# 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

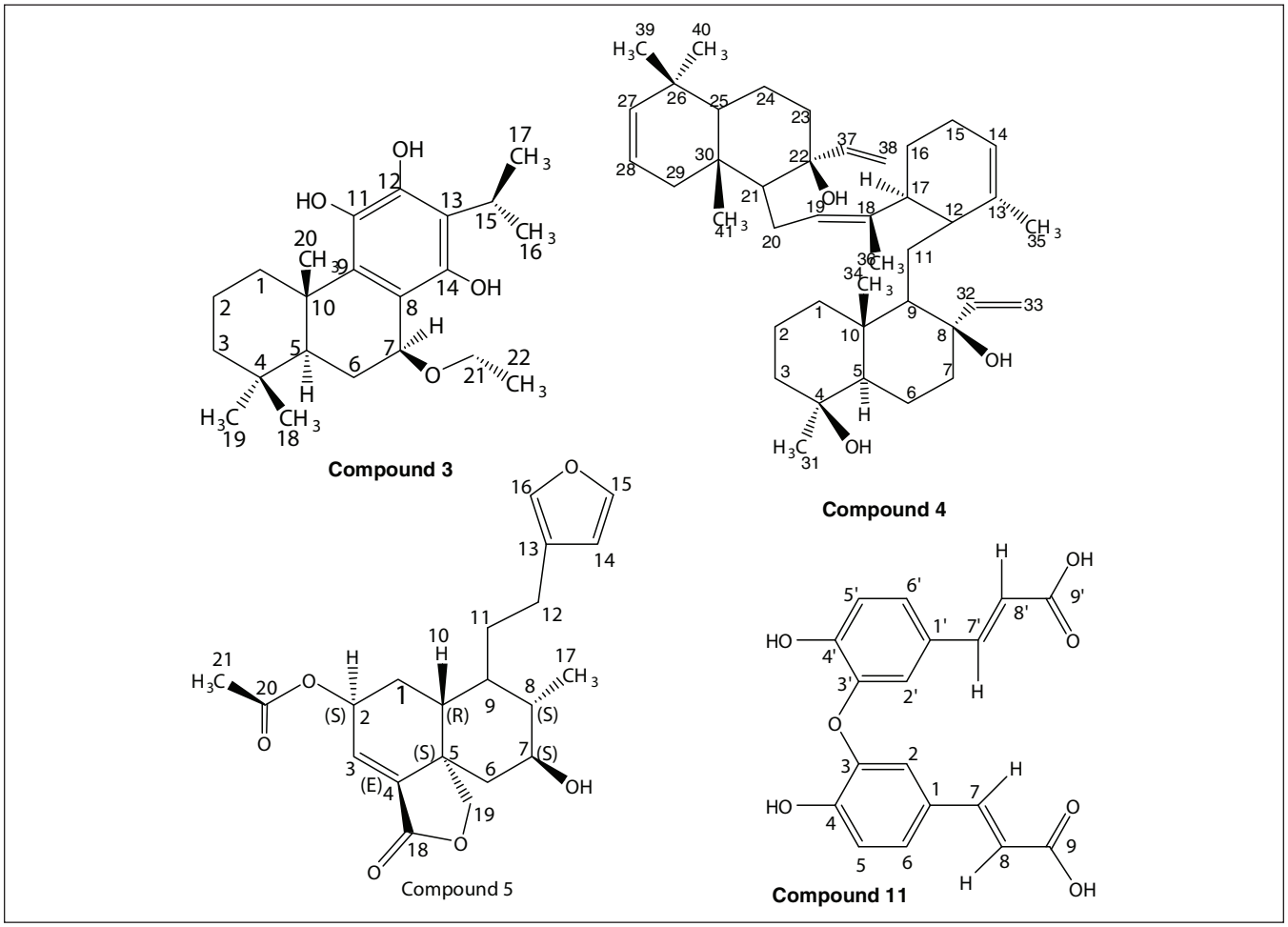


**Figure 1:** Structures of compounds 3-5 and 11.

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,