Supporting Information

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Cytotoxic Activity of New Tropinene Glycoside Isolated from *Solandra grandiflora* Sw.

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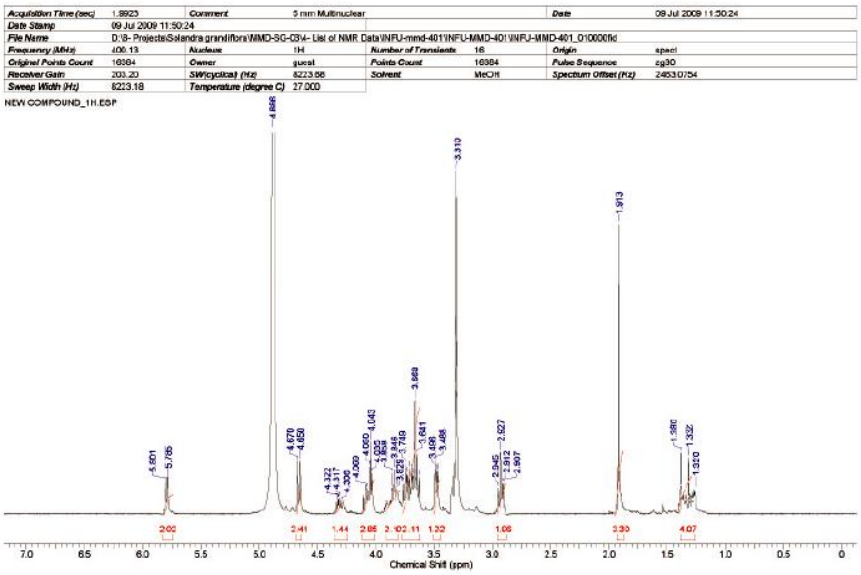
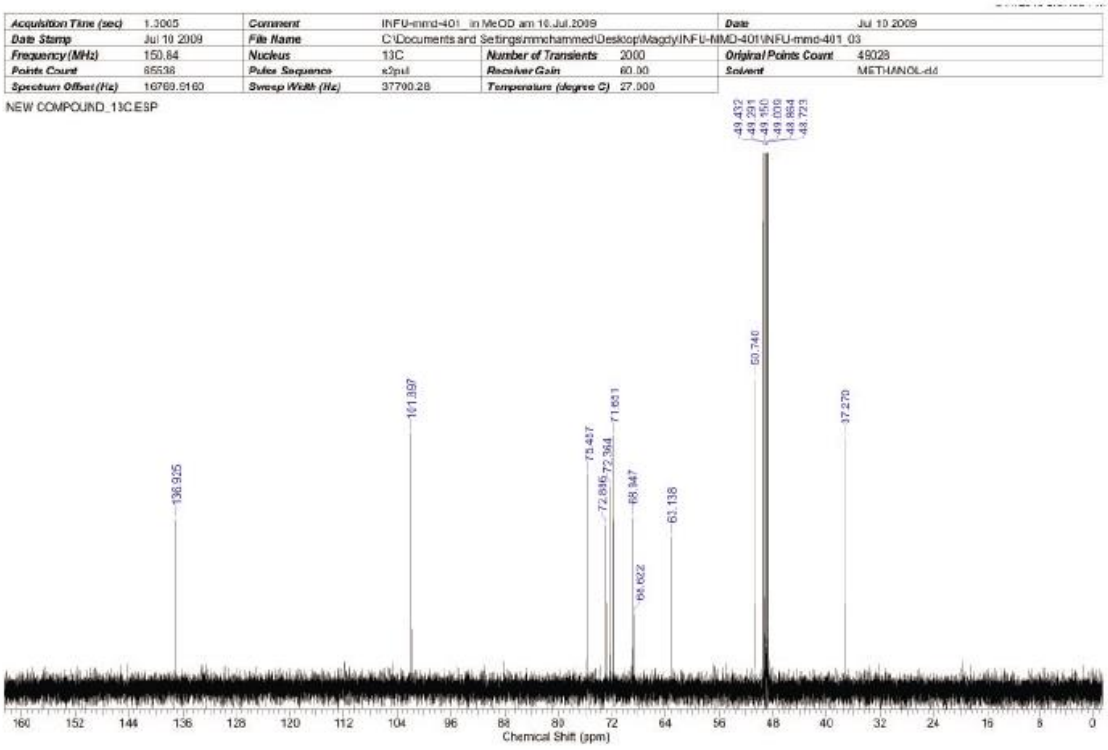
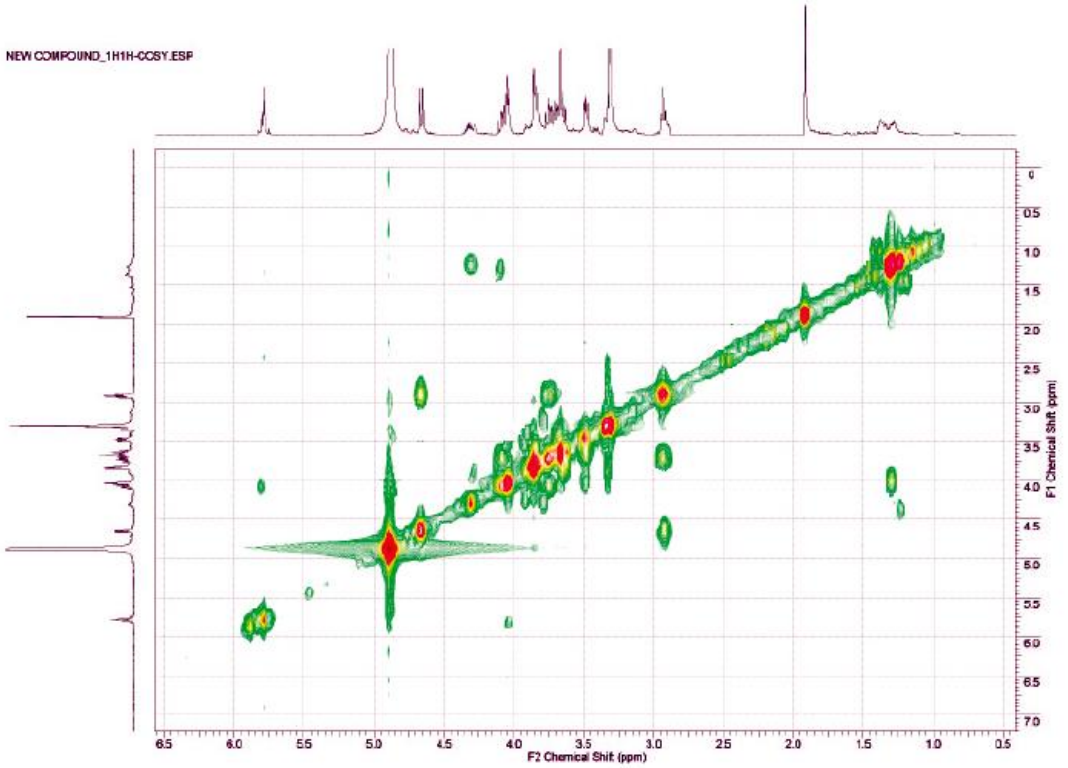


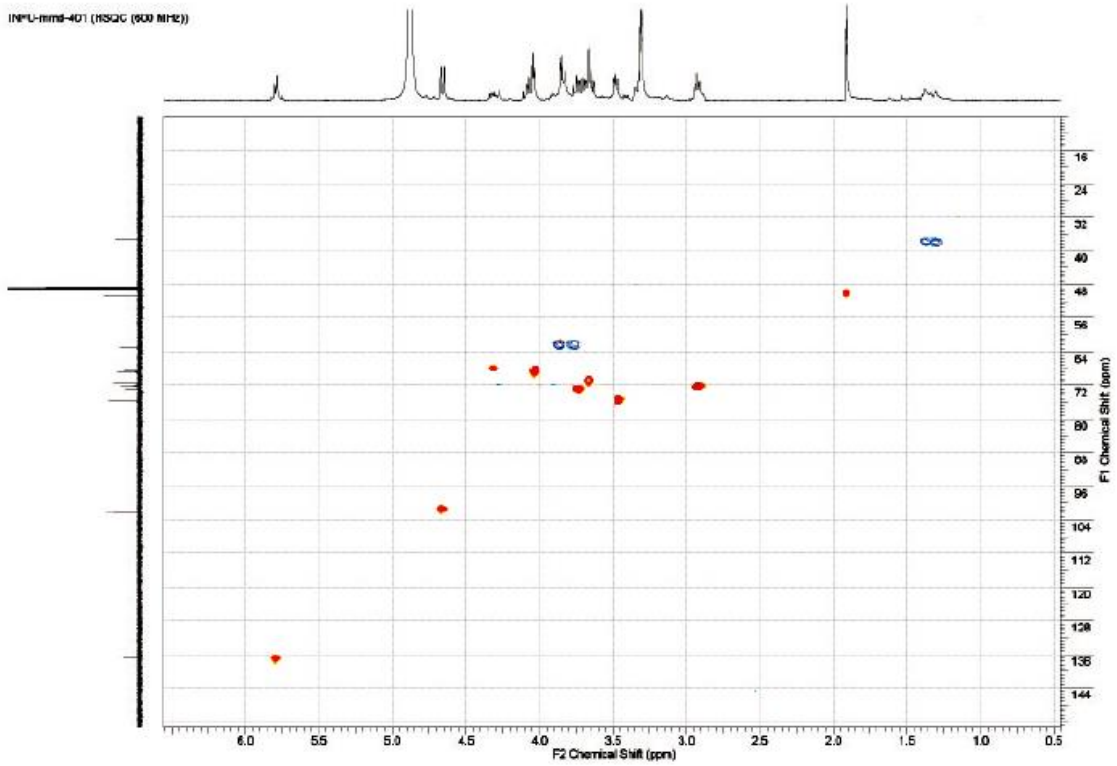
S1: ^{1}H-NMR spectrum of compound **4** (3-*O*-*β*-D-glucosyl 6-tropinene).



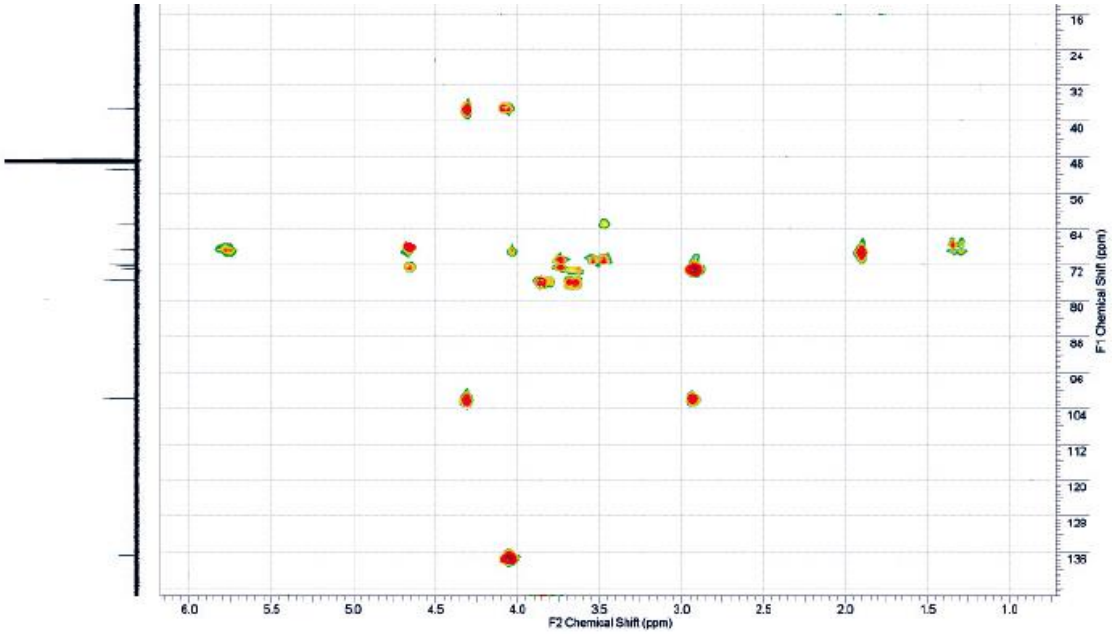
S2: ^{13}C-NMR spectrum of compound **4** (3-*O*-*β*-D-glucosyl 6-tropinene).



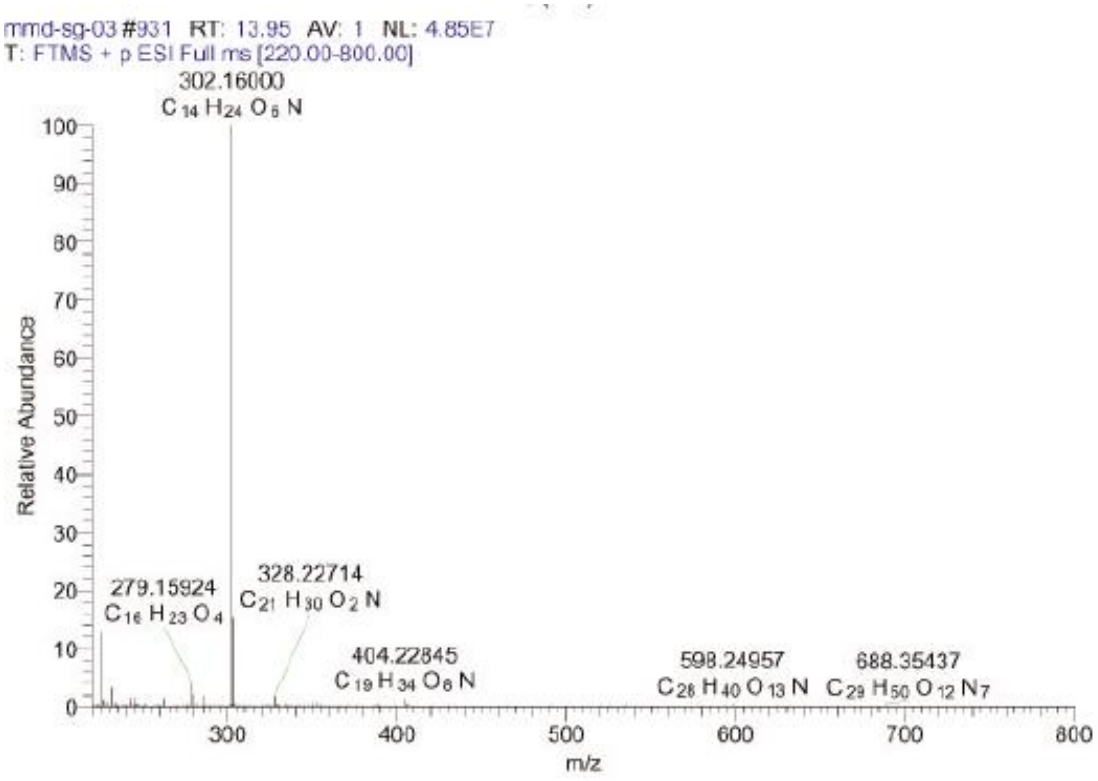
S3: COSY spectrum of compound **4** (3-*O*-*β*-D-glucosyl 6-tropinene).



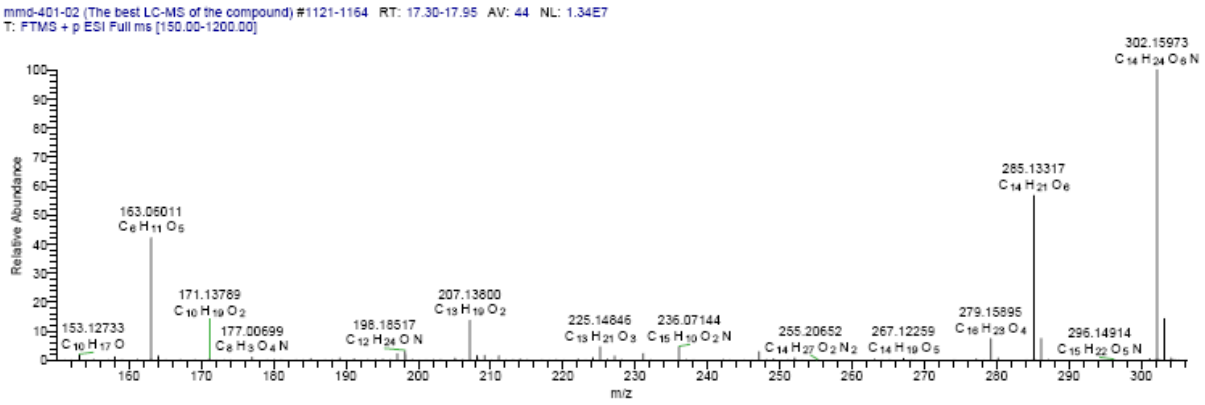
S4: HSQC spectrum of compound **4** (3-*O*-*β*-D-glucosyl 6-tropinene).



S5: HMBC spectrum of compound **4** (3-*O*-*β*-D-glucosyl 6-tropinene).



S6: HR-ESI/MS spectrum of compound **4** (3-*O*-*β*-D-glucosyl 6-tropinene).



S7: ESI/MS/MS spectrum of compound **4** (3-*O*-*β*-D-glucosyl 6-tropinene).

S8:

2. Materials and Methods

2.1. Extraction procedure

1.9 Kg of the dried powdered materials (leaves) were extracted by maceration process in a metal container using MeOH (5L x 5 times) at r.t., followed by heat-extraction with MeOH at 35 ^{o}C (5L x 2). The obtained extracts were combined together to afford 119.8 g total MeOH extract, then on cold-defatting using hexane (3L) afforded 97.3 g hexane fraction (discarded). The remaining MeOH fraction was subjected to acid-base shakeout method [1], in brief, the MeOH fraction dissolved in 2% tartaric acid (25 mL) and then extracted with EtOAc (1L) to afford the neutral fraction (10.2 g), the remaining aqueous-acid phase was made alkaline (pH = 11) with Na_2CO_3 (25 mL) followed by extraction with EtOAc (1L) to afford the alkaloidal fraction (0.5 g). Separation was achieved by RP-HPLC using solvent system composed of (A) H_2O and (B) MeOH, both of which containing 0.1% HCOOH. Elution profiles were programmed as follow: for alkaloidal fraction; 70% A isocratic (0 - 3 min), linear gradient to 100% B (3 – 20 min), after 100% B isocratic for 6 min. Afterwards, the system returned to its initial condition (70% A) within 1 min, and was equilibrated for 3 min, flow rate 3mL/min, UV 254 nm. This resulted in the isolation of three main fractions; **I** (0.124g), **II** (0.063g) & **III** (0.027g), which upon further purification using the same elution profile resulted in the isolation of two compounds **1** (20mg) & **2** (15mg) from fraction **I**, compound **3** (25mg) from fraction **II**, and compound **4** (16.2mg) from fraction **III** respectively. For the neutral fraction; 60% A isocratic (0 - 3 min), linear gradient to 100% B (3 – 20 min), after 100% B isocratic for 6 min. Afterwards, the system returned to its initial condition (60% A) within 1 min, and was equilibrated for 3 min, flow rate 8mL/min, UV 254 nm and injection volume was 5 mL. Three main fractions; **IV** (1.9g), **V** (0.9g) and **VI** (0.5g) were collected, fraction **IV** was re-fractionated using RP-HPLC with the elution profile 90% A isocratic (0 - 3 min), linear gradient to 50% B (3 – 10 min), after 50% B isocratic for 15 min. Afterwards, the system returned to its initial condition (90% A) within 4 min, and was equilibrated for 3 min, flow rate 8mL/min, UV 254 nm and injection volume was 5 mL, which resulted in the isolation of three compounds **5** (38.2mg)**, 6** (9.12mg), and **7** (10.0mg). Moreover, fraction **V** was purified on RP-HPLC with the elution profile 90% A isocratic (0 - 3 min), linear gradient to 100% B (3 – 30 min), after 100% B isocratic for 6 min. Afterwards, the system returned to its initial condition (90% A) within 21 min, and was equilibrated for 3

min, flow rate 8mL/min, UV 254 nm and injection volume was 5 mL, and resulted in the isolation of compound **8** (10.8mg). Fraction **VI** eluted with 95% A isocratic (0 - 3 min), linear gradient to 50% B (3 – 10 min), after 50% B isocratic for 1 min, followed by fast linear gradient to 100% B (10 – 25) and kept for 3 min. Afterwards, the system returned to its initial condition (95% A) within 1 min, and was equilibrated for 3 min, flow rate 8mL/min, UV 254 nm and injection volume was 5 mL, and revealed the isolation of compound **9** (15.3mg).

2.2. General instrumentation and chemicals

NMR: 1D-spectra were obtained using a pulse sequence supplied from Bruker-DRX and AVANCE-400 NMR spectrometer for (^{1}H, ^{13}C-NMR and DEPT-135), 2D-spectra (^{1}H-^{1}H COSY, HSQC and HMBC) were obtained using a pulse sequence supplied from Bruker-DRX and AVANCE-500 & -600 NMR spectrometer, and all samples were dissolved in CH_3OH-d_4 (Merck, Darmstadt, Germany) as solvent. Chemical shifts were given in values (ppm) relative to trimethylsilane (TMS) as an internal reference for both carbon and proton. The $^{3}J_{C,H}$ couplings were measured by means of pulsed field gradient HMBC spectra recorded by varying the *J*-refocusing time between t = 0.04 and 0.14 s.

Preparative reversed-phase HPLC: It was carried out using a Gilson system consisting of pump 322 with a UV detector 152 (λ = 205 nm) using a Alltech HPLC Alltima C_{18} Column (250mm x 4.6mm, 5 μm particle size) for the isolation of alkaloids (alkaloidal fraction), and a Nucleodur Gravity column from Macherey–Nagel (Düren, Germany) (250 x 16 mm, 5 μm particle size) for the isolation of phenolics (neutral fraction).

Accurate mass measurement using LC-MS: The high-resolution mass spectra were obtained with an LTQ-Orbitrap Spectrometer (Thermo Fisher, USA) equipped with a HESI-II source. The spectrometer was operated in positive mode (1 spectrum s^{-1}; mass range: 200–1000) with nominal mass resolving power of 60,000 at *m/z* 400 with a scan rate of 1 Hz with automatic gain control to provide high-accuracy mass measurements within 2 ppm deviation using an internal standard; Bis(2-ethylhexyl)phthalate: *m/z* = 391.284286. The spectrometer was attached with an Agilent 1200 HPLC system (Santa Clara, USA) consisting of LC-pump, PDA detector (λ = 205 nm), auto sampler (injection volume 10 μL) and column oven (30 ºC). MS/MS experiments were performed by CID (collision induced decay, 35 eV) mode. Following parameters were used for experiments: spray voltage 5 kV, capillary temperature 260 ºC, tube lens 70 V. Nitrogen was used as sheath gas (50 arbitrary units) and auxiliary gas (five arbitrary units). Helium served as the collision gas. The separations and purifications were performed by using a Nucleodur Gravity column (50 mm x 2mm, 1.8 μm particle size) from Macherey–Nagel (Düren, Germany) with a H_2O (+0.1% HCOOH, +10 mM NH_4Ac) (A) / acetonitrile (+0.1% HCOOH) (B) gradient (flow rate 300 μL min^{-1}). Samples were analyzed by using a gradient program as follows: 90% A isocratic for 2 min, linear gradient to 100% B over 13 min, after 100% B isocratic for 5 min, the system returned to its initial condition (90% A) within 0.5 min, and was equilibrated for 4.5 min.

2.3. Cytotoxic effect on human cell line (HepG-2 – MCF-7 – HCT-116 – A-549): Cell viability was assessed by the mitochondrial dependent reduction of yellow MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) to purple formazan [2]. All the following procedures were done in a sterile area using a Laminar flow cabinet biosafety class II level (Baker, SG403INT, Sanford, ME, USA). Cells were suspended in RPMI 1640 medium for HepG-2 – MCF-7 and HCT-116 – DMEM for A-549. The media were supplemented with 1% antibiotic-antimycotic mixture (10,000U/mL Potassium Penicillin, 10,000 μg/mL Streptomycin Sulfate and 25 μg/mL Amphotericin B), 1% L-glutamine and 10% fetal bovine serum and kept at 37 ºC under 5% CO_2. Cells were batch cultured for 10 days, then seeded at concentration of $10x10^3$ cells/well in fresh complete growth medium in 96-well microtiter plastic plates at 37 ºC for 24 h under 5% CO_2 using a water jacketed Carbon dioxide incubator (Sheldon, TC2323, Cornelius, OR, USA). Media was aspirated,

fresh medium (without serum) was added and cells were incubated either alone (negative control) or with different concentrations of sample to give a final concentration of (100-50-25-12.5-6.25-3.125-0.78 and 1.56 μg/mL). After 48 h of incubation, medium was aspirated, 40 μL MTT salt (2.5 μg/mL) were added to each well and incubated for further four hours at 37 ºC under 5% CO_2. To stop the reaction and dissolving the formed crystals, 200 μL of 10% Sodium dodecyl sulphate (SDS) in deionized water was added to each well and incubated overnight at 37 ºC. A positive control (Doxorubicin 100 μg/mL) was used as a known cytotoxic natural agent who gives 100% lethality under the same conditions [3]. The absorbance was then measured using a microplate multi-well reader (Bio-Rad Laboratories Inc., model 3350, Hercules, California, USA) at 595nm and a reference wavelength of 620nm. A statistical significance was tested between samples and negative control (cells with vehicle) using independent t-test by SPSS 11 program. DMSO is the vehicle used for dissolution of plant extracts and its final concentration on the cells was less than 0.2%. The percentage of change in viability was calculated according to the formula:

((Reading of extract / Reading of negative control) -1) x 100

A probit analysis was carried for IC_{50} and IC_{90} determination using SPSS 11 program.

References

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[2] T. Mosmann (1983). Rapid colorimetric assays for cellular growth and survival: Application to proliferation and cytotoxicity assays, *J. Immunol. Methods* **65**, 55–63.

[3] M. I. Thabrew, R. D. Hughes and I. G. McFarlane (1997). Screening of hepatoprotective plant components using a HepG2 cell cytotoxicity assay, *J. Pharm. Pharmacol.* **49**, 1132–1135.