Maiyo et al., Afr J Tradit Complement Altern Med. (2016) 13(4):105-112
doi: 10.21010/ajtcam.v13i4.15

PHYTOCHEMISTRY, CYTOTOXICITY AND APOPTOSIS STUDIES OF B-SITOSTEROL-3-*O*-GLUCOSIDE AND B -AMYRIN FROM *PRUNUS AFRICANA*

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Abstract

Background: *Prunus africana* is used traditionally in many countries for the treatment of cancer and benign prostate hyperplasia.
Materials and methods: In this study, compounds from the leaves and bark of this plant were isolated and tested for their cytotoxicity and apoptosis induction in two human cancer cell lines (hepatocellular carcinoma (HepG2) and colorectal carcinoma (Caco-2)) and a non-cancer cell line (embryonic kidney (HEK293)). GC-MS profiling of the extract was also conducted.
Results: Three compounds (β-sitosterol, β-amyrin and β-sitosterol-3-*O*-glucoside) were isolated and the cytotoxic activity of β-amyrin and β-sitosterol-3-*O*-glucoside on the HepG2, Caco-2 and HEK293 was determined using the MTT cell viability assay. Both compounds had significant cytotoxic activity towards the Caco-2 cell line with IC_{50} values of 81 μg mL^{-1} and 54 μg mL^{-1} for β-amyrin and β-sitosterol-3-*O*-glucoside, respectively while low cytotoxicity was observed on HepG2 cell lines with IC_{50} values of 206 μg mL^{-1} and 251 μg mL^{-1} for β-amyrin and β-sitosterol-3-*O*-glucoside, respectively. Apoptosis induction in cells was studied using acridine orange/ethidium bromide dual staining. In both cases, the compounds tested demonstrated selective cytotoxicity towards cancer cells with high apoptosis indices in cells exposed to β-amyrin. Low IC_{50} values of 156 μg mL^{-1} and 937 μg mL^{-1} for β-amyrin and β-sitosterol-3-*O*-glucoside, respectively, were observed in the HEK293 cell line.
Conclusion: This study reveals that the plant is rich biologically active compounds thereby validating its ethno-medicinal use.

Keywords: triterpenoids, phytosterols, MTT cell viability, apoptosis induction.

Introduction

Healthcare is of serious concern in Africa due to inaccessibility to the large majority of the population. This has created dependence on herbal remedies for treatment, particularly with regards to chronic diseases. Medicinal plants are also a source of affordable medicine to low-income earners in most parts of Africa and, even with access to modern medicine, most of the locals and rural poor continue to rely on herbal medicine.

Cancer is a leading cause of death in Africa with 70% of deaths occurring in low and middle income countries in the continent. However, due to the burden of infectious diseases, it is not a priority in the public health sector (Jamison et al., 2006). Current cancer treatments prove to be challenging with chemotherapy drugs being non-specific and with the development of multi-drug resistance. This results in chemotherapy related toxicities leading to patients succumbing to the adverse effects of the drug rather than the disease (Zhang et al., 2011). Researchers are continually searching for alternative drugs that will overcome the challenges facing modern chemotherapy with bioprospecting being a viable route for the discovery of therapeutic agents. The success of natural products in cancer chemotherapy is well documented and has led to the development of drugs such as Taxol, from the *Taxus brevifolia*, and Maytansine from *Maytenus serrata* (Cragg and Newman, 2013).
Prunus africana is an evergreen tree from the family Rosaceae. It grows widely in Africa, stretching from western to southern Africa. In Kenya, it grows in the wild in the highland regions of the country, but has also been domesticated by several communities (Gachie et al., 2012). *P. africana* is used traditionally for the treatment of colorectal, breast and skin cancer (Ochwang'i et al., 2014). The bark and leaf extracts are also used to treat fevers, gonorrhoea and stomach pains (Stewart, 2003a).

Previous studies on *P. africana* have led to the isolation of sterols and pentacyclic triterpenes (Jimu, 2011). The use of this plant has been attributed to the anti-inflammatory activity of the isolated compounds (Otieno et al., 2013). Extracts from the bark of *P. africana* are used worldwide for the treatment of benign prostate hyperplasia, which is an inflammation of the prostate gland mostly found in men over 50 years of age. The tree is also reported to contain ferulic acids, n-docosanol and n-tetracosanol which lower blood cholesterol levels (Stewart, 2003b).
Evidence and epidemiological studies suggest that phytosterols are protective against a wide range of diseases and possess anticancer activity on various cancers and only a few studies have been conducted on the anticancer potential of this plant against different cancers (Bradford and Awad, 2007). We herein report on the isolation and purification of three compounds, two of which were evaluated for cytotoxic activity on cancer cells, HepG2 and Caco-2 and for apoptosis induction as a possible form of death using immunofluorescent techniques. GC-MS profiling of the plant extracts was also conducted and the identified compounds were evaluated for their anticancer activity.

Materials and Methods

General experimental procedure

Materials

Organic solvents (deuterated and undeuterated) and high performance-liquid chromatography (HPLC) grade solvents were purchased from Sigma (St. Louis, USA) and Merck (Darmstadt, Germany) chemical companies. Silica gel (Kieselgel 60, 0.063-

doi: 10.21010/ajtcam.v13i4.15
0.200 mm, 70-230 mesh ASTM), thin layer chromatography (TLC) aluminium backed plates pre-coated with silica gel and 3-(4,5-dimethylthiazol-2-yl)- 2,5-diphenyltetrazolium bromide (MTT salt) were purchased from Merck (Darmstadt, Germany). Human embryonic kidney cells (HEK293) were obtained from the Antiviral Gene Therapy Unit (University of Witwatersrand, South Africa) while the hepatocellular carcinoma (HepG2) cells and colorectal adenocarcinoma (Caco-2) cells were purchased from Highveld Biological (PTY) LTD (Lyndhurst, South Africa). Eagle's minimum essential medium (EMEM), penicillin/streptomycin and trypsin-EDTA, were purchased from Lonza BioWhittaker (Walkersville, USA). Phosphate buffered saline (PBS) was obtained from Calbiochem® (Canada). Foetal bovine serum was sourced from Hyclone GE Healthcare (Utah, USA). Acridine orange and ethidium bromide for apoptosis studies were obtained from Promega (Madison, USA). All other reagents were of analytical reagent grade.

General experimental procedure

Organic solvents hexane, dichloromethane (DCM) and methanol (MeOH) used for extraction and purification were distilled before use. Crude extracts were subjected to column chromatography for purification, with silica gel as the stationary phase. TLC was used to profile column fractions and spots were visualised under Ultra Violet (UV) light and by use of anisaldehyde spray reagent (20% H_2SO_4 : 80% MeOH). Nuclear magnetic resonance (NMR) spectra were obtained from a Bruker Avance 400 MHz or 600 MHz spectrometer using tetramethylsilane (TMS) as an internal standard. The compounds were dissolved in deuterated solvents (chloroform ($CDCl_3$) or dimethylsulfoxide (DMSO)). Chemical shifts (δ) are expressed in ppm. UV spectra were obtained on a Perkin-Elmer Lambda 35 Ultra Violet-Visible (UV-Vis) spectrophotometer using MeOH or hexane as solvents. Infrared (IR) spectra were recorded on a Perkin Elmer Spectrum 100 FT-IR spectrometer. Gas chromatography-mass spectroscopy (GC-MS) profiling of extracts was done on an Agilent GC-MSD equipped with a DB-5SIL MS fused silica capillary column. Extracts were dissolved using HPLC reagent grade solvents. Phytocompounds were identified using the National Institute of Standards and Technology (NIST) database library (National Institute of Standards and Technology, 2008).

Phytochemical analysis

Samples of leaves and bark were collected from a traditional healer in Eldama Ravine Town (0º 03'N 35º 43'E/0.05º N 35.72º E), Koibatek District in Baringo County, Kenya. The identity of the tree was confirmed by the Kenya Forest Research Institute (KEFRI). The leaves and bark were air-dried in the shade for 2 wk, cut into smaller pieces then ground into a fine powder to yield 674.52 g and 423.45 g, respectively.

Ground leaves and bark were sequentially extracted with hexane, DCM and MeOH for 48 hr on an orbital shaker. Extracts were filtered using Whatman No. 1 filter papers, concentrated using a rotary evaporator and weighed before storing at 4 ºC in a refrigerator.

The combined hexane and DCM extract of the leaves (157.2 g) was separated using column chromatography with hexane/ethyl acetate starting with 100% hexane and stepped by 10% to 100% ethyl acetate. Ten fractions of 100 mL were collected for each solvent system. Fractions 37-40 was re-chromatographed to obtain compound **1** (112.0 mg) in fractions 13-17 with hexane/ethyl acetate (85 : 15%, v/v) after collecting 20 mL fractions. Fraction 51-55 from the crude extract yielded compound **2** (23.9 mg).

The aqueous MeOH extract of the plant was partitioned in triplicate with 200 mL DCM then 200 mL ethyl acetate to obtain a DCM fraction (25.1 g) and ethyl acetate fraction (3.1 g). The DCM fraction was subjected to column chromatography starting with 100% hexane and stepped by 20% to 100% ethyl acetate. Twenty fractions of 10 mL were collected for each solvent system. Fractions 92-100 were combined and after purification on a sephadex LH20, 1 cm column using MeOH, eluted compound **3** (19.5 mg) in fraction 3 after collecting 3 mL fractions.

Extract profiling by GC-MS

An investigation of the combined hexane and DCM extracts of the leaves was done by GC-MS analysis. After partial purification on the column, fractions with similar TLC profiles were combined and dissolved in HPLC grade DCM for GC-MS analysis. The injection volume was 1.0 µL with an injector temperature of 25 °C. Helium was used as the carrier gas with a column flow of 1.0 mL min^{-1}. The initial oven temperature of 60 °C was ramped to 260 °C at 4 °C min^{-1} for 10 min and held at 260 °C for 10 min.

Cell culture

The cancer cell lines HepG-2, and Caco-2 and the non-cancer cell line HEK293 were grown in EMEM+Glutamine medium supplemented with 10% FBS, 100 µg mL^{-1} penicillin and 100 µg mL^{-1} streptomycin. They were cultured and maintained in 25 cm^2 culture flasks at 37 °C and 5% CO_2. At 90% confluency, the cells were trypsinised by overlaying the cells with 1mL trypsin-EDTA for 2 min. Once cells were trypsinised as evidenced by cells rounding off, fresh medium was added and the cells were split as desired or plated in 48 well plates for assay purposes.

MTT cell viability assay

This is one of the most widely used assays to measure cell viability. This assay measures the ability of viable cells to reduce MTT by mitochondrial dehydrogenases to the insoluble formazan salt. The salt once solubilised forms a pink/violet colour and the absorbance can be measured spectrophotometrically (Mosman, 1983).

In this study, cells were plated on a 48 well plate with 200 µL media at a seeding density of 1.2×10^5 and incubated for 24 hr to allow attachment. Thereafter, the medium was changed and 10 µL of the compounds (dissolved in DMSO and PBS) were added to the cells at concentrations of 20, 40, 60 and 80 µg mL^{-1} in triplicate and incubated for 48 hr. Approximately 200 µL of MTT at a concentration of 5 mg mL^{-1} in PBS was added and incubated for 4 hr at 37 °C. After four hr, the medium was removed from the wells, leaving the formazan salt at the bottom. Subsequently, 200 µL of DMSO was added to solubilise the salt producing a pink colour whose absorbance was measured on a Mindray MR-96A microplate reader (Vacutec, Hamburg, Germany) at 570 nm.

doi: 10.21010/ajtcam.v13i4.15

Cytotoxicity was represented as percentage cell survival against compound concentration. IC_{50} values were calculated from graphs of percentage inhibition versus concentration that were plotted using Microsoft Excel™ (Microsoft Corporation, Redmond, USA). Data were statistical analysed using one-way analysis of variance (ANOVA) with subsequent post hoc least significant difference (LSD) test. All statistical calculations were performed using Microsoft Excel™.

Apoptosis studies

Dual staining with acridine orange and ethidium bromide is a rapid technique for studying cell apoptosis *in vitro*. It enables the identification of live, late and early apoptotic and necrotic cells. Acridine orange stains live cells by intercalating with the cell's DNA to emit green fluorescence, while the ethidium bromide stains dead cells emitting yellow to red fluorescence based on the stage of apoptosis. Approximately 100 mg mL^{-1} of both dyes was prepared in PBS. The dyes were mixed at a ratio of 1:1. Cells at a density of 1.2×10^5 were plated on 24 well plates and 20 µL of each compound at 10 µg mL^{-1} was added to the cells and incubated for 24 hr. Subsequently, old medium was removed from the plate and the cells were washed with 100 µL of cold PBS. Thereafter, 15 µL of the dye solution was added to the cells which were then viewed under an Olympus fluorescent microscope at × 200 magnifications. Apoptotic indices were calculated as shown:

Apoptotic index = number of apoptotic cells/ number of total cells counted

Results and Discussion

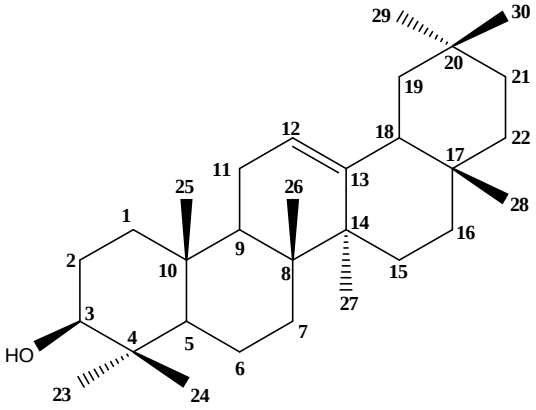
Phytochemical analysis

Compound **1** was isolated as an amorphous white solid with a mass of 112 mg and compound **3** was isolated as an amorphous white solid with a mass of 23.9 mg. The ^{1}H-NMR spectra for both compounds showed characteristic resonances for β-sitosterol. These were resonances at δ_H 5.34 (1H, t, J=5.1 Hz, H-6) for the olefinic proton and a resonance at δ_H 3.53 (1-H, m, H-3) due to the carbinylic proton. However, for compound **3**, the resonance at δ_H 4.24 (1H, d, *J*=7.7 Hz, H-1') due to the anomeric proton indicated the presence of a glycosidic linkage. This was confirmed by resonances between δ_H 2.92-4.86 which were assigned to H-2', H-3', H-4', H-5' and H-6' and the sugar identified as glucose.

The ^{13}C-NMR spectrum for compound **1** resolved 29 carbon resonances and for compound **3** resolved 35 carbon resonances with characteristic olefinic resonances at δ_C 140.9 (C-5) and δ_C 121.6 (C-6) in sitosterol. The resonance at δ_C 101.2 (C-1') in compound **3** was assigned to the anomeric carbon due to HSQC correlations. HMBC experiments confirmed the attachment of the sugar at C-3 (Figure 1) and the methylene at δ_C 61.6 was assigned to C-6' of the glucoside. The UV spectra of compounds **1** and **3** showed two bands at 204 nm and 231 nm. The IR spectra showed a broad absorption band at 3382 cm^{-1} (OH group), 2920 cm^{-1} (CH groups) and 1461 cm^{-1} (C=C). GC-MS data for compound **1** showed molecular ion peak [M^+] at m/z 414 which is in agreement with the molecular formula $C_{29}H_{50}O$. GC-MS data for compound **3** showed molecular ion peak [M^+] at m/z 576, which is in agreement with the molecular formula $C_{35}H_{60}O_6$. This data corresponded with that in literature (Arora and Kalia, 2013; Jayaprakasha et al., 2010) and confirmed compound **1** to be the aglycone β-sitosterol and compound **3** to be the glycoside, β-sitosterol-3-*O*-glucoside (Figure 1).

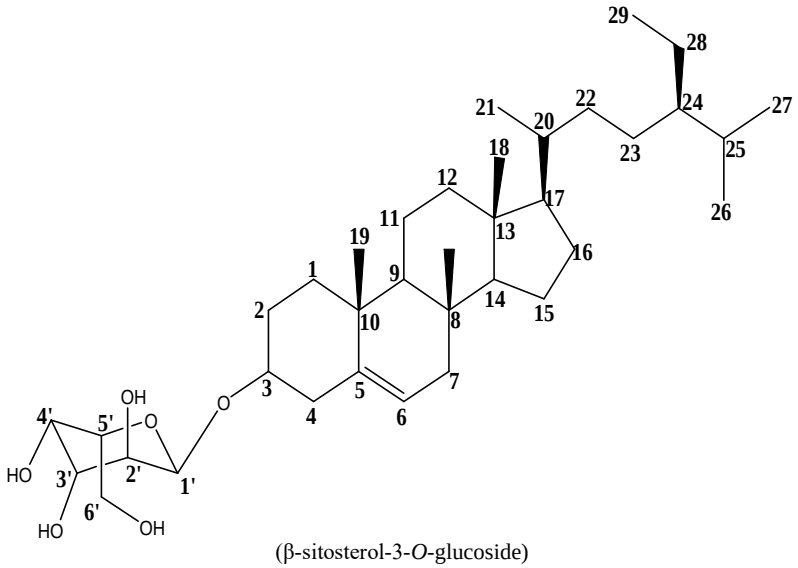
Compound **2** was isolated as a white powder with a mass of 19.5 mg. The ^{1}H-NMR spectrum of compound **2** showed characteristic resonances for a pentacyclic triterpene of the oleanane type. These were a downfield vinylic proton resonance at δ_H 5.25 (1H, t, *J*=6.6, 3.3 Hz, H-12), a carbinylic proton resonance at δ_H 3.20 (1H, dd, *J*=10.6, 4.1 Hz, H-3), a double doublet at δ_H 2.81 (1H, dd, *J*=14.3, 4.4 Hz, H-18) and seven methyl resonances between δ_H 0.77-1.19 (s, H-23 to H-30). The ^{13}C-NMR spectrum showed resonances at δ_C 143.8 (C-13) and δ_C 122.2 (C-12) due to the vinylic carbons and a resonance at δ_C 78.8 (C-3) due to the carbinylic carbon. The DEPT 90 and 135 experiments resolved 8 methyl, 10 methylene, 5 methine and 7 quaternary carbon resonances.

The UV spectrum of compound **2** gave maximum wavelength (λ_{max}) at 229 nm. The IR spectrum showed a broad absorption band at 3263 cm^{-1} (OH group) and a band at 1459 cm^{-1} (C=C). GC-MS data showed molecular ion peak [M^+] at m/z 426 which is in agreement with the molecular formula $C_{30}H_{50}O$. This data corresponded with that in literature (Mohato and Kundu, 1994) and confirmed compound **2** to be β-amyrin (Figure 1).



(β-amyrin)

doi: 10.21010/ajtcam.v13i4.15



(β-sitosterol-3-*O*-glucoside)

Figure 1: Chemical structure of compound **2** (β-amyrin) and compound **3 (**β-sitosterol-3-*O*-glucoside) isolated from *P. africana*.

GC-MS Profiling

GC-MS profiling of the extracts of leaves led to the identification of nine compounds using the NIST library (National Institute of Standards and Technology, 2008). The fragmentation pattern, name, structure and retention times were compared to those of known compounds in the library. The compounds identified were α-amyrin, phytol, vanillin, benzenedicarboxylic acid, squalene, nicotinic acid, campesterol, stigmasterol and α-tocopherol. Vanillin, a widely used flavouring agent, is also reported to successfully inhibit proliferation of HT-29 colon cancer cells by inducing apoptosis (Ho et al., 2009). Studies on phytol report significant cytotoxicity on nasopharyngeal carcinoma (KB) cell line (23 µg mL^{-1}) (Malek et al., 2009).

MTT Cell viability studies

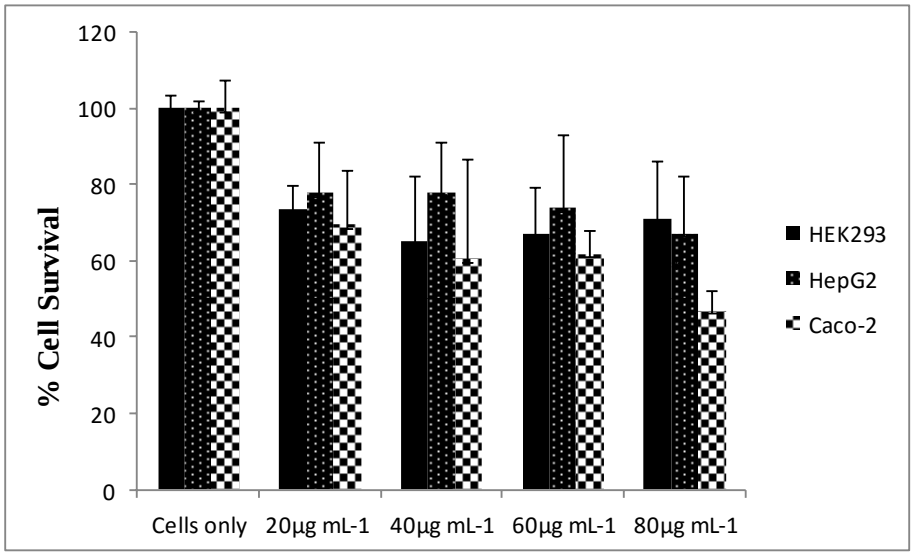
The MTT cell viability assay was used to study the cytotoxic effect of β-amyrin and β-sitosterol-3-*O*-glucoside in the two cancer cell lines HepG2 and Caco-2 and non-cancer cell line, HEK293). Compounds tested exhibited a dose dependent cytotoxicity on the cancer cell lines. Both compounds showed selective cytotoxicity to cancer cells as evidenced by the high IC_{50} values 156 µg mL^{-1} and 937 µg mL^{-1} on the control non-cancer cell line (Table 1). Significant cytotoxic activity was observed on Caco-2 cell lines on exposure to both compounds with IC_{50} values of 81 µg mL^{-1} and 54 µg mL^{-1} for β-amyrin and β-sitosterol-3-*O*-glucoside, respectively. Our results showed that β-sitosterol-3-*O*-glucoside was more potent against Caco-2 cells than β-amyrin which is in agreement with previously published studies (Jayaprakasha et al., 2010).

Table 1: IC_{50} Values of β-amyrin and β-sitosterol-3-*O*-glucoside on HEK293, HepG2 and Caco-2 cell lines

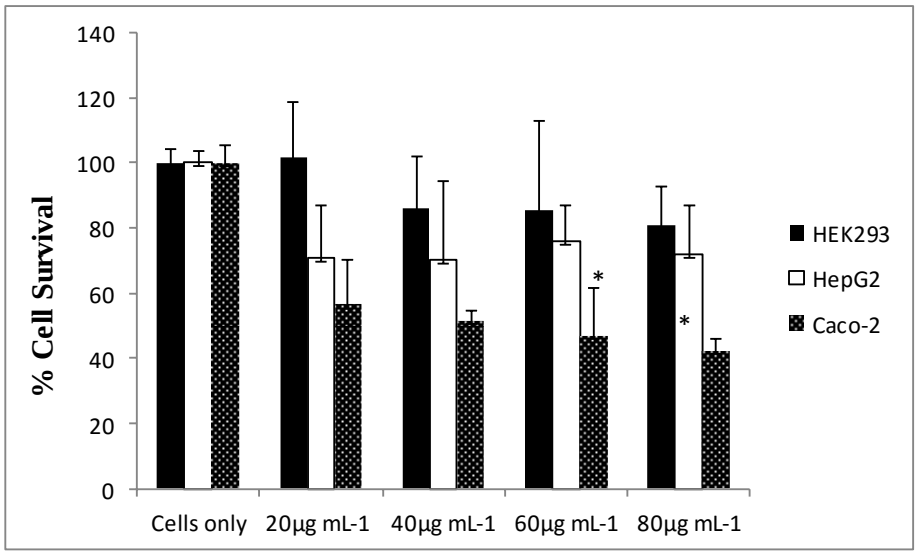
	IC_{50} values in mg mL^{-1}	
CELL LINES	**β-amyrin**	**β-sitosterol-3-*O*-glucoside**
HEK293	156	937
HepG2	206	251
Caco-2	81	54

Statistical analysis revealed that both compounds had significantly different activity across all the concentrations with β-sitosterol-3-*O*-glucoside showing higher activity (Figure 2). However, β-sitosterol-3-*O*-glucoside and β-amyrin had no significant cytotoxic effect on HepG2 cells with IC_{50} values of 251 µg mL^{-1} and 206 µg mL^{-1}, respectively at 95% confidence level. β-Sitosterol-3-*O*-glucoside exhibited significant cytotoxicity against Caco-2 cell line compared to the control untreated cells. Statistical evaluation revealed low cytotoxicity of β-amyrin across all cell lines, consistent with published reports on the weak anticancer activity of some pentacyclic triterpenes stating that derivatives of these compounds may improve activity (Barros et al., 2011).

doi: 10.21010/ajtcam.v13i4.15



β-amyrin



β-sitosterol-3-*O*-glucoside

Figure 2: MTT cell viability assay of β-amyrin and β-sitosterol-3-*O*-glucoside on HEK293, HepG2, and Caco-2. Data is represented as mean ±SD (n=3). * Greater cytotoxicity was observed in the Caco-2 cell line ($p \leq 0.05$).

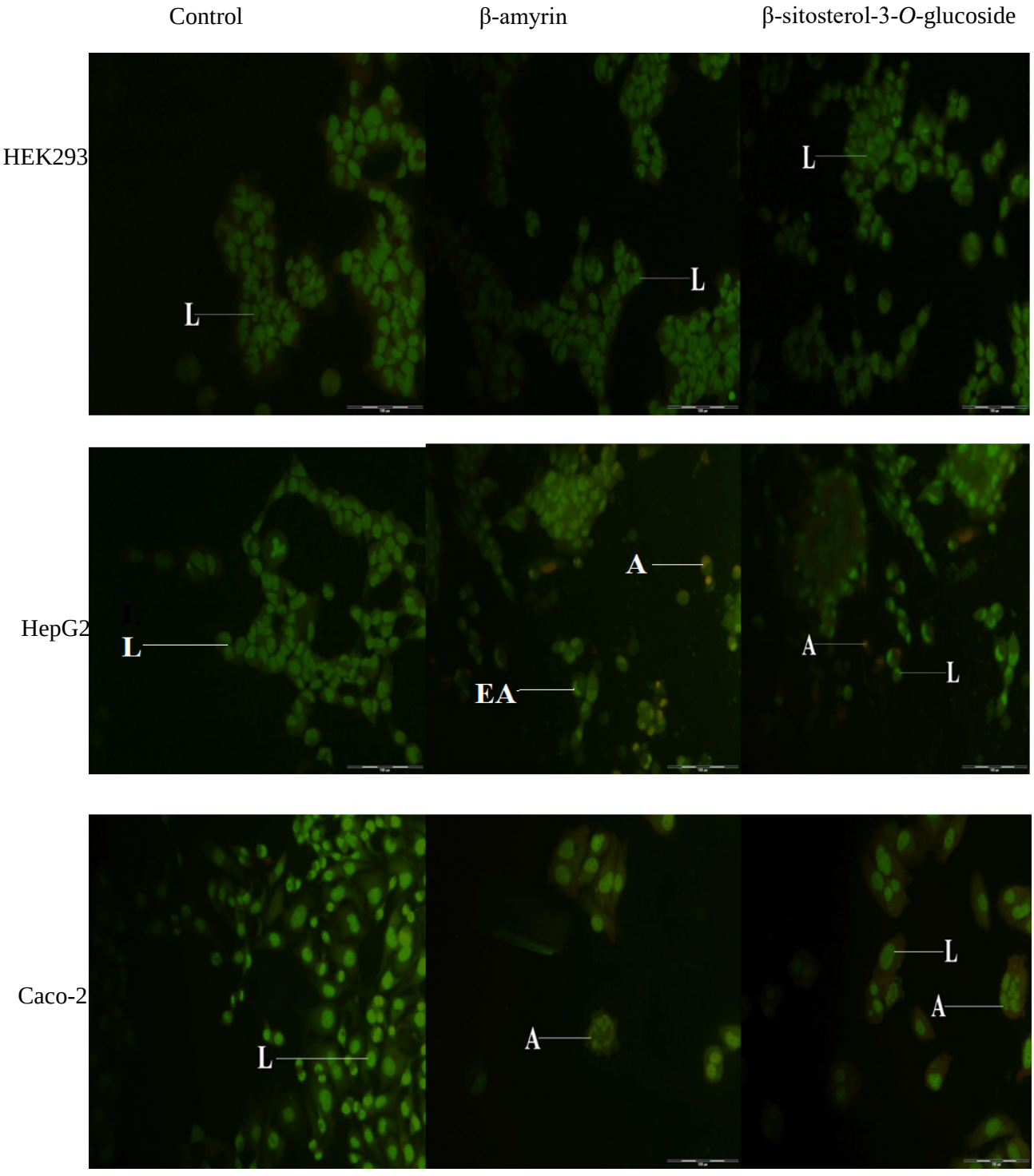
Apoptosis studies

Apoptosis studies were conducted to investigate the ability of test compounds to induce apoptosis in the cancer cell lines (Table 2, Figure 3). Dual staining with acridine orange and ethidium bromide is widely used in studying nuclear cell morphology in apoptotic cells. Examination of HepG2 and Caco-2 cell lines upon exposure of 10 µg mL^{-1} of each compound for 24 hr revealed that unlike the untreated controls, treated cells showed characteristics of apoptosis. Live cells appeared green, early apoptotic cells bright green/yellow and late apoptotic cells red/orange. Chromatin condensation, nuclear fragmentation and cell shrinkage was observed in treated cells whilst round homogenous nuclei were observed in the controls. A significant reduction in viable cell numbers was also observed, suggesting that one of the causes of cell death was apoptosis. Apoptotic bodies were not observed in the non-cancer HEK293 cell line. Apoptosis indices were higher in cells treated with β-amyrin that those treated with β-sitosterol-3-*O*-glucoside.

doi: 10.21010/ajtcam.v13i4.15

Table 2: Apoptotic index values of β-amyrin and β-sitosterol-3-*O*-glucoside on HEK293, HepG2 and Caco-2 cell lines.

CELL LINE	Apoptosis index		
	Control	**β-amyrin**	**β-sitosterol-3-*O*-glucoside**
HEK293	0.00	0.00	0.00
HepG2	0.00	0.35	0.27
Caco-2	0.00	0.33	0.13



doi: 10.21010/ajtcam.v13i4.15
Figure 3: Fluorescence images of cell treated for 24 hours with β-amyrin and β-sitosterol-3-*O*-glucoside at a concentration of 20 µg mL^{-1} showing induction of apoptosis on HEK293, HepG2 and Caco-2 L=Live A=Apoptotic EA= Early apoptotic. Scale is given as 100 µm.

In vitro cytotoxicity screening is a valuable tool in drug discovery and is used widely by researchers especially when bio-prospecting for potentially active cancer drugs. Jaiprakash and co-workers (2010) tested the inhibition of colon cancer cell line HT-29 with sitosterol glucoside isolated from *Citrus aurantium* which demonstrated potent activity with an IC_{50} value of 40 µM. β-amyrin esters exhibited potent cytotoxic activity against HL-60 leukemia cells with IC_{50} values of 1.8 to 5 µM and induced apoptosis by activating caspase 3 (Park et al., 2007). In our findings, β-sitosterol-3-*O*-glucoside and β-amyrin demonstrated dose dependent cytotoxicity across all cell lines with low cytotoxicity observed on HEK293 and HepG2 cell lines. In both compounds, greater activity was observed on the colon cancer cell line Caco-2 rather than the hepatocellular carcinoma cell line HepG2, with β-sitosterol-3-*O*-glucoside showing slightly higher activity than β-amyrin.

Apoptosis is programmed cell death and is characterised by nuclear condensation and fragmentation, cell shrinkage, chromatin condensation and membrane blebbing (Hunter et al., 2007). Apoptotic cells are eventually phagocytised *in vivo* by macrophages thereby preventing inflammation. However, apoptotic cells cultured *in vitro* ultimately undergo secondary necrosis due to the absence of phagocytes. Impaired apoptosis through up or down regulation of apoptotic proteins is implicated in a number of diseases including autoimmune and neurodegenerative diseases and in this case cancer. Mutation and down regulation of *p53* are reported in most cancers, including colon and liver cancer (Jaiprakash et al., 2010). The molecular players in apoptosis are the caspases and Bcl-2 family proteins which upon activation initiate apoptosis through the intrinsic or extrinsic pathway. The Bax/Bcl-2 protein ratio is an important indicator of how a cell will respond to apoptosis induction.

In this study, both compounds tested induced apoptosis in Caco-2 cell lines with high apoptosis indices of 0.33 and 0.13 compared to the control. Reduced cell numbers were also observed on this cell line on exposure to the compounds. However, this was not observed in HepG2 and HEK293 cell lines which showed low apoptotic indices on exposure to the compounds for 24 hr. This is in agreement with studies that reported on apoptosis induction by β-sitosterol and on the inhibition of cellular proliferation and induction of apoptosis in U937 leukemia cells by β-sitosterol by down regulation of the pro-apoptotic protein Bcl-2 and activation of caspase 3 (Park et al., 2007; Woyengo et al., 2009). This suggests that apoptosis induction by these compounds occurs via the intrinsic pathway. This has also been reported in stomach and colon cancer (Zhao et al., 2009).

Conclusion

This study investigated the secondary metabolites of *P. africana* and their potential anti-cancer activity by studying the *in vitro* cytotoxic and apoptotic activity on cancer cells. The pentacyclic triterpene, β-amyrin and a sterol, β-sitosterol with its glycoside, β-sitosterol-3-*O*-glucoside was isolated from the leaves and bark of the plant. The MTT cell viability study of β-amyrin and β-sitosterol-3-*O*-glucoside showed β-sitosterol-3-*O*-glucoside to be the more active of the two compounds against the Caco-2 cell line. Both compounds were, however, inactive against the HepG2 cell line. Apoptosis studies confirmed apoptosis induction as a mode of cell death with β-amyrin exhibiting higher apoptotic indices in both cancer cell lines than β-sitosterol-3-*O*-glucoside. The compounds tested showed selective cytotoxic and apoptotic activity as evidenced by the high IC_{50} values and low apoptotic indices on HEK293, the control non-cancer cell line. Triterpenes, phytosterols and phenolic compounds were identified in the hexane and DCM extracts of the leaves by GC-MS analysis. This study reveals that the plant is rich in phenolics, pentacyclic triterpenes and sterols, classes of compounds that are known to be biologically active, which validates its ethno-medicinal use.

Acknowledgements

The authors wish to acknowledge the University of KwaZulu-Natal, Durban, South Africa for financial support.

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