aromatic carbons at (δ 113.50 (C-8), 129.41 (C-9), 139.15 (C-11), 155.01 (C-12), 121.15 (C-13) and 157.25 (C-14), and two sp^3 at δ 33.72 (C-4) and 39.72 (C-10). The above data suggested that **(3)** is an abieta-8,11,13-trien type diterpenoid derivative.[20-24] Location of the ethyl moiety at C-7 of (**3**) was deduced from the upfield shift of H-7 (δ 4.40, $^{\delta}\Delta_H$ -0.33 ppm), and the significant downfield shift of C-7 (δ 69.92, $^{\delta}\Delta_C$ + 7.35 ppm) in (**3**) compared to those of H-7 (δ 4.73) and C-7 (δ 62.57) in (**1**), respectively. Consequently, the structure of (**3**) was established as 7α-ethoxy-11, 12, 14-trihydroxy-8, 11, 13-abietatriene. A new example of the isolation of abeitane type diterpene from nature and was named as Salviatane B.

Table 1: ^{1}H and ^{13}CNMR spectral data of compound 3 (400 MHz for ^{1}H, and 100 MHz for ^{13}C, Acetone-d_6) and Compound 5 (500 MHz for ^{1}H and 100 MHz for ^{13}C NMR, DMSO-d_6).

	Compound 3			Compound 5	
Position	**^{1}H (J in Hz)**	**^{13}C**	**DEPT**	**^{1}H (J in Hz)**	**^{13}C***
1	α 1.45, m β 2.71, m	36.58	CH_2	α 1.30, m β 1.50, m	24.00
2	α 1.52, m β 1.73, m	19.58	CH_2	5.87, dd, 10.5, 3.4	66.00
3	α 1.44, m β 1.46, m	41.98	CH_2	6.66, dd, 10.5, 1.9	137.00
4	–	33.72	C	–	139.00
5	α 1.61, d, 12.8	46.39	CH	–	41.00
6	α 2.08, m β 1.99, dd, 13.0, 1.8	23.84	CH_2	α 2.35, m β 1.80, m	40.50
7	4.40, dd, 8.5, 1.8	69.92	CH	5.40, ddd, 4.9, 3.6, 2.1	74.00
8	–	113.50	C	2.25, m	41.50
9	–	129.41	C	1.45, m	32.00
10	–	39.72	C	2.30, ddd, 10.5, 10.5, 6.5	43.00
11	–	139.15	C	α 1.20, m β 1.40, m	30.00
12	–	155.01	C	α 1.90, m β 2.15, m	20.00
13	–	121.15	C	–	131.00
14	–	157.25	C	6.50, d, 1.5	109.00
15	3.18, hept., 7.0	24.88	CH	7.46, t, 1.5	144.00
16	1.23, d, 6.8	16.11	CH_3	7.53, d, 1.5	141.00
17	1.18, d, 6.8	18.82	CH_3	1.15, d, 7.2	12.00
18	0.92, s	22.22	CH_3	–	171.00
19	0.93, s	33.49	CH_3	α 3.94, dd, 7.2, 2.2 β 5.35, d, 7.2	73.00
20	1.22, s	20.13	CH_3	–	170.50
21	3.65, q, 7.0	65.60	CH_2	2.00, s	21.00
22	1.12, t, 7.0	20.46	CH_3	–	–

*Data obtained from HMQC and HMBC.

Compound 4: was obtained as a white crystal solid and gave on TLC violet color with vanillin/H_2SO_4. $(\alpha)^{25}_D$ –25 (c.0.1, $CHCl_3$). It had molecular formula of $C_{41}H_{64}O_3$ as determined by its positive ion ESI-MS at m/z 643 $(M + K)^+$ and from ^{13}C, and DEPT-NMR spectral data (Table 2). Analysis of the IR spectrum of (**4**) suggested that it contained a hydroxyl (3395 cm^{-1}), olefinic (1660, 895 cm^{-1}) and geminal dimethyl (1387, 1367 cm^{-1}) functionalities. The ^{1}HNMR spectrum of (**4**) (Table 2) showed the existence of seven tertiary methyl groups as singlet at δ_H (0.77 (3H, Me-34), 0.82 (3H, Me-31), 0.88 (3H, Me-41), 1.22 (3H, Me-39), 1.23 (3H, Me-40), 1.60 (6H, Me-35, Me-36)), twelve methine proton signals including 6 olefenic functionalities at δ_H 5.35 (brs, H-14), 5.14 (brs, H-19), 5.10 (d, J = 7.2 Hz, H-27), 5.16 (d, J = 7.3 Hz, H-28), 5.91 (dd, J = 17.3, 10.7 Hz, H-32), 5.95 (dd, J = 17.3, 10.7 Hz, H-37). The remaining six methine protons were observed at δ_H 1.10 (m, H-5) 1.99 (m, H-9), 2.05 (m, H-12), 2.04 (dd, J = 4.3, 2.2 Hz, H-17), 1.87 (m, H-21), 1.66 (m, H-25), and fourteen methylene protons including two olefinic signals at δ_H 5.32 and 4.98 (each 1H, dd, J = 17.3, 1.8 Hz, CH_2-33) and δ_H 5.19, 4.95 (each 1H, dd, J = 17.3, 1.8 Hz, CH_2-38). The ^{13}C NMR spectrum (Table 2) confirmed the previous data through the resonances displayed at δ_C 15.99, 16.09, 13.96, 25.84, 17.74, 22.17, 122.28, 125.07, 135.15, 125.75, 147.12, 146.91, 56.14, 51.06, 33.54, 64.04, 51.06, 56.14, and 111.26, respectively. Inspection of the DEPT-^{13}CNMR spectra (135° pulse sequence) also revealed the presence of seven methyls, fourteen methylenes; including 2 vinyl carbons at δ 111.26 (C-33 and C-38), and twelve methines; including 6 sp^2 at δ 122.28 (C-14), 125.07 (C-19), 135.15 (C-27), 125.07 (C-28), 147.12 (C-32), 146.91 (C-37). Among the eight signals of quaternary carbons observed in ^{13}C NMR spectrum, two olefinic at δ_C 136.39 (C-13), 135.43 (C-18) and three oxygenated quaternary at δ_C 73.27 (C-4), 72.82 (C-8 and 22) were recognized. These spectral data and by literature comparisons, compound (**4**) was recognized as a member of *bis*-labdane type diterpene[25-27] and showed the resemblance of the signal multiplicities, chemical shifts and coupling constants observed for the vinyl groups, methines and side chain carbons in both units, suggesting a close structural relationship between them (Figure 1). Comparison of the IR, ^{1}H and ^{13}C NMR spectral data of (**4**) with those of the *bis*-labdane type diterpenoid (Lancelatine and Lanceolatol) isolated from the roots of *Cuuinghamia laceolata*[25] concluded that the difference between (**4**) and Lancelatine and Lanceolatol was the presence of hydroxyl group at C-4 and

Table 2: ^{1}H and ^{13}CNMR spectral data of compound 4 (400 MHz for ^{1}H, and 100 MHz for ^{13}C, Acetone-d_6).

Position	^{1}H (J in Hz)	^{13}C	DEPT	Position	^{1}H (J in Hz)	^{13}C	DEPT
1α	1.66, m	39.90	CH_2	21	1.87, m	50.06	CH
1β	1.76, ddd, 12.7, 4.5, 4.3						
2	1.67, m	19.46	CH_2	22	–	72.82	C
3	1.40, m	43.07	CH_2	23	1.97, m	40.46	CH_2
4	–	73.27	C	24α	2.10, m	27.26	CH_2
				24β	2.08, m		
5	1.01, m	56.14	CH	25	1.66, m	56.14	CH
6α	1.20, m	27.26	CH_2	26	–	33.53	C
6β	1.27, m						
7	2.11, m	40.43	CH_2	27	5.10, d, 7.2	135.15	CH
8	–	72.82	C	28	5.16, d, 7.3	125.75	CH
9	1.99, m	51.06	CH	29α	1.29, m	43.36	CH_2
				29β	1.90, m		
10	–	37.85	C	30	–	37.85	C
11α	1.48, m	23.34	CH_2	31	0.82, s	16.09	CH_3
11β	1.65, m						
12	2.05, m	33.54	CH	32	5.91, dd,17.3, 10.7	147.12	CH
13	–	136.39	C	33α	5.23, dd, 17.3, 1.8	111.26	CH_2
					4.98, dd, 10.7, 1.8		
				33β			
14	5.35, brs	122.28	CH	34	0.77, s	15.99	CH_3
15α	1.16, m	27.46	CH_2	35	1.60, s	17.74	CH_3
15β	2.06, m						
16α	1.95, m	24.45	CH_2	36	1.60, s	22.17	CH_3
16β	1.98, m						
17	2.04, dd, 4.3, 2.2	46.04	CH	37	5.95, dd,17.3, 10.7	146.91	CH
18	–	135.43	C	38α	5.19, dd, 17.3, 1.8	111.26	CH_2
				38β	4.95, dd, 10.7, 1.8		
19	5.14, brs	125.07	CH	39	1.22, s	25.84	CH_3
20	2.00, m	22.11	CH_2	40	1.23, s	22.17	CH_3
				41	0.88, s	13.96	CH_3

methyl group at C-26 in (**4**) versus carboxyl and hydroxymethyl groups in the same position in Lancelatine and Lanceolatol, respectively.

The other difference was the presence of two vinyl and two hydroxyl groups at C-8 and C-22 in (4) versus methyline groups in Lanceolatol.[25] Compound (4) was constructed of two different monomers (12*E*-8-vinyl-12,14-labdane-diene-4,8-diol (unit 1) and 12*E*-8-vinyl-2,12,14-labdane-triene-8-ol (unit 2), and both of them were constructured by Diels-Alder cycloaddition reaction of the two different diterpene monomer units (Figure 2). There are two ways in which the Diels-Alder cycloaddition reaction could occur, either head to head or head to tail. Furthermore, one monomer should act as diene and the other as dienophile in the Diels-Alder cycloaddition reaction. Then there are four possibilities for the structure of compound (4). In the ^{1}H and ^{13}C NMR spectra, the chemical shift difference of two methyline protons (δ_H 5.23 (H-33α) and 4.95 (H-33β)), connected with vinyl carbon at δ_C 111.26 (C-33), were more different than that of two protons attached to δ_C 111.26 (C-38), (δ_H 5.19 (H-38α) and 4.98 (H-38β)). This is because one proton is shielded and the other is deshielded by the double bond between C-18 and C-19. Therefore, the monomer containing the methylene protons at this vinyl carbon (δ_H 5.23 (H-33α), 4.95 (H-33β)), should be the dienophile in the Diels-Alder cycloaddition. The chemical shifts of (C-11 to C-21) in the ^{1}H and ^{13}C NMR spectra (Table 2), showed close similarities to those of Lancelatine, Lanceolatol and Lanceolatic acid,[25,27] confirmed that the link pattern of the (4) was the same as these *bis*-labdane type diterpenoids, and should be head to head Diels-Alder cycloaddition. Based on these spectral data the structure of (4) was established as 12, 15 (22-hydroxy-28-vinyl labdane-27-ene) 8-vinyl-4, 8-labdane-diol, a new natural *bis*-labdane type diterpenoid and was named as Salvianol A. Interestingly, this is the first example of the isolation of a *bis*-labdane type diterpene from *genus Salvia*.

Compound 5: was isolated as colorless prisms, $(\alpha)^{25}_{D}$ - 115° (c.0.20, $CHCl_3$). The molecular formula was deduced as $C_{21}H_{26}O_6$ from the molecular ion peak observed at m/z 374 $(M)^+$ in the EI-MS. The IR spectrum showed the presence of a hydroxyl group (3450 cm^{-1}), α, β-unsaturated carbonyl group (1755 cm^{-1}), lactone ring (1760 cm^{-1}), and a furan ring (1490, 876 cm^{-1}) absorptions. The UV spectrum of (**5**) exhibiting a maximum at 240 nm indicated the presence of an α, β-unsaturated carbonyl group. The ^{1}H NMR spectrum of (**5**) (Table 1), exhibited 25 non-exchangeable protons, including one secondary methyl group at δ 1.15 (3H, d, J = 7.2 Hz, H_3-17), one tertiary methyl group at δ 2.00 (3H, s, H_3-21), and four olefinic protons; three were assigned to the β-substituted furan ring[28,29] based on a pattern arising from aromatic resonances at (δ 6.50 (1H, d, J =1.5 Hz, H-14), 7.46 (1H, t, J =1.5 Hz, H-15), and 7.53 (1H, d, J =1.5 Hz, H-16)), and an olefinic proton assigned at δ 6.66 (1H, dd, J =10.5, 1.9 Hz, H-3), as well as two methine proton signals shifted downfield at δ 5.40 (1H, ddd, J = 4.9, 3.6, 2.1 Hz), and 5.87 (1H, dd, J =10.5, 3.4 Hz), were assigned to H-7 and H-2, respectively. The ^{1}H NMR spectrum also showed signals corresponding to an oxymethylene protons at δ 3.94 (1H, dd, J =7.2, 2.2 Hz, H-19α), and 5.35 (1H, d, J = 7.2 Hz, H-19β). The ^{13}C NMR data of (**5**) {obtained from HMQC and HMBC spectra} (Table 1) showed the presence of 21 carbon atoms in (**5**) from which 16 are protonated (two methyls at δ 12.00 (C-17), and 21.00 (C-21), five methylenes including an oxygenated carbon at δ 73.00 (C-19), five methines including two oxygenated carbons at δ 66.00 (C-2) and 74.00 (C-7), and four olefinic carbons at δ 109.0 (C-14), 131.0 (C-13), 141.0 (C-16), 144.0 (C-15)), arising from the β-substituted furan ring.[29-31] The remaining five carbons are quaternary including two carbonyls at δ 170.5 (C-20), and 171.0 (C-18). The methyl group at (δ 21.0) and the ester carbonyl group at (δ 170.5) suggested the presence of an acetoxyl moiety in the molecule of (**5**). The olefinic carbons and the two carbonyl groups accounted for five of the nine unsaturations, thus implying that (**5**) consisted of four-ring system with a structure related to a tetracyclic clerodane diterpenoid[29,32] and was nearly similar to that of Salvigresin isolated previously from *Salvia greggii*.[33] On the bases of the interpretation of HMQC which gave the

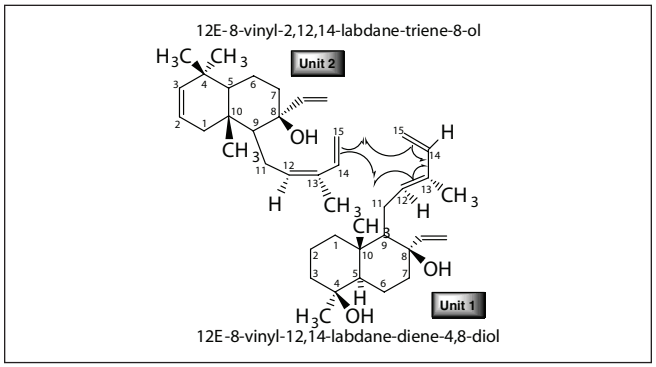

Figure 2: The structure of the two units of 4.

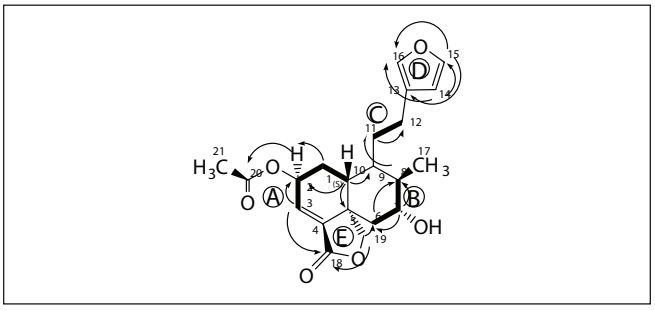

Figure 3: HMQC and HMBC correlations of partial structure of Salviaclerodan A.

corresponding carbon assignments and HMBC experiments, which used for detection of quaternary carbon atoms and all possible two-and three-bond inter-and intra-residue correlations. HMQC of (**5**) suggested the presence of five partial structures; A-E (Figure 3), in addition to the quaternary carbon at δ 41.0 (C-5), two methyl groups at δ 12.0 (CH_3-17) and 21.0 (CH_3-21) and the above two carbonyl carbons. The connectivity of these partial structures was deduced from HMBC spectrum of (**5**). The methyl group singlet at δ 2.0 (H_3-21) showed correlation to the carbonyl carbon at δ 170.5 (C-20), the methine proton at δ 2.30 (H-10) was correlated to the carbon at δ 66.0 (C-2) of segment **A**. The methine carbon at δ 41.50 (C-8) of the segment **B** and the methylene carbon at δ 30.0 (C-11) of segment **C**. The olefinic proton at δ 6.66 (H-3) of the segment **A** with carbonyl group at δ 171.0 (C-18). The methylene protons at δ 3.94 and 5.35 (H_2-19) of the segment **C** were correlated to the quaternary carbons at δ 139.00 (C-4), 41.0 (C-5) and 171.00 (C-18) of the segment **E**, respectively. On the other hand the connectivities observed between H-14 (δ 6.50) and the olefinic carbons at δ 141.0 (C-16) and 144.0 (C-15); H-15 (δ 7.46) and the olefinic carbon at δ 131.0 (C-13), and between H-16 (δ 7.53) and (C-13 and C-16) of the segment **D**, confirmed the presence of *β*-substituted furan ring.[29-32, 34, 35] The correlations observed between H-2 (δ 5.87) and carbonyl group at δ 170.50 (C-20) indicated that the acetyl group was attached at C-2. The relative stereochemistry of the chiral centers in structure (**5**) could be assigned readily on the basis of signal multiplicities, chemical shifts and coupling constants in the ^{1}H NMR spectrum. Large coupling constants between H-2 and H-10 (10.5 Hz) required that H-2 and H-10 be found in axial α-and β-orientations, respectively. Furthermore, diaxial couplings between H-7 and H-8 (4.9 Hz) required that both hydroxyl and methyl groups must be equatorial. Therefore H-7 and H-8 must be in α-and β-orientations, respectively. The most difficult stereochemical assignment was at H-9. Observation of the multiplet couplings between H-9 and the two H-11 hydrogens, implied that H-9 could be in α-or β-orientation. The structure of (**5**) was closely related to salvigresin isolated from *Salvia gregii*.[25, 28] The most notable difference with the salvigresin is the missing of an acetyl, a methyl, and a hydroxyl group from positions, 7, 9 and 12, respectively in (**5**). Therefore by use of a combination of ^{1}H NMR, HMQC and HMBC, the structure of **(5)** was established as (5*S*, 7*R*, 8*R*, 9*S*, 10*R*)-2*S*-acetoxy-15, 16-epoxy-cleroda-3, 13(16), 14-trien-18, 19-olide, a new natural clerodane type diterpene and was named as Salviaclerodan A.

Compound 11: was isolated as a light yellow powder and gave on TLC violet and yellow color with vanillin/H_2SO_4 and pauly's reagents,[36] respectively. $(\alpha)^{25}_D$ - 10.7^0 (c 1.0, $CHCl_3$). The molecular formula of (**11**) was determined to be $C_{18}H_{14}O_7$ on the bases of a positive FAB-MS m/z 343 $(M + H)^+$, along with a significant fragment peak at *m/z* 307 $(M + H - 2H_2O)^+$. The UV absorptions at 291 and 325 nm showed that (**11**) has a conjugated aromatic system. Absorption bands at 3430 cm^{-1} (hydroxyl), 1710 cm^{-1} (conjugated carbonyl) and 1590 cm^{-1} (aromatic), functional groups were suggested by IR spectrometry. 1D (^{1}H NMR) spectrum, in conjunction with detailed two dimensional (2D) analysis by the ^{1}H-^{1}H correlation spectroscopy (COSY), heteronuclear multiquantum coherence (HMQC), and heteronuclear multiple bond connectivity (HMBC) (Table 3) were performed on compound (**11**) and revealed the presence of two aromatic rings, two pairs of *trans* olefinic protons and two carboxylic groups (a carbon signal at δ_C 168.50, reasonably assigned to a carboxylic carbonyl carbon).

The ^{1}H NMR data also revealed the presence of ten protons (five proton signals integrated for two protons each) in addition to a singlet peak integrated for two protons at δ 10.99, confirmed (**11**) is a dimmeric structure of cinnamic acid derivative and indicating that the linkage between the two acid moieties by one ether bond. Examination of assignments (signal multiplicities, chemical shifts and coupling constant) in the ^{1}H NMR spectrum achieved for all protons of (**11**) were in agreement for those reported for two (*E*)-caffeic acid moieties.[37-39] Two *trans*-olefinic protons (AB-system, J =16 Hz) at δ 7.38 (2H, H-7, H-7') and δ 6.15 (2H, H-8, H-8'), six aromatic protons belonging to two set of typical ABX- spin system; (signals for 1,3,4-trisubstituted aromatic ring protons were observed at δ 7.11 (2H, d, J = 1.9 Hz, H-2, H-2'), δ 6.75 (2H, d,

Table 3: ^{1}H and ^{13}C NMR spectral data of compound 11 (500 MHz for ^{1}H, and 100 MHz for ^{13}C, DMSO-d_6).

Position	^{1}H (*J* in Hz)	COSY	^{13}C NMR*	HMBC
1,1'	–	–	126.50	–
2,2'	7.11, d, 1.9	–	115.00	C-3, 3' ; C-4, 4'; C-6, 6'
3,3'	–	–	148.50	–
4,4'	–	–	146.50	–
5,5'	6.71, d, 8.2	H-6, 6'	116.50	C-1, 1'; C-3, 3'; C-4, 4'
6,6'	6.92, dd, 8.2, 1.9	H-5, 5'	121.00	C-2, 2'; C-4, 4'
7,7'	7.38, d, 16.0	H-8, 8'	142.50	C-2, 2'; C-6, 6'; C-9, 9'
8,8'	6.15, d, 16.0	H-7, 7'	116.00	C-1, 1'; C-9, 9'
9,9'	10.99, brs	–	168.50	–

*Data obtained from HMQC and HMBC.

J = 8.2 Hz, H-5, H-5') and δ 6.92 (2H, dd, *J* =8.2, 1.9 Hz, H-6, H-6')), meaning that one of C-3 or C-4 hydroxyl groups were substituted in the dimeric structure of (*E*)-caffeic acid . These observations were also confirmed by ^{1}H-^{1}H-COSY-NMR spectrum which showed connectivities between H-7 and H-8 and between H-7' and H-8'. Furthermore, protonated carbons and quaternary carbons were assigned by HMBC. Thus long range correlations between the (H-7 (δ 7.38) and C-7 (142.50)/C-8 (116.00)) unsubstituted double bond and the carbons of the unsubstituted caffeic acid moiety δ 115.00, (C-2) and δ116.50 (C-6), showed that the caffeic acid units was involved only by an ether linkage. Moreover, the H-7/H-8 and H-7'/H8' are also coupled with acid carbonyl carbons C-9/ C-9' which resonated at δ 168.50. Short and long range correlations allowed to distinguish the C-1, C-2, C-3, C-4, C-5 and C-6 signals of each residual phenolic rings, and to attribute H-2 at (δ 7.11, d, *J* =1.9 Hz), H-5 at 6.71 (d, *J* = 8.2 Hz) and H-6 at δ (6.92, dd, *J* = 8.2, 1.9 Hz) in each ring. Long range correlations of both H-5 protons with both substituted C-3 carbons indicated that the linkage between caffeic acid moieties was 3, 3' bond. FAB-MS of (**11**) confirmed also the location of the dimeric structure on C-3, C-3' by a fragment at m/z 307 $(M + H - 2H_2O)^+$, clearly confirmed the loss of two molecules of water from two free carboxylic acids. Additionally C-3/C-3' signal of (**11**) was shifted downfield to (δ 148.50) in comparison with those reported for unsubstituted caffiec acid C-3 δ 144.50 (Δ + 4.0 ppm) dimmer and tetramer (Yannanic acid) derived by oxidative coupling of two molecules of rosmarininc acid isolated previously from *Salvia yannanesis*.[39] Therefore the structure of (**11**) was a new caffeic acid dimmer, and was named 3, 3'-didehydrodicaffeic acid.

Results of cytotoxic activity: Cell lines SNU-398, Hep G2, PLC/PRF/5, HT-1376, UM-UC-3, Hs 388.T, Hs 751.T were the most sensitive of all lines examined to the activities of *S. lanigra* and *S. splendens* extracts (MeOH, Acetone and *n*-BuOH) (Table 4). These extracts showed also moderate cytotoxic activities when tested against ovary adenocarcinoma (NIH:OVCAR-3 and SK-OV-3). Cell line MES-SA/MX2 was the least sensitive of all lines examined to the activities of these extracts. However, neither extract, showed any cytotoxic activity (>100) against kidney hypernephroma (SW 156), kidney carcinoma (A-498) and uterine sarcoma (MES-SA) cells in a concentration-dependent manner at the recommended NCI (USA) doses.[40] The diterpenes containing 11, 14-dione; Horminone (**1)** and 7-O-ethoxy-horminone (**2**), 11, 12-dihydroxyl groups; salviatane B (**3**), *bis*-labdane type diterpene; salvianol A (**4**) and 7-hydroxy clerodane derivative; salviaclerodan A (**5**) showed the most potent cytotoxic activities of the active compounds isolated and identified, showing significant activity (ED_{50} = 2.25 to 18.70 μg/mL), with human liver hepat-cellular carcinoma (SNU-398, Hep G2), human liver hepatoma (PLC/PRF/5), urinary bladder transitional cell carcinoma (UM-UC-3) and Lymph node Hodgkin's disease; Hodgkin's lymphoma (Hs 388.T, Hs 751.T) cell lines (Table 5). Salvigenin (**9**) was generally more potent than apigenin (**10**) and less potent than diterpenes **(1-5)**, possessing the most cytotoxic activities with human liver hepato-cellular carcinoma (SNU-398, Hep G2), human liver hepatoma (PLC/PRF/5), urinary bladder carcinoma (HT-1376), stomach gastric carcinoma (Hs 746T, Hs 740.T), ovary adenocarcinoma (NIH:OVCAR-3, SK-OV-3) (Table 5).

Results of antioxidant activity: Among the three different extracts, acetone extract expected to have the highest activity in DPPH• quenching (65.5%) while MeOH extract (59.8%) and *n*-BuOH extract (57.2%) showed slightly less DPPH• activity than D, L-α-tocopherol (72.3%) but slightly more than BHT (52.6%) (**Table 6**). The results obtained with compounds (**1-5**, **9** and **10**); salviatane B (**3)** (82.4 %), Horminone (**1)** (78.8%), and 7-O-ethoxy-horminone (**2)** (74.5%) showed the highest DPPH• quenching than those of such typical antioxidants D, L-α-tocopherol (72.3%) and BHT (52.6%). Salvigenin (**9)** (58.5%) and apigenin (**10)** (55.5) showed slightly less DPPH• activity than D, L-α-tocopherol (72.3%), but highest than BHT (52.6%). Salvianol A (**4)** and salviaclerodan A (**5**) showed less DPPH• quenching activity than D, L-α-tocopherol and BHT (Table 6).

Horminone (1): A pale yellow amorphous solid; UV λmax (MeOH) nm: 270, 402; IR υmax (KBr) cm^{-1}: 3395, 1665, 1640, 1607; ^{1}H NMR (400 MHz, acetone-d_6) δ 4.73 (1H, *dd*, *J* = 3.6, 1.4 Hz, H-7), 3.65 (2H, m, H2-21), 3.17 (1H, hept, *J* = 7.1 Hz, H-15), 1.23 (3H, s, H3-20), 1.21 (3H, *d*, *J* = 7.1 Hz, H_3-16), 1.17 (3H, d, J= 7.1 Hz, H_3-17), 0.94 (3H, s, H_3-19), 0.91 (3H, s, H_3-18); ^{13}C NMR (100 MHz, acetone-d_6) δ 188.67 (C-14, s), 185.03 (C-11, s), 153.16 (C-12, s), 147.85 (C-9, s), 143.76 (C-8, s), 124.51 (C-13, s), 62.57 (C-7, d), 46.17 (C-5, d), 41.99 (C-3, t), 39.85 (C-10, s), 36.58 (C-1, t), 33.63 (C-4, s), 33.55 (C-19, q), 27.20 (C-6, t), 24.69 (C-15, d), 22.13 (C-18, q), 20.28 (C-20, q), 20.16 (C-17, q), 19.56 (C-2, t), 18.74 (C-16, q); positive ESIMS *m/z* 333 $[M + H]^+$, 1030 $[3M + H + K]^+$.

7-*O*-ethylhorminone (2): A yellow amorphous powder; UV λmax (MeOH) nm: 273, 402; IR υ_{max} (KBr) cm^{-1}: 3400, 1665, 1645; ^{1}H NMR (400 MHz, acetone-d_6) δ 4.40 (1H, *dd*, *J* = 3.4, 1.8 Hz, H-7), 3.18 (1H, hept, *J* = 7.0 Hz, H-15), 1.23 (3H, s, H_3-20), 1.21 (3H, *d*, *J* = 7.0 Hz, H_3-16), 1.17 (3H, *d*, *J* = 7.0 Hz, H_3-17), 1.11 (3H, *t*, *J* = 7.0 Hz, H_3-22), 0.93 (3H, s, H_3-19), 0.92 (3H, s, H_3-18); ^{13}C NMR (100 MHz, acetone-d_6) δ 186.98 (C-14, s), 185.00 (C-11, s), 152.79 (C-12, s), 148.04 (C-9, s), 142.18 (C-8, s), 124.78 (C-13, s), 69.88 (C-7, d), 65.63 (C-21, t), 46.35 (C-5, d), 41.93 (C-3, t),

Table 4: Cytotoxicity of *S. lanigra* and *S. splendens* extracts against selected Liver, Kidney, Urinary bladder, Stomach, Ovary, Lymph node, Spleen and Uterus tumor cell lines.

	Salvia lanigra extracts ED_{50} (µg/mL)			*Salvia splendens* extracts ED_{50} (µg/mL)		
Cell Line	**MeOH**	**Acetone**	***n*-BuOH**	**MeOH**	**Acetone**	***n*-BuOH**
Liver hepatocellular carcinoma						
SNU-398	14.22 (± 0.12)	9.83 (± 0.05)	10.04 (± 0.08)	16.56 (± 0.18)	7.42 (± 0.05)	9.14 (± 0.05)
Hep G2	8.56 (± 0.05)	6.95 (± 0.03)	8.12 (± 0.05)	7.82 (± 0.09)	9.15 (± 0.07)	12.16 (± 0.10)
Liver hepatoma						
PLC/PRF/5	13.35 (± 0.10)	10.66 (± 0.08)	18.10 (± 0.15)	17.50 (± 0.16)	19.85 (± 0.18)	17.58 (± 0.15)
Kidney hypernephroma						
SW 156	>100	>100	>100	>100	>100	>100
Kidney carcinoma						
A-498	>100	>100	>100	>100	>100	>100
Urinary bladder carcinoma						
HT-1376	34.92 (± 0.28)	19.78 (± 0.17)	22.73 (± 0.20)	43.12 (± 0.30)	17.85 (± 0.15)	20.73 (± 0.20)
Urinary bladder transitional cell carcinoma						
UM-UC-3	20.18 (± 0.18)	23.45 (± 0.22)	26.12 (± 0.25)	16.35 (± 0.15)	15.25 (± 0.15)	20.14 (± 0.20)
Stomach gastric carcinoma						
Hs 746T	>100	65.91 (± 0.58)	>100	85.60 (± 0.75)	78.48 (± 0.70)	90.85 (± 0.80)
Hs 740.T	>100	>100	>100	>100	80.15 (± 0.76)	87.45 (± 0.80)
Ovary adenocarcinoma						
NIH:OVCAR-3	66.15 (± 0.55)	58.40 (± 0.45)	77.92 (± 0.68)	72.30 (± 0.60)	85.10 (± 0.75)	88.12 (± 0.77)
SK-OV-3	70.95 (± 0.60)	65.78 (± 0.50)	>100	77.18 (± 0.65)	60.90 (± 0.45)	93.24 (± 0.80)
Lymph node (Hodgkin's lymphoma)						
Hs 388.T	14.95. (± 0.15)	8.50 (± 0.05)	16.13 (± 0.16)	18.25 (± 0.16)	11.45 (± 0.08)	10.33 (± 0.08)
Hs 751.T	7.93 (± 0.05)	5.65 (± 0.03)	7.72 (± 0.04)	9.12 (± 0.08)	5.18 (±0.03)	6.55 (± 0.05)
Uterus (uterine sarcoma)						
MES-SA	>100	>100	>100	>100	>100	>100
MES-SA/ MX2	75.38 (± 0.68)	78.78 (± 0.70)	83.90 (± 0.85)	81.17 (± 0.60)	85.53 (± 0.80)	88.85 (± 0.85)

Values are presented as mean ± SE of 2 test sample observation, compared with that of control group ($p < 0.05$) for all value

Table 5: Cytotoxicity of isolated compounds (1-5, 9 and 10) from *S. lanigra* and *S. splendens* against selected Liver, Kidney, Urinary bladder, Stomach, Ovary, Lymph node, Spleen and Uterus tumor cell lines.

Cell Line	ED_{50} (µg/mL)						
	1	2	3	4	5	9	10
Liver hepatocellular carcinoma							
SNU-398	8.28 (± 0.05)	9.03 (± 0.05)	3.39 (± 0.02)	5.80 (± 0.05)	11.75 (± 0.08)	66.50 (± 0.50)	10.62 (± 0.06)
Hep G2	5.68 (± 0.03)	5.70 (± 0.04)	2.25 (± 0.02)	12.54 (± 0.15)	15.95 (± 0.10)	73.11 (± 0.55)	17.24 (± 0.12)
Liver hepatoma							
PLC/PRF/5	4.95 (± 0.02)	6.82 (± 0.05)	3.10 (± 0.01)	11.97 (± 0.10)	18.55 (± 0.08)	82.25 (± 0.66)	21.66 (± 0.14)
Kidney hypernephroma							
SW 156	>100	>100	>100	>100	>100	76.50 (± 0.50)	50.52 (± 0.32)
Kidney carcinoma							
A-498	>100	>100	>100	>100	>100	55.98 (± 0.50)	43.87 (± 0.26)
Urinary bladder carcinoma							
HT-1376	22.19 (± 0.15)	20.08 (± 0.10)	17.35 (± 0.10)	27.03 (± 0.15)	32.65 (± 0.18)	49.88 (± 0.30)	29.38 (± 0.18)
Urinary bladder transitional cell carcinoma							
UM-UC-3	16.55 (± 0.12)	13.30 (± 0.08)	7.40 (± 0.04)	10.14 (± 0.08)	25.35 (± 0.13)	>100	>100
Stomach gastric carcinoma							
Hs 746T	>100	80.55 (± 0.68)	>100	>100	75.90 (± 0.65)	>100	34.60 (± 0.22)
Hs 740.T	> 100	>100	>100	64.95 (± 0.58)	70.87 (± 0.60)	91.59 (± 0.76)	28.11 (± 0.16)
Ovary adenocarcinoma							
NIH:OVCAR-3	75.14 (± 0.65)	62.55 (± 0.50)	58.17 (± 0.45)	80.85 (± 0.80)	78.65 (± 0.70)	69.95 (± 0.55)	6.78 (± 0.03)
SK-OV-3	70.95 (± 0.60)	>100	80.25 (± 0.75)	73.50 (± 0.75)	>100	>100	20.74 (± 0.11)
Lymph node (Hodgkin's lymphoma)							
Hs 388.T	18.70. (± 0.15)	12.90 (± 0.06)	5.15 (± 0.03)	8.20 (± 0.05)	21.25 (± 0.18)	96.56 (± 0.70)	48.99 (± 0.15)
Hs 751.T	4.90 (± 0.05)	8.54 (± 0.03)	3.10 (± 0.04)	6.74 (± 0.05)	25.60 (±0.03)	83.15 (± 0.65)	70.13 (± 0.53)
Uterus (uterine sarcoma)							
MES-SA	>100	>100	>100	>100	>100	>100	66.70 (± 0.15)
MES-SA/ MX2	>100	>100	>100	>100	>100	>100	53.90 (± 0.53)

Values are presented as mean ± SE of 2 test sample observation, compared with that of control group ($p < 0.05$) for all value

Table 6: Effects of *Salvia lanigra* extracts (MeOH, Acetone and *n*-BuOH), isolated compounds (1-5, 9 and 10) and positive controls on the *in vitro* Free Radical Generation.

Bioassay	DPPH• % decoloration Sample 1000 µg/ml	Bioassay	DPPH• % decoloration Sample 1000 µg/ml
MeOH extract	59.8 ± 2.95	Compound 4	35.8 ± 1.50
Acetone soluble	65.5 ± 3.10	Compound 5	42.3 ± 1.85
n-BuOH soluble	57.2 ± 2.75	Compound 9	55.5 ± 2.44
Compound 1	78.8 ± 3.35	Compound 10	58.5 ± 2.82
Compound 2	74.5 ± 3.15	D and L-α-tocopherol	86.4 ± 3.50
Compound 3	82.4 ± 3.30	BHT	62.4 ± 2.75

Values are presented as mean + SE of 3-test sample observation.
$P < 0.05$ for all values.

39.76 (C-10, s), 36.55 (C-1, t), 33.71 (C-4, s), 33.47 (C-19, q), 24.87 (C-15, d), 23.77 (C-6, t), 22.20 (C-18, q), 20.37 (C-22, q), 20.05 (C-20, q), 19.55 (C-2, t), 18.81 (C-17, q), 16.08 (C-16, q); positive ESIMS *m/z* 360 $[M]^+$, 361 $[M + H]^+$, 315 $[M - CH_3CH_2O]^+$, 297 $[M - CH_3CH_2O - H_2O]^+$, 255 $[M + H - isopropyl - H_2O]^+$.

Salviatane B (3): A yellow amorphous solid; UV λmax (MeOH) nm: 280, 245; IR υ_{max} (KBr) cm^{-1}: 3420, 1615, 1580, 1502; ^1H NMR (400 MHz, acetone-d_6) and ^{13}C NMR (100 MHz, acetone-d_6) data are listed in Table 1; ESIMS *m/z* 363 $[M + H]^+$, 317 $[M + H - CH_3CH_2O]^+$.

Salvianol A (4): A white crystal solid; $[\alpha]^{25}_D$ - 25 (c.0.1, $CHCl_3$); UV λ_{max} (MeOH) nm: 280, 245; IR υ_{max} (KBr) cm^{-1}: 3395, 1660, 1387, 1367, 895; ^1H NMR (400 MHz, acetone-d_6) and ^{13}C NMR (100 MHz, acetone-d_6) data are listed in Table 2; ESIMS *m/z* 643 $[M + K]^+$.

Salviaclerodan A (5): A colorless prisms; $[\alpha]^{25}_D$ - 115° (c.0.20, $CHCl_3$); UV λmax (MeOH) nm: 240; IR υ_{max} (KBr) cm^{-1}: 3450, 1760, 1755, 1490, 876; ^1H NMR (500 MHz, DMSO-d_6) and ^{13}C NMR (100 MHz, DMSO-d_6) data are listed in Table 3; EIMS *m/z* 374 $[M]^+$.

Ursolic acid (6): An amorphous solid [MeOH]; IR υ_{max} (KBr) 3455, 2955, 1710, 1632, 1160 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 4.99 (1H, brs, H-12), 2.92 (1H, *dd*, *J* = 10.3, 4.5 Hz, H-3), 1.08 (3H, s, H_3-27), 0.89 (3H, s, H_3-23), 0.84 (3H, s, H_3-25), 0.74 (3H, s, H_3-26), 0.70 (3H, s, H_3-24), 0.68 (3H, *d*, *J* = 6.8 Hz, H_3-29), 0.63 (3H, *d*, *J* = 6.8 Hz, H_3-30); ^{13}C NMR (75.0 MHz, DMSO-d_6) δ 179.22 (C-28, *s*), 137.76 (C-13, *s*), 124.17 (C-12, *d*), 76.57 (C-3, *d*), 54.59 (C-5, *d*), 52.19 (C-18, *d*), 46.98 (C-9, *d*), 45.53 (C-17, *s*), 41.50 (C-14, *s*), 39.23 (C-8, *s*), 38.95 (C-19, *d*), 38.66 (C-20, *d*), 38.32 (C-1, *t*), 38.20 (C-4, *s*), 36.46 (C-10, *s*), 36.40 (C-22, *t*), 32.49 (C-7, *t*), 30.17 (C-21, *t*), 29.76 (C-23, *q*), 28.30 (C-15, *t*), 27.49 (C-2, *t*), 26.67 (C-16, *t*), 23.68 (C-27, *q*), 23.00 (C-30, *q*), 22.70 (C-11, *t*), 17.80 (C-6, *t*), 16.61 (C-26, *q*), 16.52 (C-29, *q*), 15.29 (C-24, *q*), 14.92 (C-25, *q*); EIMS *m/z* 456 $[M]^+$, 248, 203.

Oleanolic acid (7): An amorphous solid [MeOH]; IR υ_{max} (KBr) 3430, 2950, 1695, 1640 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6) δ 5.15 (1H, brs, H-12), 2.99 (1H, dd, *J* = 10.0, 5.0 Hz, H-3), 1.08 (3H, s, H_3-27), 0.88 (3H, s, H_3-30), 0.86 (6H, s, H_3-23, 25), 0.83 (3H, s, H_3-29), 0.71 (3H, s, H_3-26), 0.65 (3H, s, H_3-24); ^{13}C NMR (100.0 MHz, DMSO-d_6) δ 178.58 (C-28, *s*), 143.83 (C-13, *s*), 121.49 (C-12, *d*), 76.79 (C-3, *d*), 54.77 (C-5, *d*), 47.06 (C-9, *d*), 45.67 (C-17, *s*), 45.44 (C-19, *t*), 42.12 (C-14, *s*), 41.80 (C-18, *d*), 41.30 (C-8, *s*), 38.37 (C-4, *s*), 38.03 (C-1, *t*), 36.59 (C-10, *s*), 33.30 (C-21, *t*), 32.82 (C-29, *q*), 32.39 (C-22, *t*), 32.07 (C-7, *t*), 30.39 (C-20, *s*), 28.22 (C-23, *q*), 27.19 (C-2, *t*), 26.94 (C-15, *t*), 25.58 (C-27, *q*), 23.36 (C-30, *q*), 22.59 (C-16, *t*), 22.49 (C-11, *t*), 18.00 (C-6, *t*), 16.84 (C-26, *q*), 16.02 (C-24, *q*), 15.09 (C-25, *q*); EIMS *m/z* 456 $[M]^+$, 248, 203.

β-sitosterol (8): A colorless needles; IR υ_{max} (KBr) cm^{-1}: 3450, 1635, 1160; ^1H NMR (300 MHz, DMSO-d_6) δ 5.20 (1H, *m*, H-6), 3.25 (1H, *m*, H-3), 1.16 (3H, *s*, H_3-19), 0.88 (3H, *t*, *J* = 7.0 Hz, H_3-29), 0.84 (3H, *d*, *J* = 6.0 Hz, H_3-21), 0.74 (3H, *d*, *J* = 6.6 Hz, H_3-27), 0.72 (3H, *d*, *J* = 6.6 Hz, H_3-26), 0.58 (3H, *s*, H_3-18); EIMS *m/z* 414 $[M]^+$.

Salvigenin (9): A yellowish-white amorphous powder; UV λ_{max} (MeOH) nm: 275, 340; IR υ_{max} (KBr) cm^{-1}: 3410, 1700, 1585; ^1H NMR (400 MHz, acetone-d_6) δ 12.90 (1H, *brs*, 5-OH), 8.03 (2H, *d*, *J* = 8.6 Hz, H-2`,6`), 7.14 (2H, *d*, *J* = 8.6 Hz, H-3`,5`), 6.85 (1H, *s*, H-3), 6.71 (1H, *brs*, H-8), 3.98 (3H, *s*, 6-OMe), 3.91 (3H, *s*, 7-OMe), 3.80 (3H, *s*, 4'-OMe); ^{13}C NMR (100 MHz, acetone-d_6) δ 183.48 (C-4), 164.95 (C-2), 163.77 (C-5), 160.10 (C-4'), 154.06 (C-9), 153.95 (C-7), 133.52 (C-6), 129.06 (C-2', 6'), 124.30 (C-1'), 115.39 (C-3', 5'), 106.53 (C-10), 104.43 (C-3), 91.92 (C-8), 60.50 (6-OMe), 56.79 (7-OMe), 55.99 (4'-OMe); positive ESIMS *m/z* 329 $[M + H]^+$, 679 $[2M + Na]^+$, 1007 $[3M + Na]^+$.

Apigenin (10): A yellowish-white amorphous powder; UV λ_{max} (MeOH) nm: 271, 337; IR υ_{max} (KBr) cm^{-1}: 3430, 1710, 1595; ^1H NMR (400 MHz, DMSO-d_6) δ 12.98 (1H, *brs*, 5-OH), 10.50 (1H, *brs*, 4'-OH), 7.90 (2H, *d*, *J* = 8.8 Hz, H-2',6'), 6.90 (2H, *d*, *J* = 8.8 Hz, H-3',5'), 6.72 (1H, *s*, H-3),

6.40 (1H, *brs*, H-8), 6.10 (1H, *brs*, H-6); ^{13}C NMR (100 MHz, DMSO-d_6) δ 183.48 (C-4), 164.95 (C-2), 161.89 (C-5), 157.80 (C-4'), 158.08 (C-9), 164.75 (C-7), 98.50 (C-6), 128.00 (C-2', 6'), 128.30 (C-1'), 116.00 (C-3', 5'), 103.95 (C-10), 102.50 (C-3), 94.00 (C-8); negative-ion MALDI-TOFMS *m/z* 270 $[M]^+$, 269 $[M - H]^+$, 538 $[2M - 2H]^+$.

3,3'-dehydrodicaffeic acid (11): A light yellow powder; $[\alpha]^{25}_D$ - 10.7^0 (c 1.0, $CHCl_3$); UV λ_{max} (MeOH) nm: 325, 291; IR υ_{max} (KBr) cm^{-1}: 3430, 1710, 1590; ^{1}H NMR (500 MHz, DMSO-d_6) and ^{13}C NMR (100 MHz, DMSO-d_6) data are listed in Table 4; FABMS *m/z* 343 $[M + H]^+$, 435 [M + glycerol]$^+$, 307 $[M + H - 2H_2O]^+$.

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