

# Anti-proliferative activities of *Byrsocarpus coccineus* using ovarian cancer cell lines

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## Research

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# Abstract

Background: Ovarian cancer is one of the most lethal tumors of gynecologic malignancies, due to lack of early detection, and a high rate of metastasis. The standard treatment is surgery and cytotoxic chemotherapy, but due to its cost and side effects, medicinal plants are widely used in developing countries. *Byrsocarpus coccineus* plant preparation, has been administered to patients traditionally in the management of tumour in Nigeria. In this study, we investigated the anti-proliferative effects of *B. coccineus* ethanol leaf extract against OVCAR3 and SW626 ovarian cancer cell lines. After treatment of the two cell lines with the extracts, analyses were carried out to determine inhibition of proliferation and expression of cell cycle markers, pro-apoptotic and anti-apoptotic markers.

Results: Results showed that *B. coccineus* ethanol leaf extract, significantly inhibited cell migration and colony formation in OVCAR3 and SW626 treated cells in a dose-dependent manner. Results also show that *B. coccineus* ethanol leaf extract modulated the expression of p53 gene, cell cycle progression, anti-apoptotic and pro-apoptotic genes.

Conclusions: These results suggest that *B. coccineus* have anti-proliferative properties and could induce apoptosis. Further investigation will be carried out to isolate bioactive compounds for the treatment of ovarian cancer.

## Background

Ovarian cancer is the fifth most aggressive and lethal cancer worldwide in women [1], due to lack of early detection and a high rate of metastasis [2]. Epithelial ovarian carcinomas make up to 90% of malignant ovarian cancer and it is the most aggressive [3]. In 2019, 22,530 new ovarian cancer cases and 13,980 deaths due to ovarian cancer were estimated to occur in the United States [4]. The standard treatment for ovarian cancer is surgery and chemotherapy [5], but relapse occurs in most women [1].

Due to poor prognosis, cost and side effect of treatment, phytochemicals have become a growing source of alternate medicine in Africa. *Byrsocarpus coccineus* plant extract preparation is administered to patients traditionally in the management of cancer in Africa. *B. coccineus* known as 'Amuje wewe or Ado kanti-kanti' is a scandent shrub and it is indigenous to Nigeria (West Africa). Studies have shown that the plant has anti-plasmodia [6], antimicrobial [7], and anti-diarrhea activity [8]. Fractions of *B. coccineus* have been reported to modulate cytochrome P450 (CYP) enzyme activity, cytokine production and anti-proliferation in colon cancer cell line [9]. Studies have also shown that the plant extract has cytotoxic activity against human breast and prostate carcinoma cell lines [10]. Activation of p53 (a tumor suppressor protein) signaling pathway inhibits cancer cell proliferation by cell cycle arrest and induction of apoptosis through the intrinsic and extrinsic pathway [11]. The p53 protein, is a key regulator of apoptosis and has been implicated in the development of ovarian cancer[1].

Therefore, the study is to justify the folkloric use as anti-tumour plant and propose a mechanism/pathway of action of the extracts by investigating p53 involvement in cell cycle arrest and

induction of apoptosis [11].

## Results

### Cell proliferation assay

The anti-proliferation effects of *B. coccineus* ethanol leaf extract against OVCAR3 and SW626, were determined by treating the two cell lines with different concentrations of the extract. The cells were treated for 24 h, 48 h and 72 h and DMSO was used as control. The effect of the extract on cell viability was most effective dose dependently in the two cell lines, after 48 h when compared to the control. The optimal IC50 values were determined to be OVCAR3 446.5 µg/mL and SW626 486.94 µg/mL. The results indicate that *B. coccineus* ethanol leaf extract inhibits the proliferation of OVCAR3 and SW626 (Table 1).

### Live/Dead cell staining

Cytotoxic effect of *B. coccineus* ethanol leaf extract was confirmed, after treating OVCAR3 and SW626, by live/dead staining assay. The live nuclei are stained blue, whereas the dead nuclei or detached cells are stained green. Images of both cell lines (untreated and treated) taken using immunofluorescence microscope at x4 showed a high degree of dead nuclei in the treated cells, after incubating for 48 h when compared to untreated cells (Fig. 1).

### Cell migration

The effect of *B. coccineus* ethanol leaf extract on cell migration of OVCAR3 and SW626 cells was investigated using the scratch assay. This result showed that *B. coccineus* extract was able to inhibit cell migration in the two ovarian cancer cell lines (Fig. 2). In the baseline control the average gap distance was 69.25µm. After scratching and incubating OVCAR3 and SW626 cells for 24 h, 48h and 72 h, there was no significant difference in the gap distance between treated cells when compared to the 0 h, but there the gap closed completely in the untreated cells when compared to the control. It could be inferred from the results, that *B. coccineus* ethanol leaf was able to inhibit cell migration in OVCAR3 and SW626.

### Colony formation

To investigate the effect of *B. coccineus* on colony formation, a clonogenic assay was carried out using OVCAR3 and SW626 cells. After treating the two cell lines and incubating for 10 days, the results showed that *B. coccineus* could inhibit colony formation in OVCAR3 and SW626 cells, when compared to the control groups (Fig. 3).

### Flow cytometry

#### Annexin V/PI apoptosis detection

Annexin V/PI was used to determine apoptosis in OVCAR3 and SW626 cells, after treatment with *B. coccineus* 48h, staining with PI and analysed using the flow cytometer. The flow cytometer data analysis

shows that apoptotic cells were found more in the right lower (early apoptotic) quadrant (Q3) in the treated cells when compared to the control (Fig 4). This implies that *B.coccineus* was able to induce apoptosis in the two cell lines.

### ***B. coccineus* up-regulates pro-apoptotic and down-regulates anti-apoptotic markers at the mRNA level in OVCAR3 and SW626 cell lines**

The effect of *B. coccineus* ethanol extract on expression of anti-apoptotic and pro-apoptotic genes, cell cycle progression and p53 gene were investigated to determine if *B. coccineus* could induce apoptosis in OVCAR3 and SW626 cells.

The result of RT-PCR quantitative analysis of the fold change expression relative to control in OVCAR3 and SW626 treated cells (with known concentration) shows the mRNA expression of p53 gene, anti-apoptotic and pro-apoptotic genes, PARP, Caspase 3, cyclin D1, CDK4, CDK2, p19 and p21 (Fig. 5). GAPDH was used to normalize changes in the expression of protein.

Relative to the control in OVCAR3 and SW626 treated cells, RT-PCR shows a significant down regulation of McL-1 and BCL-xL and a significant up regulation of BAX, BID and BAD genes in the two cell lines. In addition the apoptotic marker caspase-3, PARP were significantly up regulated in the two cell lines. This result suggest that *B. coccineus* induces apoptosis in OVCAR3 and SW626 treated cells by inducing p53.

The result of RT-PCR analysis of OVCAR3 and SW626 treated cells relative to control, shows up regulation of p53, a tumor *suppressor gene* and p19, p21 which are CDK family inhibitors in OVCAR3 and SW626 cells. In addition, the result shows the down regulation of cyclin D1/CDK4 complex which regulates the G1/S phase, in OVCAR3 and SW626 cells. However CDK2 was not significantly down regulated in both cell lines. This result suggest that *B. coccineus* up-regulated the expression of p53 which led to cell cycle arrest and eventually apoptosis.

### **Immunomodulatory properties of *B. coccineus* on OVCAR3 and SW626 cells**

Tumor necrosis factor alpha (TNF- $\alpha$ ) and IL-10 are multifunctional inflammatory cytokine which are involved in apoptosis and cell survival. The result shows that TNF- $\alpha$  was significantly up-regulated in OVCAR3 and SW626 treated cells. IL-10 was significantly up-regulated in OVCAR3 cells, but the extract had no significant effect on SW626 treated cells (Table 2).

### ***B. coccineus* induced the regulation of pro- and anti-apoptotic protein OVCAR3 and SW626 cells**

To corroborate the anti-proliferation effects of *B. coccineus* ethanol leaf extract against OVCAR3 and SW626 treated cells, the anti- and pro-apoptotic protein markers were analysed using western blot after treating the two cell lines with known concentration of the extract.

Figure 6 shows that pro-apoptotic markers McL-1 and Bcl-xL were down regulated in SW626 and OVCAR3 treated cells, but anti-apoptotic markers BID and BAD were up regulated in SW626 when

compared to the control. This corroborates the RT-PCR results which shows that *B. coccineus* ethanol leaf extract was able to induce apoptosis by inhibiting Bcl-xL and Mcl-1 anti-apoptotic proteins and activating BID and BAD pro-apoptotic proteins in OVCAR3 and SW626 treated cells when compared to the control.

## Discussion

*B. coccineus* plant extract preparation is administered to patients traditionally in the management of various ailments including tumour in West Africa [10]. This study was able to show that *B. coccineus* ethanol leaf extract could inhibit cell proliferation by regulating some cell cycle regulators and inducing apoptosis using OVCAR3 and SW626 ovarian cancer cell lines.

This study shows that *B. coccineus* extract has anti-proliferative properties after treating for 48 h and 72 h by reducing cell viability significantly. This is corroborated by previous studies which show that *B. coccineus* has anti-proliferative activities using breast cancer cell lines and prostate cancer cell lines [10].

*B. coccineus* extract was shown in this study, to inhibit cell migration in OVCAR3 and SW626 cell lines using the scratch assay [13]. In the treated cells it was observed that cells did not migrate to the gap after incubating for 24 h, 48 h and 72h in two cell lines but in the untreated cells, cell migration to the gap was observed after incubating for 24 h, 48 h and 72h in two cell lines. These results show that *B. coccineus* was able to inhibit colony formation in the two cell lines after treating for 10 days. *B. coccineus* significantly inhibited cell migration and colony formation in the two cell lines.

Apoptosis is important in the prevention of proliferation of cancer cells. Studies have shown that most cytotoxic agents with anti-proliferative activity can regulate apoptosis [14]. The flow cytometry result indicates that *B. coccineus* induced apoptosis in OVCAR3 and SW626 ovarian cancer cell lines.

Apoptosis is one of the important functions of p53 and it leads to disruption of tumour progression [14]. The tumour suppressor gene (p53), is important in cell cycle progression and apoptosis. Up-regulation of p53 leads to induction of apoptosis [11]. Up-regulation of p53 by *B. coccineus* probably resulted in the up-regulation of pro-apoptotic markers (BAX, BID and BAD) and the down-regulation of anti-apoptotic markers (BCL-2, and MCL-1) as shown in this study. Caspase-3 (aspartate-specific cysteine proteases) and PARP genes were also up-regulated after treating the two cell lines with *B. coccineus*.

p53 regulates apoptosis through the extrinsic pathway, by up-regulating BID which inhibits anti-apoptotic proteins (Bcl-2, Bcl-XL and MCL-1) and intrinsic pathway by up-regulating BAX (See Additional file 1). p53 also activates BAD which result in the release of BAX and its translocation to the mitochondria. BAX increases the permeability of the outer mitochondria membrane which leads to an efflux of cytochrome c. Cytochrome c then binds to Apaf-1 and initiates the initiator caspase 9 and this in turn initiates executioner caspases, such as caspase 3, 6 and 7. Caspase-3 cleaves PARP which leads to DNA fragmentation and then apoptosis [15]. Up-regulation of caspase-3 and PARP in the treated cells of the two cell lines further confirms the induction of apoptosis.

Activation of p53 in OVCAR3 and SW626 cells treated with *B. coccineus*, arrest cell cycle by inducing the activation of p21 (See Additional file 1). Result shows that p21 was up-regulated and cyclin D and CDK4 were significantly down regulated in OVCAR3 and SW626 cell lines. p21, a CDK family inhibitor inhibits the cell cycle at the G1 to S phase by binding to cyclin D/CDK4 and cyclin E/Cdk2 thereby preventing its progression [11]. The binding of p21 to the CDK4 and CDK2 results in their down regulation and this prevents the phosphorylation of pRb and leads to pRb forming a complex with E2F1. This results in inhibition of DNA replication and arrest of cell cycle [16]. Studies have shown that p21 can independently regulate pro-apoptotic proteins like BAX and anti-apoptotic protein like Bcl-2 without the expression of p53 [17].

TNF and IL-10, are multifunctional cytokines produced by the immune system and they have pro- and anti-cancer effect [18]. TNF- $\alpha$  was significantly up-regulated in OVCAR3 and SW626 treated cell lines when compared to untreated cells. Studies have shown that TNF- $\alpha$  induces apoptosis through the extrinsic pathway by binding to TNFR which recruits the adaptor protein TNF receptor-associated death domain (TRADD) and Fas-associated death domain protein (FADD). This adaptor proteins then transmit signals from TNFR to activate the caspase which leads to apoptosis (19). TNF- $\alpha$  also induces apoptosis through the intrinsic pathway by cleaving of Bcl-2 from BID which induces apoptosis via the intrinsic pathway (20). Accumulation of p53 has been reported in some cells which TNF- $\alpha$  has been shown to induce apoptosis, implicating p53 in TNF- $\alpha$  mediated apoptosis (21).

IL-10 which has both immunosuppressive and anti-angiogenesis effects has been shown in previous studies to stimulate TNF and other molecules of the immune system (22). IL-10 was significantly up-regulated in OVCAR3 treated cells, but not in SW626 treated cells when compared to the control in this studies. IL-10 expression inhibits angiogenesis by down-regulating some molecules of the immune system like TNF- $\alpha$  which are needed for angiogenesis. This suggests that IL-10 did not play a major role in inducing apoptosis in SW626 treated cells [23].

## Conclusion

It can be inferred in this study that *B. coccineus* ethanol leaf extract induces apoptosis probably by the expression of p53 and p21 genes and inhibit cyclinD1/CDK4 complex in OVCAR3 and SW626 cell lines. The extract also inhibited ovarian cancer cell migration and colony formation. The *B. coccineus* also exhibited immune-modulatory properties by expressing TNF- $\alpha$  in the two cell lines. All the results taken together suggest that *B. coccineus* induces apoptosis by up-regulating p53 which results in cell cycle arrest and induce apoptosis.

The p21 protein binds directly to cyclin-CDK4 complexes that drive forward the cell cycle and inhibits their kinase activity thereby causing cell cycle arrest to allow repair to take place.

## Methods

## Preparation of plant extract

Plant samples (wild type) was collected from Southern part of Nigeria, and was authenticated by Prof. J. D. Olowokudejo, at the Dept. of Botany University of Lagos, Nigeria, with the Voucher Number 7491. The fresh leaves of *B. coccineus* were air dried and the powdered sample was extracted by maceration in ethanol. The filtrate was collected and evaporated to dryness using the evaporator at reduced pressure, to get the ethanol extract. Stock concentration of 20mg/mL was prepared by dissolving 200mg of ethanol leaf extract in 1ml of DMSO and 9mL of PBS. Stock solution was stored at -20 °C.

## Materials

All chemicals used for this study were of analytical grade. All the primary and horseradish peroxidase-conjugated secondary antibodies were obtained from Cell Signalling Technology (Danvers, MA). Annexin V Apoptosis Detection Kit with PI was purchased from Bio-legend (San Diego, CA). The two human epithelial, adherent ovarian cancer cell lines OVCAR3 and SW626 were obtained from ATCC (Manassas, VA).

## Cultures

OVCAR3 cells were grown in RPMI media supplemented with 50 mL of Fetal Bovine Serum (FBS), 5 mL of HEPES Buffer, 5 mL of Non-essential amino acid solution, 5 mL of penicillin/streptomycin solution and 0.5 mL of vitamin Solution. SW626 is a grade 111 adenocarcinoma. SW626 cells were grown in L-15 media and supplemented with 50 mL of Fetal Bovine Serum (FBS) and 5 mL of Penicillin/Streptomycin.

## MTT assay

3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was carried out to determine cell viability of OVCAR3 and SW626 cell lines after treatment with *B. coccineus* ethanol leaf extract. OVCAR3 and SW626 growing cells were trypsinized with 0.25% Trypsin-EDTA (Fisher Scientific, Pittsburgh PA). 10,000 cells/well were seeded in 96 well plates in triplicates for the two cell lines for 48 h and 72 h time points and incubated for 24 h at 37°C. The cells were treated with different concentrations of *B. coccineus* ethanol leaf extract ranging from 0.020 mg/ml - 5 mg/ml, DMSO was added to control and media to blank, the plates were incubated for 48 h and 72 h time points. 20µL of 5 mg/ml MTT solution was then added to each well and incubated for 2 h, 100µL of DMSO was added to dissolve the formazan crystals, and the optical density was read at 570 nm using a micro-plate reader (Spectramax M5, Molecular devices, Sunnyvale, CA) [12].

## Live/Dead cell staining

Live/Dead cell staining was carried out as per manufacturer's protocol (NucGreen and NucBlue molecular probes by life technology), by seeding 100,000 cells/well for 24 h, treating with plant extract for 48 h and adding Nuc blue for live cells and Nuc green for dead cells. Images were taken using immunofluorescence microscope at x4.

## **Annexin V/PI apoptosis detection**

FITC annexin V apoptosis detection kit with PI (Biolegend, San Diego, CA) was used to investigate if plant extract could induce cell apoptosis in OVCAR3 and SW626 ovarian cancer cell lines. OVCAR3 and SW626 cells were seeded and treated with plant extract for 48 h, after which the cells were trypsinized with 0.25% trypsin. The hemocytometer (Countess II FL, Life Technology) was used to count the cells and  $0.5 \times 10^7$  cells/ml were added to 5  $\mu$ L of FITC Annexin V and 10  $\mu$ L of Propidium Iodide solution. The mixture was vortexed gently and kept in the dark 30min at room temperature then FACS buffer (400  $\mu$ L) was added to each sample tube. The flow cytometer with guava easy Cyte HT (EMD Millipore, Billarica) was used to analyze 50,000 cells [11].

## **Cell Migration Assay**

To investigate the effect of plant extract on OVCAR3 and SW626 ovarian cancer cell line migration, 40,000 cells/well were seeded, in 24well plate and place in the incubator for 24h. Cells were treated with extract and a monolayer of the cells was scratched with a 10  $\mu$ l plastic pipette tip to create a uniform wound. The scratch width was observed after 24 h, 48 h and 72 h of incubation under a phase-contrast microscope ((EVOS XL Core), at  $\times 10$  magnification and photographs were taken [13].

## **Clonogenic assay**

Clonogenic or colony formation assay is based on the cells ability to form a colony from one cell. The effect of the plant extract on OVCAR3 and SW626 colony formation was investigated to determine. 1600 cells/well were seeded in 24 well plate and place in the incubator for 24h. Cells were treated with extract and incubated at 37 °C for 10 days. Colonies were fixed with methanol for 20 min, stained with 0.1% crystal violet, and visualize using a phase-contrast light microscopy at  $\times 4$ , formation of 50 cells and above will be regarded as a colony [13].

## **RNA insolation**

RNA was isolated from OVCAR3 and SW626 cells treated with plant extract to determine its effect on apoptotic and cell cycle gene expression. The two cells were treated for 24 h and 48 h after which the cells were lysed with lysis buffer. The binding, washing and elution of cells were done as per protocol using PureLink RNA Mini Kit. RNA was quantified using Nanodrop 2000. The primers sequences of all the genes used in this study were synthesized at the National Center for Biotechnology Information (NCBI) gene bank database. The following are the sense and antisense primers sequences used in this study; MCL-1: For 5'-AAGAGGCTGGGATGGGTT TG-3' and Rev 5'-CAGCAGCACATTCTGATGC-3'; BCL-2: Rev 5'-GATAACGGAGGCTGGGATGC-3'and Rev 5'- TCACTTGTGGCCAGATAGG-3'; BID: For 5'-AGCACAGTGCGGATTCTGTC- 3'and Rev 5'-ACCGTTGTTGACCTCACAGT-3'; BAD: For 5'-CGAAGGGATGGGGGAGGA- 3' and Rev 5' -GGCGAGGAAGTCCCTTCTTA-3'; BAX: 5'- AAAGTGGT GCTCAAGGCC-3'and 5'-CTTCAGTGA CTGGC CAGG-3'; BAK:5'- TTTACCGCCATCAGCAACCT-3' and 5'-ATAGGCA TTCTCTGCCGTGG-3'; PARP:5'-GC TTCAGCCTCCTTGCTACA-3'and 5'-TTCGCCACTTC

ATCCACTCC-3'; Cyclin D1: For 5'-TGCATCTACACCGACAACCTC-3' and Rev 5'- TGGAGAGGAAGTGTTC AATG-3'; CDK2: For 5'-CGAGCTCCTGAAATCCTCCTG-3 and Rev 5'- GGCGAGTCACCATCTCAGCAA-3'; P21: For 5'-TGCCGAAGTCAGTTCCTTGT-3' and Rev 5'- GTTCTGACATGGCGCCTCC-3'; P19: For 5'-TAGTCTTACGACCCTGGTGC-3' and Rev 5'- GTGCATATCTGGCTCGGGTA-3'; TNF alpha: For 5'-ATGAGCACTGAAAGCATGATCC and Rev 5'- GAGGGCTGATTAGAGAGAGGTC-3'; IL10: For 5'-TCAAGGCGCATGTGAACT-3' and Rev 5'- GATGTCAAACCTCACTCATGGCT-3'; P53: For 5'-TTTTCCCCTCCCATGTGCTC-3' and Rev 5'- TGGACGGTGGCTCTAGACTT. The control was 18S primer (5'-GGCCCTGTAATTGGAATGAGTC-3' and 5'- CCAAGATCCAACCTACGAGCTT-3'). SYBR® Green PCR master mix reagents (Biorad, USA) were used for RT-PCR and CFX-manager software (CFX96 Real-Time System; Bio-Rad). All experiments were repeated three times.

## Western blot analysis

Western blotting was employed to analyze pro- and anti-apoptotic protein levels. Seeded OVCAR3 and SW626 cells were treated for 24 h, 48 h and 72 h time points, harvested and lysed with RIPA buffer with 1X protease inhibitor (Thermo Scientific, Rockford, IL). BCA (bicinchoninic acid) protein assay kit (Thermo Scientific, Rockford, IL) was used to determine the cell lysates. Protein concentrations, 30 µg of cell lysates was denatured by heating in Laemmli buffer and resolved by 4–12% polyacrylamide gels (Life Technologies, Carlsbad, CA). The cell lysates containing protein were transferred to PVDF membranes, and blocked in 5% non-fat dry milk (Biorad, USA) with TBS-T (Fisher Scientific, Pittsburgh, PA) containing 0.1% Tween 20 for 1 h at 25 °. The membrane was then probed at 4 °C overnight with primary antibodies (in 5% non-fat milk with Tris-Buffered Saline-Tween 20) against pro-apoptotic (BAD, BID), anti-apoptotic (BCL-XI, MCL-1) and reprobated with glyceraldehyde 3-phosphate dehydrogenase (GAPDH; Cell signaling, USA) at 1:1000 dilution (Cell Signaling Technology MA, USA). After washing, the membrane was incubated with horseradish peroxidase (HRP)-conjugated secondary antibodies for 1 h at 25 ° and 1:2000 dilution. The membrane was washed three times, and chemiluminescent reagent (Thermo fisher Scientific, Rockford, IL) was added to the membrane to detect and visualize the proteins using Image Quant LAS4000 (GE Healthcare- Biosciences, Pittsburgh, PA). Image-J software (NIH) was used to quantify the intensity of the bands [3].

## Statistical analysis

Statistical analysis was done by using one-way analysis of variance (ANOVA) and the unpaired *t*-tests. Results are expressed as standard errors of means ( $\pm$ SEM). P values less than 0.05 were statistically significant.

## Abbreviations

GAPDH: glyceraldehyde 3-phosphate dehydrogenase; HRP: horseradish peroxidase; FBS: Fetal Bovine Serum; MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; NCBI: National Center for Biotechnology Information; TNF- $\alpha$ : Tumor necrosis factor alpha

# Declarations

## Ethics approval and consent to participate

Ethical approval for the present study was obtained from the Health Research Ethics Committee of the College of Medicine of the University of Lagos.

## Consent for publication

Not applicable.

## Availability of data and materials

Not applicable.

## Competing interests

The authors declare that they have no competing interests.

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## Authors' contributions

EOAT, ARA, SS, and SR conceived, designed, analyzed the data and review, while UCE carried out the study and wrote the manuscript under the supervision of EOAT, ARA, SS and SR. All authors read and approved the manuscript.

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# References

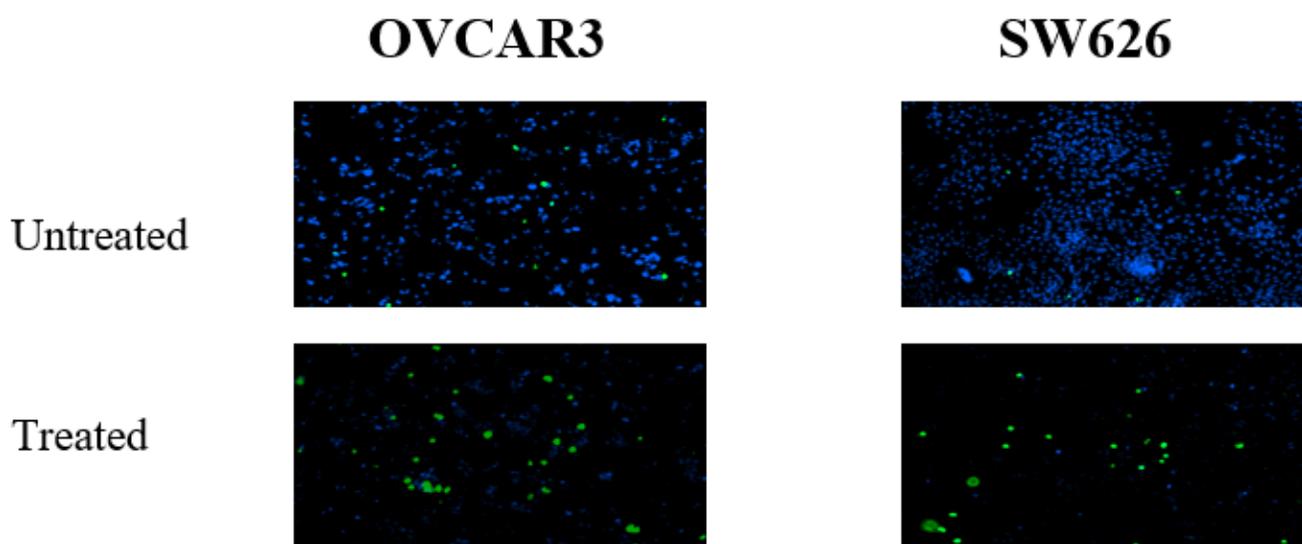
1. Ottevanger PB. Ovarian cancer stem cells more questions than answers. *Semin Cancer Biol.* 2017;44(February):67–71.
2. Webb PM, Leader G, Jordan SJ, Head T, Causes C. Best Practice & Research Clinical Obstetrics and Gynaecology Epidemiology of epithelial ovarian cancer. *Best Pract Res Clin Obstet Gynaecol.* 2016;1–12.
3. Singh SK, Mishra MK, Singh R. Hypoxia-inducible factor-1 $\alpha$  induces CX3CR1 expression and promotes the epithelial to mesenchymal transition (EMT) in ovarian cancer cells. *J Ovarian Res.* 2019;12(1):1–10.
4. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2019 (US statistics). *CA Cancer J Clin.* 2019;69(1):7–34.
5. Jayson GC, Kohn EC, Kitchener HC, Ledermann JA. Ovarian cancer. *Lancet.* 2014;384(9951):1376–88.
6. Akpan J, Akuodor G, Ezeokpo B, Essien A, Bassey A, Ezeonwumelu JO. In vivo antiplasmodial activity of byrsocarpus coccineus leaf extract in mice infected with plasmodium berghei. *Ibnosina J Med Biomed Sci.* 2012;4(3):78.
7. A. A. Ahmadu\*1, H. S. Hassan1 MUA and INA. Flavonoid Glycosides From Byrsocarpus Coccineus Leaves. *Schum and Thonn (Connaraceae).* *Afr J Trad CAM.* 2004;4(3):257–60.
8. Ejeh SA, Onyeyili P, Abalaka SE. Anti-diarrhea activity of the aqueous root bark extract of Byrsocarpus coccineus on castor oil-induced diarrhea in Wistar rats. *Vet World.* 2017 Jul 5;10(7):743–7.
9. Connaraceae T, Akindele AJ, Ezenwanebe KO, Anunobi CC, Adeyemi OO. Hepatoprotective and in vivo antioxidant effects of Byrsocarpus coccineus Schum . *J Ethnopharmacol.* 2010;129(1):46–52.
10. Fadeyi SA, Fadeyi OO, Adejumo AA, Okoro C. In vitro anticancer screening of 24 locally used Nigerian medicinal plants. *BMC Complement Altern Med.* 2013;13(79):0–9.
11. Singh SK, Banerjee S, Acosta EP, Lillard JW, Singh R. Resveratrol induces cell cycle arrest and apoptosis with docetaxel in prostate cancer cells via a p53/p21 WAF1/CIP1 and p27 KIP1 pathway. *Oncotarget.* 2017;8(10):17216–28.
12. Banerjee S, Singh SK, Chowdhury I, Lillard JW, Singh R. Combinatorial effect of curcumin with docetaxel modulates apoptotic and cell survival molecules in prostate cancer. *Front Biosci - Elit.* 2017;9(2):235–45.
13. Pan HC, Jiang Q, Yu Y, Mei JP, Cui YK, Zhao WJ. Quercetin promotes cell apoptosis and inhibits the expression of MMP-9 and fibronectin via the AKT and ERK signalling pathways in human glioma cells. *Neurochem Int.* 2015;80:60–71.
14. Fridman JS, Lowe SW. Control of apoptosis by p53. *Oncogene.* 2003;22(56 REV. ISS. 8):9030–40.
15. Loreto C, La Rocca G, Anzalone R, Caltabiano R, Vespasiani G, Castorina S, et al. The role of intrinsic pathway in apoptosis activation and progression in Peyronie's disease. *Biomed Res Int.* 2014;2014.
16. Luo Y, Hurwitz J, Massague J. Cell-cycle inhibition by independent CDK and PCNA binding domains in p21. *Nature.* 1995;375(May):159–61.

17. Agrawal S, Agarwal ML, Stark GR, Chisolm GM, Chatterjee-kishore M. Expression of p21 waf1 Modulates Oxysterol-Induced Apoptosis. *Mol Cell Biol.* 2002;22(7):1981–92.
18. Wong ML, Bongiorno PB, Rettrori V, Mccann SM, Licinio J. Interleukin (IL) 1 $\beta$ , IL-1 receptor antagonist, IL-10, and IL-13 gene expression in the central nervous system and anterior pituitary during systemic inflammation: Pathophysiological implications. *Proc Natl Acad Sci U S A.* 1997;94(1):227–32.
19. Goeddel C and D V. TNF-R1 Signaling: A Beautiful Pathway Guoqing. *Science* (80- ). 2002;296(5573):1635–6.
20. Wang X, Lin Y. Tumor necrosis factor and cancer, buddies or foes? *Acta Pharmacol Sin.* 2008;29(11):1275–88.
21. Rokhlin OW, Gudkov A V., Kwek S, Glover RA, Gewies AS, Cohen MB. p53 is involved in tumor necrosis factor- $\alpha$ -induced apoptosis in the human prostatic carcinoma cell line LNCaP. *Oncogene.* 2000;19(15):1959–68.
22. Koca YS, Bulbul M, Barut I. The Diagnostic Roles of Cytokines in Hepatobiliary Cancers. *Biomed Res Int.* 2017;2017:1–5.
23. Sheikhpour E, Noorbakhsh P, Foroughi E, Farahnak S, Nasiri R, Neamatzadeh H. A survey on the role of interleukin-10 in breast cancer: A narrative. *Reports Biochem Mol Biol.* 2017;7(1):30–7.

## Tables

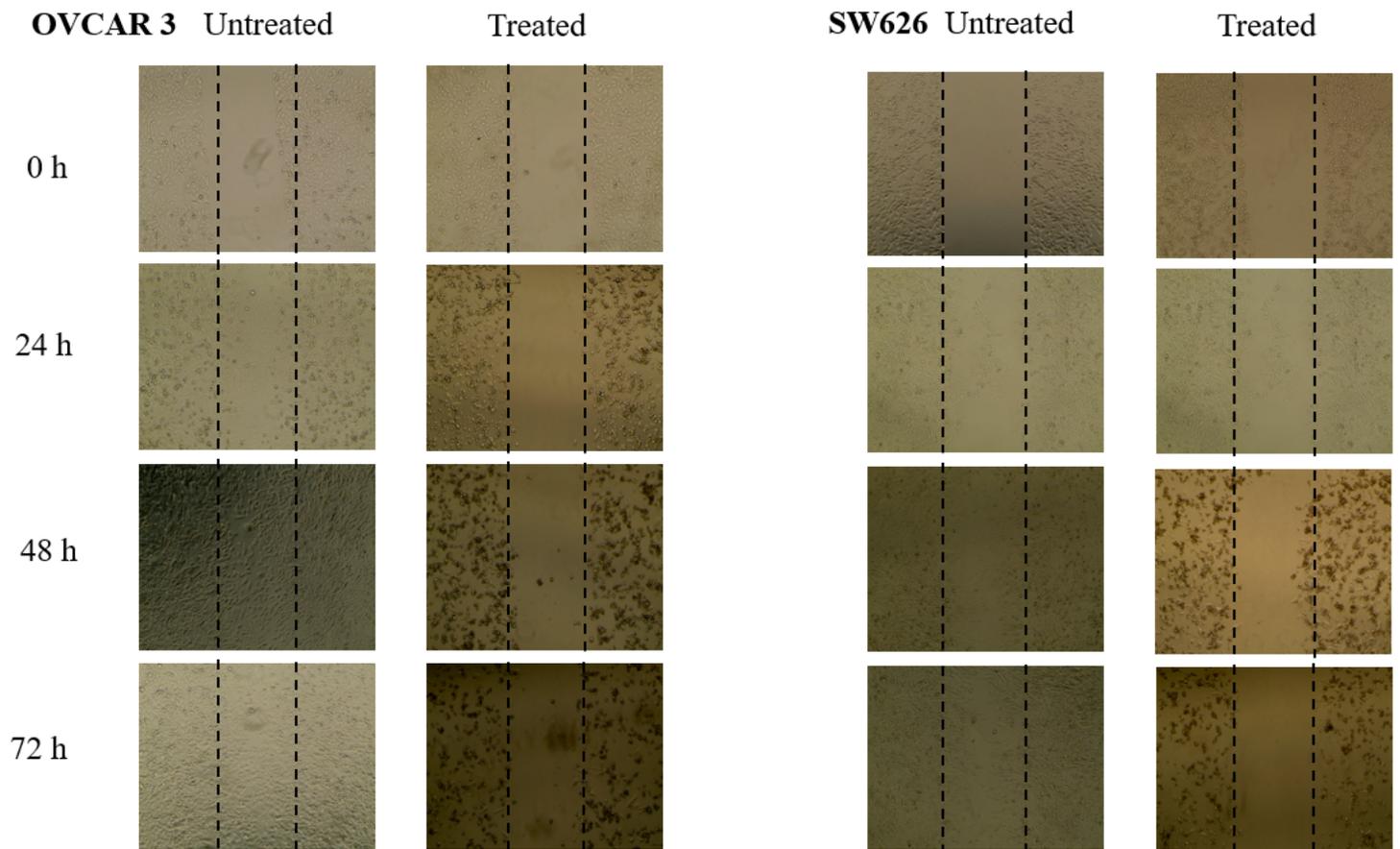
Due to technical limitations, the tables are only available as a download in the supplemental files section.

## Figures



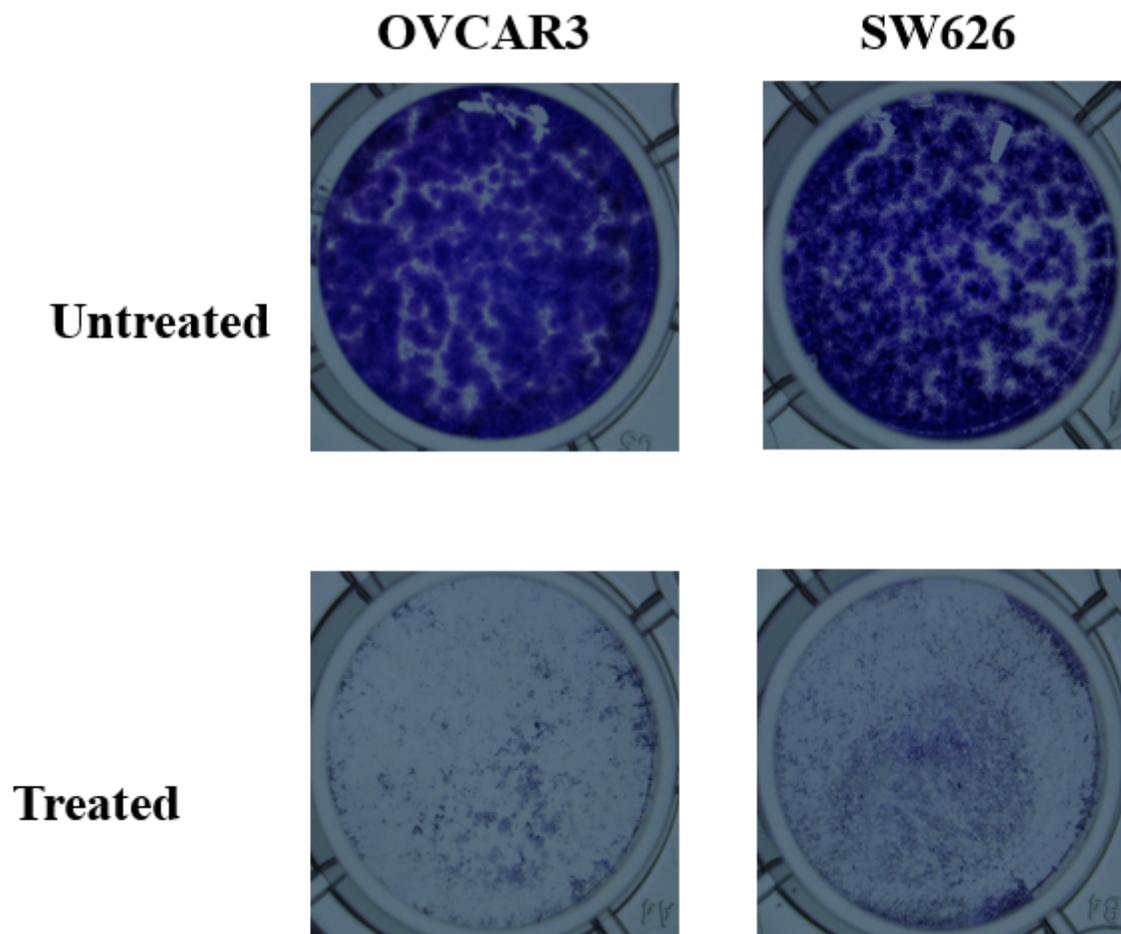
**Figure 1**

Immunofluorescence staining live and dead cells images



**Figure 2**

Cell migration assay for OVCAR3 and SW626; images were captured after treatment for 0, 2, 24 and 48 h with *B. coccineus* ethanol leaf extract at IC50



**Figure 3**

Colony formation images from treated and untreated OVCAR3 and SW626 cells

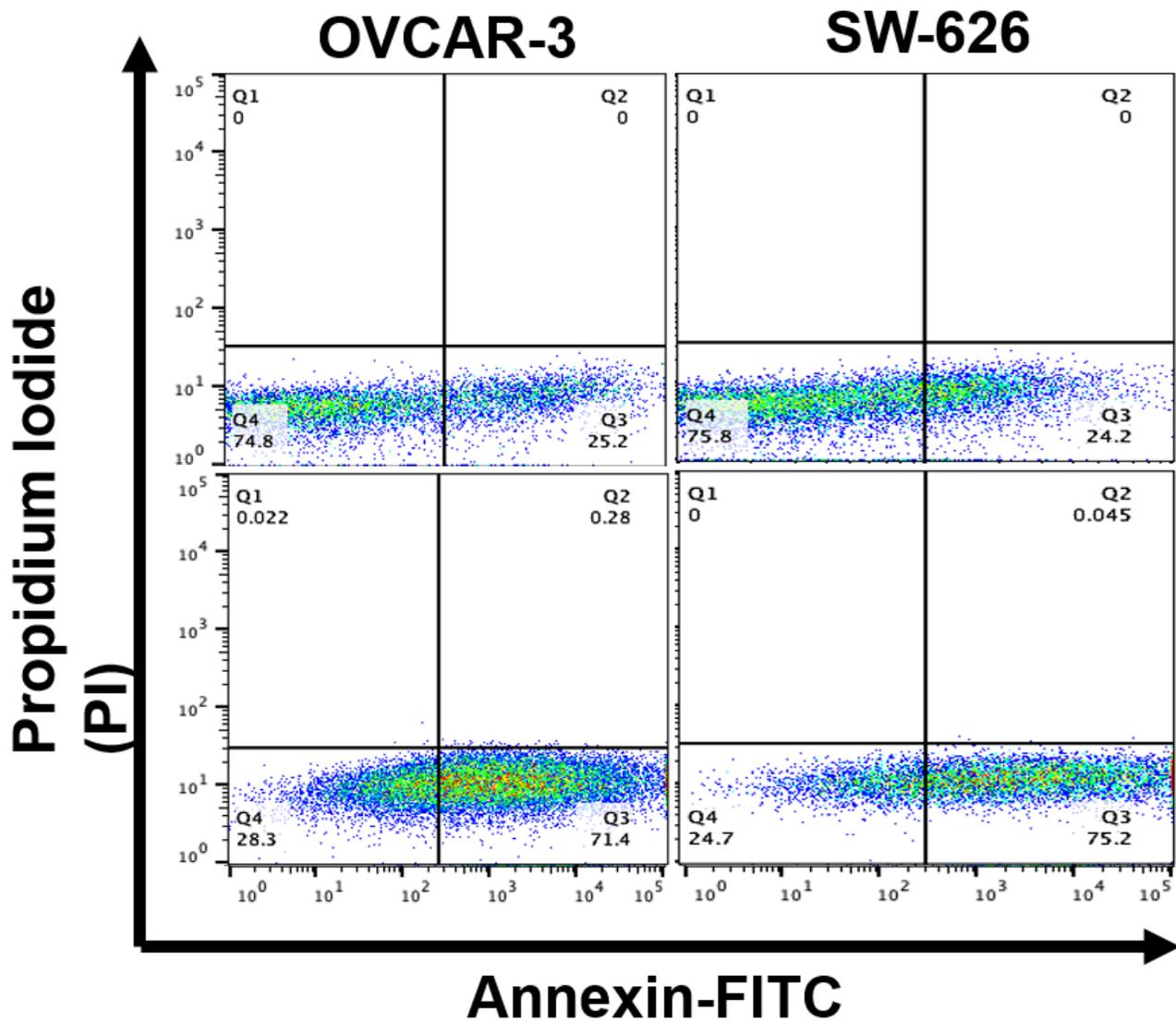
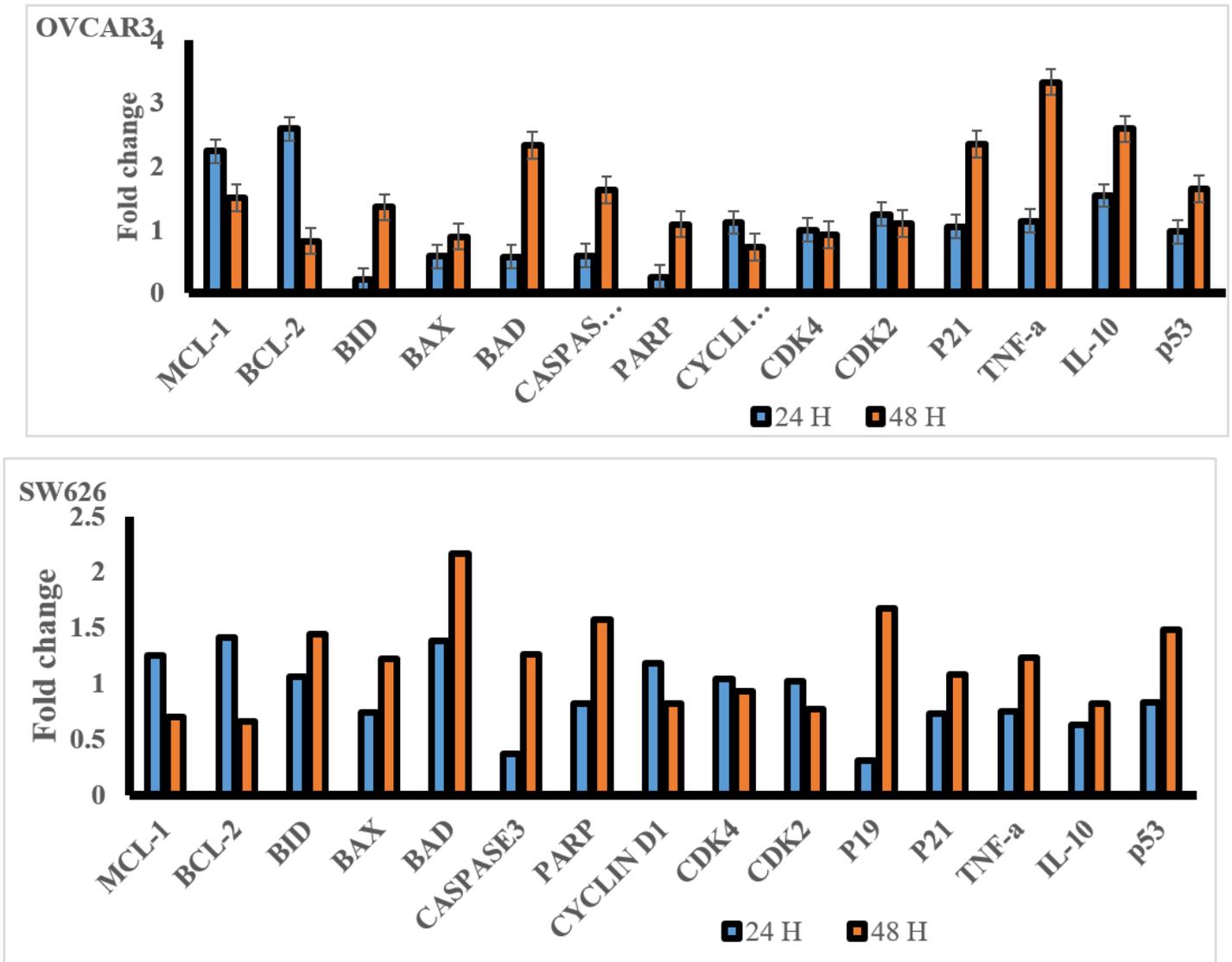


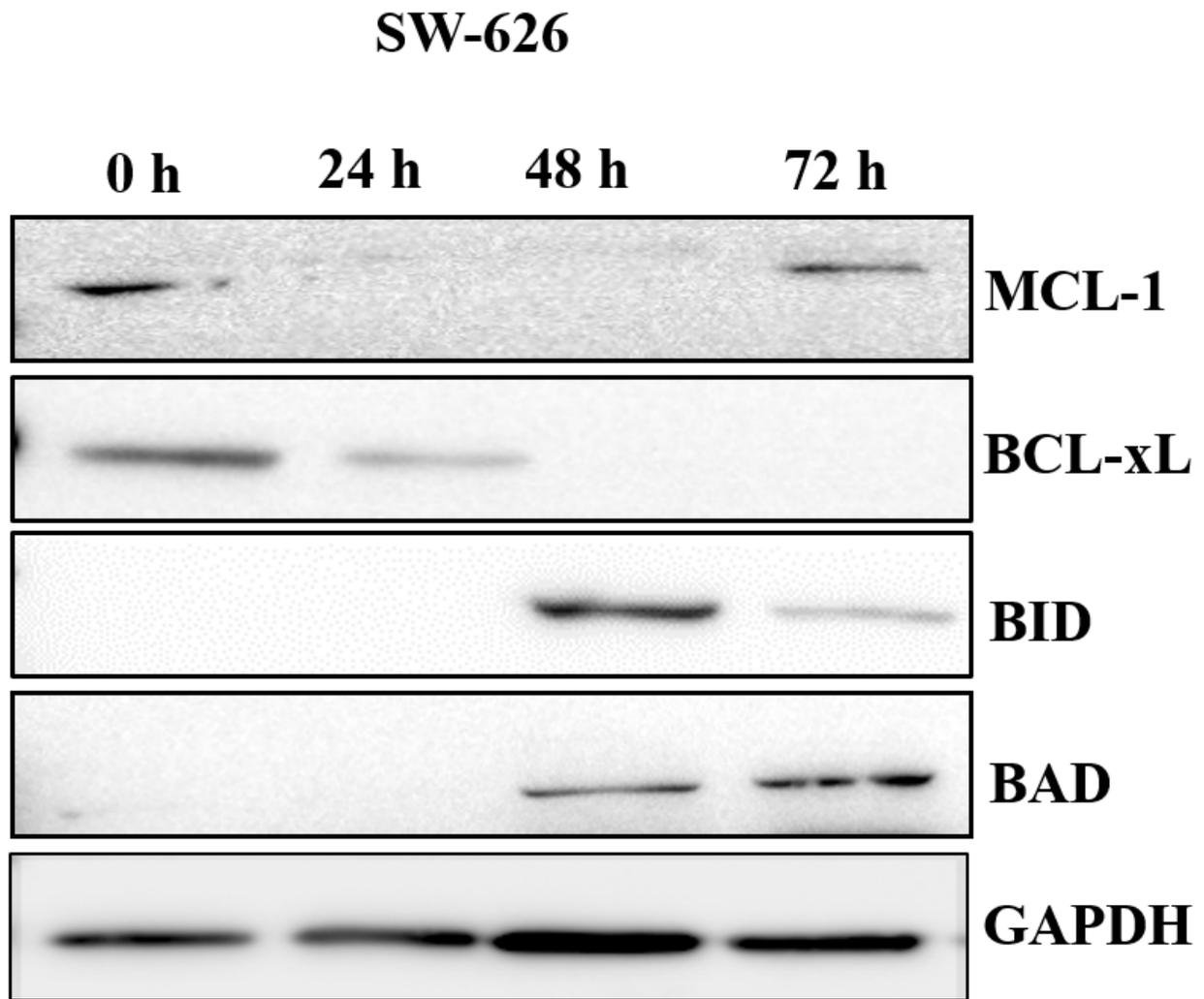
Figure 4

Determination of apoptosis in OVCAR3 and SW626 treated and untreated cells, using Annexin V-FITC /PI staining and flow cytometry. On each quadrant is the percentage of cells. Q1 (necrotic): Annexin(-)/PI(+), Q2 (late): Annexin(+)/PI(+), Q3(early) (Annexin(+))/(PI(-) and Q4 (viable): Annexin(-)/PI(-).Data are from three separate experiments  $\pm$  S.D



**Figure 5**

RT-PCR analysis of fold change expression relative to control in OVCAR3 and SW626 treated cells



**Figure 6**

Protein expression of SW626 and OVCAR3 cells treated with *B. Coccineus* ethanol leave extract at different time points and untreated cells

## Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [Additionalfile1.docx](#)
- [Table2.PNG](#)
- [Table1.PNG](#)