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Isolation and Characterisation of Two Quercetin Glycosides with Potent Anti-Reactive Oxygen Species (ROS) Activity and an Olean-12-en Triterpene Glycoside from the Fruit of *Abelmoschus esculentus* (*Malvaceae* Juss.)

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ABSTRACT

Abelmoschus esculentus (okra) is used in the traditional treatment of cancer, hyperlipidaemia and hyperglycaemia. We, therefore, investigated its composition and potential cytotoxic or antioxidant properties that might underlie its phytotherapeutic applications. Its methanolic fruit extract yielded compounds **1**, **2** and **3,** identified through NMR, UV and MS analyses as Olean-12-en-3-*O*-β-D-glucopyranoside, isoquercitrin (quercetin glycoside) and 5,7,3′,4′-tetrahydroxy-flavonol-3-O-[*β*-D-glucopyranosyl-(1→6)]-*β*-D-glucopyranoside (quercetin diglycoside), respectively. Following 48 h exposure, oleane glycoside was mildly toxic to the HeLa and the MRC5-SV2 cancer cells, isoquercitrin was not toxic except at 100 µg/ml in HeLa, and quercetin diglycoside elicited no toxicity. In a 2′,7′-dichlorofluorescein diacetate (DCFDA) assay of intracellular levels of reactive oxygen species (ROS), hydrogen peroxide increased ROS levels, an effect not affected by oleane glycoside but protected against by isoquercitrin and quercetin diglycoside, with IC_{50} values, respectively, of 2.7 ± 0.5 µg/ml and 1.9 ± 0.2 µg/ml (3 h post-treatment) and 2.0 ± 0.8 µg/ml and 1.5 ± 0.4 µg/ml (24 h post-treatment.) This is the first report of an oleanene skeleton triterpenoid in this plant. The work provides some insight into why the plant is included in remedies for cancers, cardiovascular complications and diabetes, and reveals it as a potential source of novel therapeutics.

Keywords: *Abelmoschus esculentus*; okra; antioxidant; reactive oxygen species; cytotoxicity

Introduction

Okra or ladies' fingers (*Abelmoschus esculentus*), a cultigen, is one of the most popular and widely utilized species of the family Malvaceae [1] and is an economically important vegetable crop naturalized and grown in tropical and sub-tropical parts of the world. Today it is widely cultivated for its edible green fruits, which are harvested when immature and are popular for their slimy mucilage. *A. esculentus* grows up to 6 feet in height, with sturdy stems and long, broad, serrated, deeply-lobed leaves. Worldwide, Okra production is estimated at 6 million tonnes per year.[2] Its mucilage finds application as a suspending agent in some pharmaceutical formulations.[3] *A. esculentus* has found unique industrial and medical applications. Medicinally, the mucilage finds uses as a plasma replacement or blood volume expander.[4] Lectin, a protein extracted from okra, binds carbohydrates on the surface of cancer cells, causing significant apoptosis, and was thus found to selectively inhibit breast cancer cell proliferation.[5] *A. esculentus'*s application in the treatment of stomach ulcers has been attributed to the presence of rhamnogalacturonan polysaccharides, which disrupt the adhesion of *Helicobacter pylori* bacteria to stomach tissue.[6] Okra was found to be effective at binding bile acids, being 34% as effective as cholestyramine.[7] Sabitha *et al.* [8] indicated that the consumption of okra may help to reduce hyperlipidaemia and hyperglycaemia in diabetics, thus helping to prevent cardiovascular disease and other co-morbidities associated with diabetes.[9]

So far, there have been a number of phytochemical investigations to probe the extracts of *A. esculentus* for bioactive compounds. A wide array of compounds have been reported that include (3β,21β)-19,21-epoxylup-20(29)-en-3-yl acetate, (3β)-9,18-dihydroxyolean-12-en-3-yl acetate,[10] polyphenolic groups of compounds like flavonoids, hydroxycinnamic derivatives,[11] quercetin-3-O-sophoroside, 5,7,3′,4′-tetrahydroxy flavonol-3-O-[β-D-rhamnopyranosil-(1 → 2)]-β-D-glucopyranoside and isoquercitrin, [12] rutin, procycanidin B1

and B2,[13] 5,7,3′,4′-tetrahydroxy-4″-O-methyl flavonol−3-O-β-D-glucopyranoside and 5,7,3′,4′-tetrahydroxy flavonol−3-O-[β-D-glucopyranosyl-(1 → 6)]-β-D-glucopyranoside [14] and oligomeric proanthocyanidins.[15] A variety of other compounds have been identified from the different parts of the plant [16] and some identified constituents have been shown to be bioactive, e.g., abscisic acid, which has been demonstrated to have hypoglycaemic effect, with potential as a nutraceutical for the treatment of diabetes.[17, 18] Despite the identification of these phytochemicals in okra, Islam [19] concluded that scientific reports on *A. esculentus's* anticancer activity are very sparse. This necessitates continuous study of the plant. Therefore, in continuation of our studies to explore anti-cancer potentials of Nigerian vegetables,[20] we isolated and characterized bioactive phytochemicals from the plant species and evaluated them for their potential to combat mechanisms that lead to carcinogenesis, especially oxidative stress.

Results and Discussion

Isolation of compounds

Isolation of compounds began with solid phase extraction on Phenomenex cartridges to achieve some levels of initial purification of the extract that had been adsorbed onto a non-polar adsorbent. The mobile phase was made up of increasing proportions of methanol in water until 100% methanol was achieved. This approach enabled separation of highly polar constituents from moderately polar ones, which were eluted with increasing proportions of methanol. Chromatographic procedures were harnessed in effecting the purification and final isolation of the compounds from the crude extract. For instance, further purification harnessed chromatographic separation on silica gel, which maximizes on polarity of the phytoconstituents, as well as gel permeation on Sephadex LH-20, a size exclusion approach

that separates constituents based on particle size. Ferric chloride spray was used to selectively get out phenolic constituents, which are reputed to be good free radical scavengers with good UV activity. However, compound 1 was isolated as a prominent spot in a fraction, based on the yield of the fraction which later turned out to give positive reaction with Lieberman-Burchard reagent.

Structural elucidation of isolated compounds

Compound **1** was isolated as white amorphous powder. It tested positive with Liebermann-Burchard test for triterpenoids. The compound gave a signal for *m/z* 1176.1453 $[2M]^+$ for a dimer of molecular formula $C_{36}H_{60}O_6$. The NMR spectra result displayed 30 carbon atoms in addition to a unit of sugar, indicating the presence of a triterpenoid glycoside. The ^{1}H NMR spectrum showed the presence of eight angular methyl groups at δH: 0.65 (3H, s), 0.78 (3H, s), 0.81 (3H, s), 0.82 (3H, d, *J*=1.67 Hz), 0.90 (3H, s), 0.96 (3H, s), 0.98 (3H, s) and 1.00 (3H, s). A vinyl proton signal was observed at δH 5.34, which correlated with the carbon signal at δc 121.7 in the HSQC spectrum. ^{13}C NMR spectrum displayed 30 signals with 6 oxygenated methine carbon signals at δc; 77.4 (C-3), 77.3 (C-2′), 77.3 (C-4′), 73.9 (C-5′), 70.6 (C-3′) and one oxygenated methylene carbon signal at δc 61.2 (C-6′). One quaternary olefinic carbon signal was observed at δc 140.5 and this signal was observed to have a long-range correlation with the proton signal at δH 0.94 (H-27). A signal for the anomeric proton of sugar was observable at δH 4.23, with a coupling constant of 7.8 Hz, indicating a β-D-Glucose. This signal has a long-range correlation with the carbon signal at δc 77.4 (C-3) on the triterpenoid skeleton, which allowed placement of the sugar unit at position 3 of the ursene moiety. Compound **1** was therefore identified as Olea-12-ene 3-*O*-β-D-Glucopyranoside based on comparison with literature values (NMR data) [21] (Figure 1 and Supplementary Table 1).

Compound **2** was isolated as a yellow powder. The UV spectrum at 257 and 356 was characteristic of quercetin flavonoid. HRTOFMS in the negative mode displayed a signal at *m/z* 463.0880 $[M-H]^-$, (cal. 463.0877) for a molecular formula $C_{21}H_{19}O_{12}$. The ^1H NMR spectrum displayed signals at δH: 7.73 (1H, d, *J*=2.1 Hz), 7.60 (1H, dd, *J*= 8.5, 2.1 Hz), 6.88 (1H, d, *J*=8.6 Hz), 6.41 (1H, d, *J*=2.1 Hz) and 6.22 (1H, d, *J*=2.1 Hz). An anomeric proton signal was observed at δH 5.22 (1H, d, *J*=7.4 Hz), the large coupling constant indicating a β-glycoside. Other signals were observed between δH 3.25 and 3.65. On comparison with literature data (NMR spectra), [22] compound **2** was identified as isoquercitrin (quercetin glycoside) (Figure 1).

Compound 3 was isolated as a brown powder. Its UV spectrum displayed strong absorption at 257 and 357 nm, characteristic of quercetin flavonoid. The high resolution TOF ES MS gave a signal at *m/z* 649.1378 $[M+Na]^+$ (calculated 649.1381) for a molecular formula $C_{27}H_{30}O_{13}Na$. The proton NMR spectrum displayed five aryl proton signals at δH: 7.72 (1H, d, *J*=2.0), 7.68 (1H, dd, *J*=8.4, 2.0 Hz), 6.88 (1H, d, *J*=8.2 Hz), 6.42 (1H, d, *J*=1.9 Hz), 6.22 (1H, d, *J*=1.8 Hz). Two anomeric proton signals were observed at δH 5.25 (1H, d, *J*=7.3 Hz) and 4.17 (1H, d, *J*=7.6 Hz). The large coupling constant values for the anomeric protons indicated a β-orientation for each of the sugars. The other sugar protons were observed at δH 3.99 (1H, d, *J*=11.9 Hz) and 3.77 (1H, dd, *J*=12.1, 2.4 Hz) and a series of overlapping proton signals between δH 3.02 and 3.69. On comparison of the NMR data with the literature values, Compound **3** was identified as 5,7,3′,4′-tetrahydroxy-flavonol-3-O-[*β*-D- glucopyranosyl-1→6)]-*β*-D-glucopyranoside (quercetin diglycoside). [14] (Figure 1).

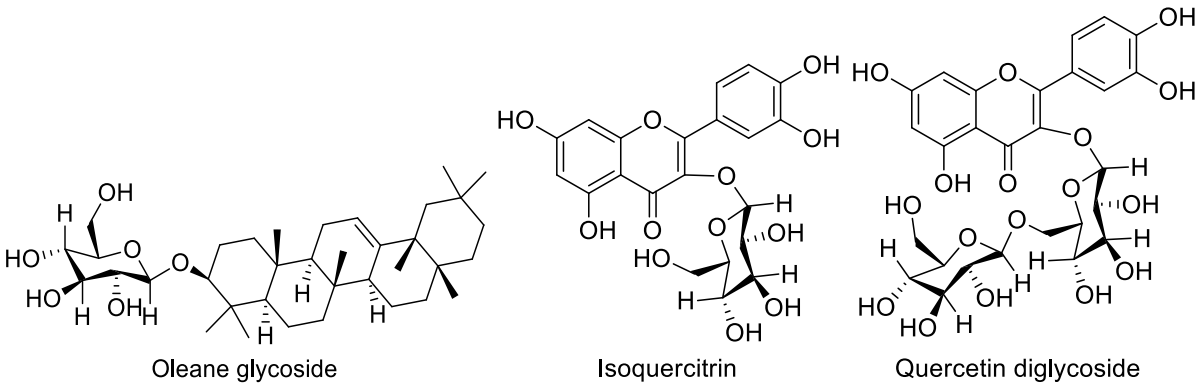

Figure 1: Chemical structures of the three isolated compounds

Mild cytotoxicity of compound 1 upon prolonged exposure and non-cytotoxicity of compounds 2 and 3

We assessed the potential toxicity of the three isolated compounds in two different cancer cell lines, the HeLa cell line (a model of cervical cancer) and the MRC5-SV2 cell line (a model of lung cancer). Generally, following long-term incubation (48 h) with the cells, compound 3 was not toxic to the cells and compounds 1 and 2 elicited only mild toxicity (IC_{50}>>100 µg/ml). In HeLa cells, following 48 h exposure, the positive control compound, doxorubicin (up to 20 µM), an anti-cancer drug, elicited concentration-dependent toxicity, which was statistically significant from 0.5 µM (Figure 2). However, compounds 1 and 2 only elicited mild toxicities, while compound 3 was not toxic. The effects of the compounds on a second cancer cell line are shown in Figure 3. Similar to what was obtained in HeLa cells, when tested on the MRC5-SV2 cells, doxorubicin induced concentration-dependent toxicity, significant from 0.5 µM, while compound 1 induced mild toxicity and compounds 2 and 3 were not toxic at all (Figure 3). The three compounds are glycosides and not aglycones. Compound 1 belongs to the class of triterpenes (it is a triterpene glycoside - a saponin). Generally, while triterpenes are usually found to be toxic [23, 24], there are also reports of non-toxic triterpenoids [25, 26]. Compounds 2 and 3 are quercetin glycosides. Many flavonoids are known to exist in nature in the form of their glycosides rather than as aglycones, and glycosylated flavonoids have been demonstrated

to be less toxic than their aglycones [27, 28], which could explain the non-toxic effects of compounds 2 and 3, at least, in part.

The observation that the tested compounds were relatively non-toxic led us to attempt to investigate their potentially beneficial biological effects, and as it is known that plants contain a significant number of molecules that are antioxidant in nature, through which they could combat disease mechanisms, we first investigated whether these compounds could protect against a build-up of ROS that leads to oxidative stress.

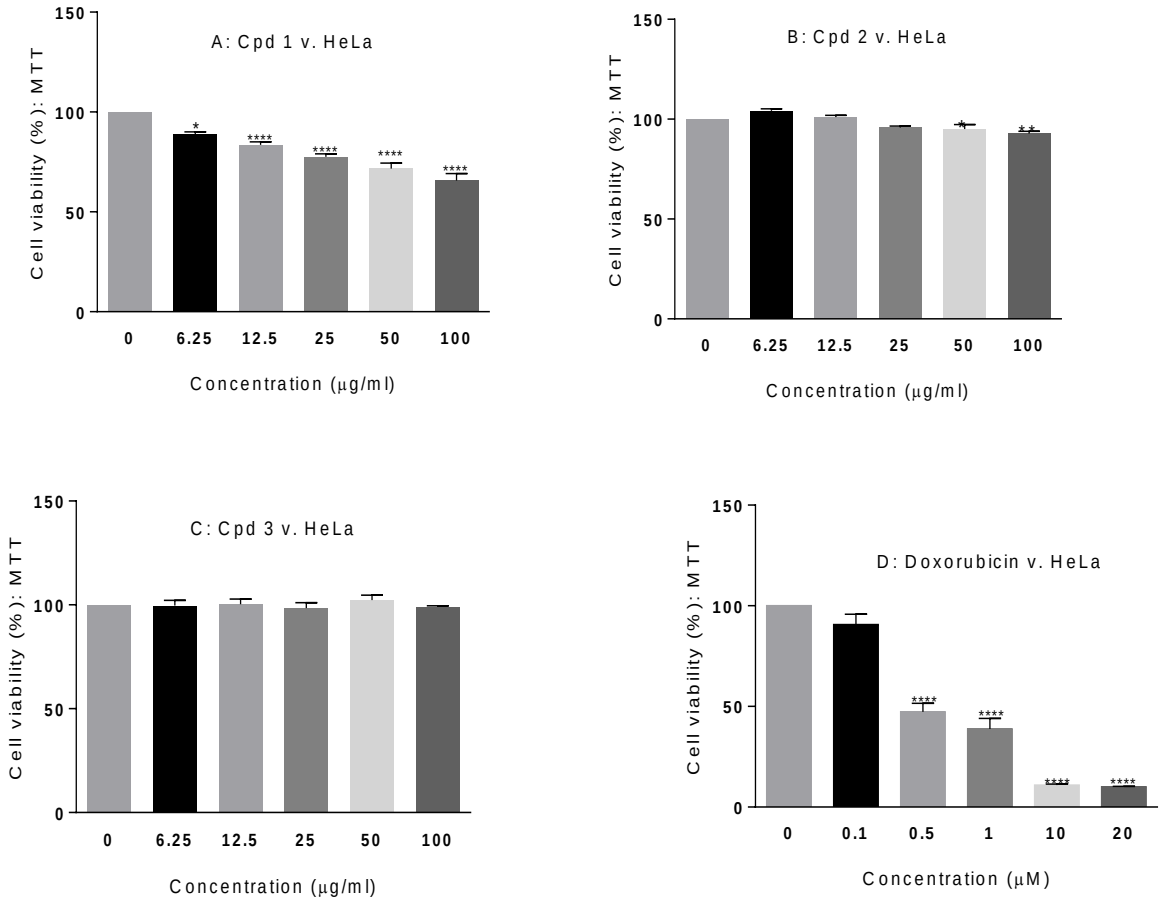

Figure 2: Cell viability (%) in HeLa cells following 48 hours exposure to Compounds 1, 2, 3 and doxorubicin. Each bar represents mean ± SEM (n=3); *p<0.05, **p<0.01, ****p<0.0001 vs. control using one-way ANOVA followed by Dunnett's *post hoc* multiple-comparisons test.

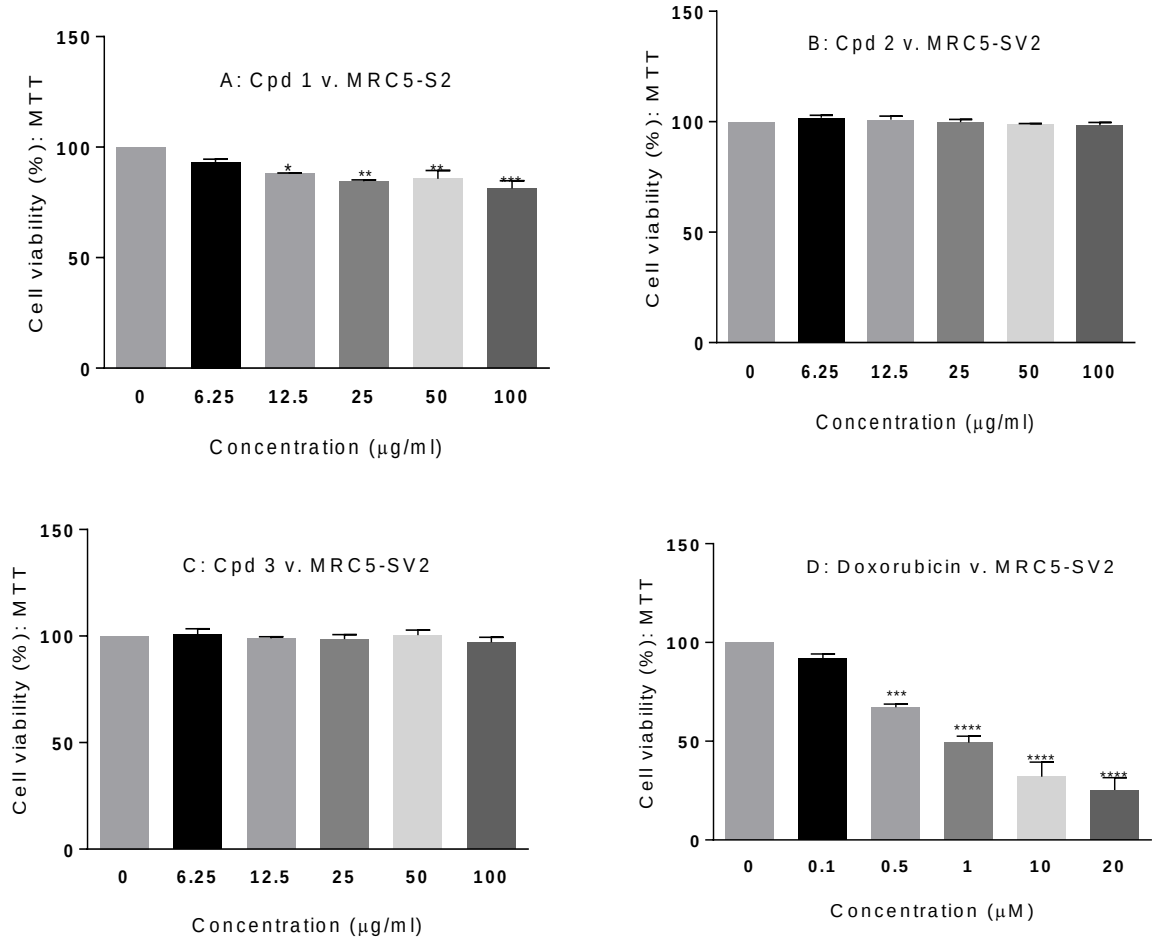

Figure 3: Cell viability (%) in MRC5-SV2 cells following 48 hours exposure to Compounds 1, 2, 3 and doxorubicin. Each bar represents mean ± SEM (n=3); *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001 vs. control using one-way ANOVA followed by Dunnett's *post hoc* multiple-comparisons test.

Compounds 2 and 3, but not Compound 1, potently protected against peroxide-induced intracellular build-up of oxidative stress in a ROS (DCFDA) Assay

In view of the relative non-toxicity of the compounds and our observation that compound **1** is an oleane analogue while compounds **2** and **3** are flavonoids, we investigated their ability to protect against ROS induction. We employed hydrogen peroxide as the oxidative stressor, as

it has ability to release active oxygen species, especially due to its potential to produce the highly reactive hydroxyl radical through the Fenton reaction. [29, 30] In the presence of hydrogen peroxide (100 µM), ROS levels were significantly increased nearly 2.5-fold compared to the negative control ($P<0.01$), when assessed at an early time point (3 h), and the profile was sustained in the longer term (24 h) (Figure 4). Compound **1** did not modulate the hydrogen peroxide-induced increase in ROS, as levels of ROS remained sustained significantly well above the control levels, whether at 3 h or 24 h post-induction. However, compounds 2 and 3 each reduced peroxide-induced ROS levels in a concentration-dependent manner, with their highest concentration (100 µM) bringing ROS levels to negative control levels (indicating complete protection against ROS induction). Notably, the protective effects of compounds 2 and 3 against peroxide-induced ROS were sustained over the 24 h post-induction period (Figure 4). The IC_{50} values for compounds 2 and 3, respectively, were 2.7 ± 0.5 µg/ml and 1.9 ± 0.2 µg/ml for the 3 h time point, and 2.0 ± 0.8 µg/ml and 1.5 ± 0.4 µg/ml for the 24 h time point. These values in the low micromolar range suggest compounds 2 and 3 to be potent and thus promising antioxidants that deserve to be further investigated in models of diseases where oxidative stress or its downstream effects (such as lipid peroxidation) play a significant mechanistic role, including cancers.

Our findings demonstrate a clear correlation of chemical structures/nature with biological effects. Compounds 2 and 3, which are quercetin analogues (flavonoids and polyphenols) are potent antioxidants, which we rationalise was as a result of their ability to scavenge free radicals, as flavonoids and polyphenols have been shown to possess significant antioxidant actions linked to their ability to scavenge radicals [31] as well as superoxides and peroxynitrite.[32] On the other hand, compound 1, a triterpene, did not have any antioxidant ability, as would normally be expected, since it is non-phenolic.

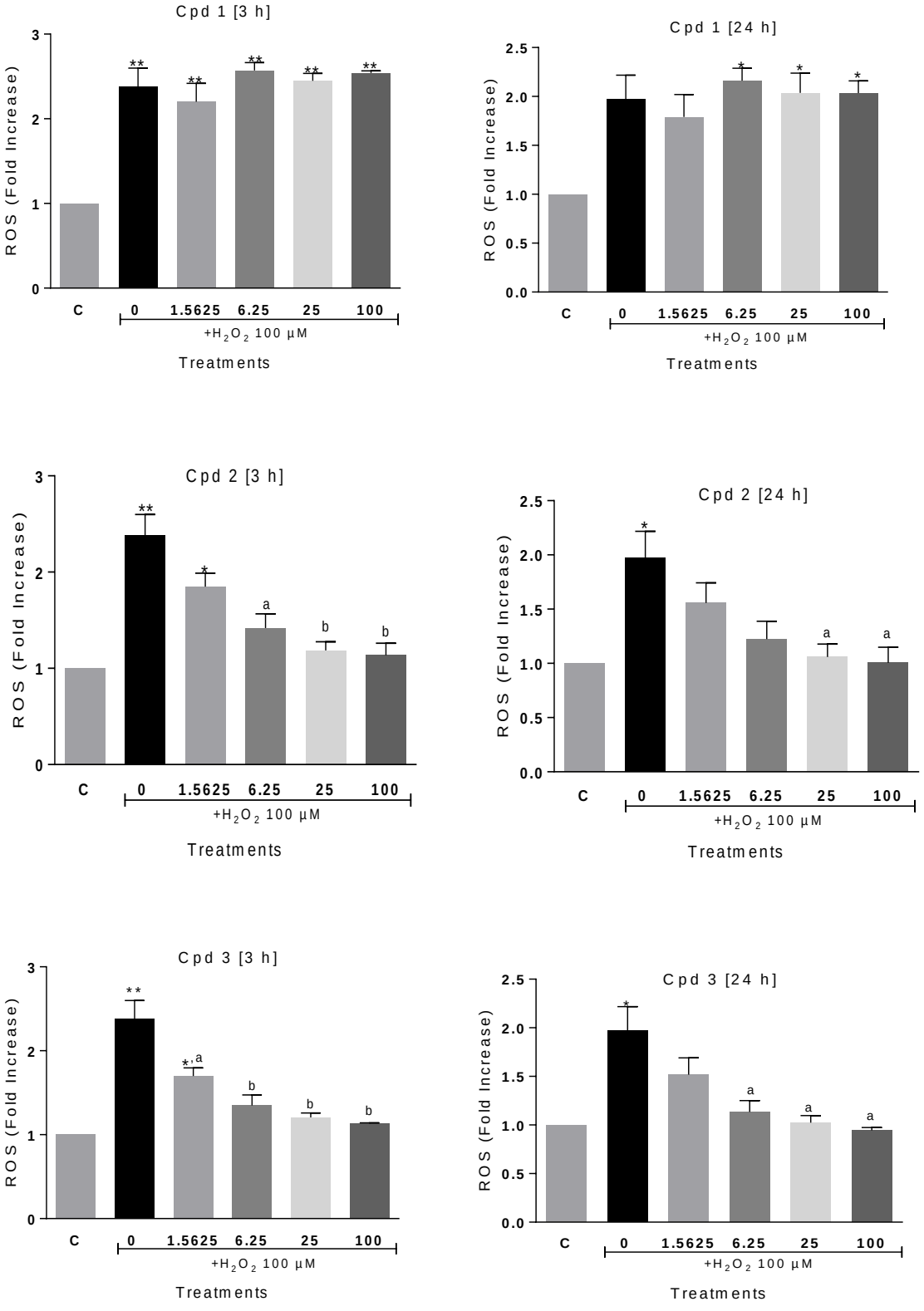

Figure 4: Cellular ROS levels (fold increases compared to negative control) following 3 h and 24 h exposure of HeLa cells to hydrogen peroxide (100µM) in the absence or presence of each of Compounds 1, 2 and 3 (up to 100µM). Each bar represents Mean ± SEM of

duplicate values of an experiment that was run three times with similar results; $^{*}p<0.05$, $^{**}p<0.01$ versus vehicle control (C). $^{a}p<0.05$, $^{b}p<0.01$ versus 'peroxide alone' treatment, using one-way ANOVA followed by Dunnett's *post hoc* multiple-comparisons test.

Our work provides further inspiration to investigate medicinal plants and other natural sources of small molecules for novel chemical structures that could become, or ultimately generate, drug leads. It is known that a significant proportion of current anti-cancer drugs are either natural products or have been derived from natural product drug leads. Molecules such as compounds 2 and 3 in this work, which are not toxic but potently protective against ROS, might be useful in cancer chemoprevention by preventing a build-up of ROS, which could lead to oxidative stress that could contribute to the development of cancer. They can also be used to treat a wide range of diseases for which oxidative stress is a major causal or contributory factor. This can include their use as components of an anti-cancer remedy.

Conclusion

This study identified for the first time the presence of an oleanene skeleton triterpenoid (oleane glycoside - compound 1) in *A. esculentus*. This non-toxic compound did not protect against a build-up of ROS within the cell, unlike the two quercetin glycosides (isoquercitrin and flavonol diglycoside, as compounds 2 and 3, respectively), also identified from the plant, which were confirmed to be potently anti-ROS. The anti-ROS effects of isoquercitrin and flavonol diglycoside lend some credence to the rationale for including okra in traditional remedies for the treatment of conditions such as diabetes, cardiovascular complications and cancers, which have been reported to be underpinned, at least, in part, by raised levels of cellular oxidative

stress. The findings also affirm plants as an enduring source of novel compounds or inspirations for creating synthetic compounds for drug development.

Experimental Section

General

Solvents used for extraction and chromatography were redistilled before use. Open column chromatography was performed with Kieselgel 60 (ASTM 230–400 mesh, 0.040–0.063 mm particle size, Merck). Size exclusion column chromatography was achieved using Sephadex LH-20 (Pharmacia, Sweden) pre-swollen in the specified solvent before loading onto the column. All Thin Layer Chromatography (TLC) analyses were performed at ambient temperature using analytical silica gel 60 $GF_{254+366}$ pre-coated aluminium backed plates (Merck, 0.25 mm thick). The resulting spots on TLC plates were visualized under UV light (254 nm) and detected by the use of vanillin/H_2SO_4 and ferric chloride spray reagents as appropriate. ^{1}H and ^{13}C NMR spectra (for both 1D and 2D experiments) were obtained on the Bruker AV400 (IconNMR) Spectrometer at 400 and 100 MHz, respectively, while the HRMS analyses were carried out on an Agilent Quadrupole Time-of-Flight LCMS machine at the School of Chemistry and Physics of the Kwazulu-Natal University in Pietermaritzburg, South Africa.

Plant collection

The fruit of *Abelmoschus esculentus* was collected from the Obafemi Awolowo University Central market in August 2018 and identified by Mr A.A. Ogunlowo, Curator of the Department of Pharmacognosy, Faculty of Pharmacy, Obafemi Awolowo University. A voucher specimen with number FPI 2221 was deposited in the Faculty Herbarium. The vegetative part was air-dried at room temperature and milled. The powdered plant parts (1.0 kg) were extracted with

100% Methanol (2.5 L) and the crude extract was concentrated *in vacuo* to give 164.8 g of the crude extract.

Isolation of compounds

The crude extract (50.4 g) was dissolved in 100 mL of water and subjected to solvent-solvent partitioning with *n*-hexane. The aqueous fraction was concentrated to dryness *in vacuo* and 2.1 g of the aqueous fraction was subjected to solid phase extraction on Phenomenex cartridges using the following solvent mixtures: MeOH:H_2O (20:80), **AE 1** (0.942 g); 50:50, **AE 2** (0.568 g); 80:20, **AE 3** (0.207 g) and 0:100, **AE 4** (0.184 g). Fraction AE 3 (9.86 % w/w) of the crude extract, eluted with 80 % v/v aqueous methanol, showed UV active spots and spots that tested positive with ferric chloride spray. The fraction (0.2 g) was adsorbed on silica gel, mesh 200-400 and allowed to dry. The dry powder was packed into a column (i.d 30 x 3) with a layer of plain silica gel below as the separating phase. The column was eluted with solvents of increasing polarity from 100 % v/v *n*-hexane through 100 % v/v ethyl acetate up to 15% v/v ethanol in ethyl acetate and eluted under gravity on an open column. The content of each tube was analysed on thin layer chromatography. Fractions with similar chromatographic profile (colour with ferric chloride spray reagent, retardation factor, *etc*.) were bulked together to give fractions AE 3A - AE 3C. Fraction AE 3B (0.082 g, with UV and ferric chloride positive spots) was dissolved in 3 mL of ethyl acetate : methanol (9:1) and was layered carefully on a column (i.d 15 x 2.5 cm) of Sephadex LH-20 previously swollen overnight in ethyl acetate : methanol (9:1). The column was eluted using isocratic gradient of 10 % v/v methanol in ethyl acetate to give compound **3 (**brown powder, 0.034 g, R_f 0.44 (solvent 1)), which came out first, followed later by compound **2 (**yellow powder, (0.085 g, R_f 0.52 (solvent 1)). Fraction AE 2 (0.5 g), isolated with 50 % aqueous methanol, was purified on silica gel with increasing proportions of methanol in ethyl acetate, to give seven sub-fractions, AE 2A - AE 2G. Fraction AE 2E (0.2 g) with a prominent spot which was only faintly UV active but gave positive result with Lieberman-

Burchard test was subjected to further purification on an open silica gel column (i.d 20 x 3 cm) with 100 ml each of ethyl acetate : methanol (100:0, 90:10, 80:20, 60:40) as mobile phase. Eluate analysed on thin layer chromatographic plate was bulked together. Semi-purified fraction of the Lieberman-Burchard test positive spot was subjected to repeated purification on silica gel with 100 mL each of ethyl acetate : methanol (97.5:2.5, 95:5, 90:10, 85:15 and 80:20). The compound was finally purified on a column of Sephadex LH-20 with isocratic gradient of ethyl acetate: methanol (9:1) to give compound **1** (white amorphous power, 0.024 g, R_f 0.58 (mobile phase was Ethyl acetate: methanol: water : acetic acid (10:2:1: 0.2)). The isolated compounds were subjected to NMR (1D and 2D analyses), UV and MS analyses.

Cell culture

HeLa cell (immortalized human cervical cell line) and MRC-5 SV2 cell (human foetal lung fibroblast line transfected with the virus SV40 – a model of lung cancer) were grown in culture and maintained in Dulbecco's Modified Eagle Medium (DMEM) (4.5 g/L D-glucose) supplemented with 10% Foetal Calf Serum (FCS), 1% L-Glutamine (2mM), and 1% antibiotic-antimycotic solution (penicillin/streptomycin/amphotericin B) at 37°C in a humidified atmosphere of 5% CO_2.[33]

Cell viability assay to assess cytotoxicity

Compounds **1**, **2** and **3** at concentrations up to 100 µg/ml were evaluated for their potential to alter the viability of HeLa and MRC-5 SV2 cells. The procedure was similar to what we previously reported.[33] Briefly, cells were seeded into opaque 96-well plates ($7.5x10^4$ cells/ml, 100 µl added to each well) and incubated for 24 hours at 37°C and 5% CO_2 to allow the cells to adhere. After 24 hours, the medium was discarded and the wells were treated with 100 µl of

the different concentrations of extracts prepared in growth medium. A set of untreated (control) cell-containing wells (vehicle only and growth medium only) was included in each plate, as well as cells treated with doxorubicin at concentrations up to 20 µM. Doxorubicin as an anti-cancer agent was used as a positive control.

Following incubation with each compound for up to 48 hours, 10 µl of MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, 5mg/ml in PBS) was added to each well. After 2 hours of incubation with MTT at 37°C, the content of each well was aspirated and 100 µl of DMSO was added to each well to solubilise the formed formazan. Absorbance was then read at 570 nm on a microplate reader (CLARIOstar, BMG Labtech, UK). Each experiment was repeated three times with each treatment run in triplicate. The mean viability of the negative control culture was set to 100% and the mean viability of each treatment was normalised to it.

Reactive Oxygen Species (ROS) assay using DFCDA

HeLa cells were used for the experiment, seeded into dark, clear-bottom 96-well microplates at a density of $2.5x10^6$ cells per ml ($2.5x10^5$ cells per well, with 100µl in each well). The cells were incubated at 37°C and allowed to adhere overnight. The medium was thereafter aspirated from each well, followed by rinsing with 1X buffer provided in the assay kit (Abcam, Cat. No. ab113851). The buffer was aspirated and the cells stained by adding 100 µl of diluted 2',7' – dichlorofluorescein diacetate (DCFDA) solution. Stained cells were incubated for 45 min at 37°C in the dark. After 45 min, DCFDA solution was removed from stained cells, the cells were rinsed with buffer, the buffer was aspirated and the cultures were treated with hydrogen peroxide in the absence or presence of each compound, tested at a range of concentrations (up to 100 µg/ml). The Fluorescence Intensity (FI) (Ex/Em = 485/535 nm) of the wells was read on

a microplate reader (CLARIOstar, BMG Labtech, UK) at 3 and 24 h following treatment. Background wells (untreated or diluent-treated stained cells), as well as blank wells (medium only), were included in each experiment. Each experiment was repeated three times, with each treatment run in duplicate. Cellular ROS was analysed as fold changes compared to the negative control.

Data representation and statistics

Values are expressed as Mean ± SEM (standard error of the mean), except where otherwise stated. Statistical analyses were conducted using GraphPad Prism (Version 8.3.1) (GraphPad Software Inc., CA, USA). One-way Analysis of Variance with post-hoc test for multiple comparisons was used to assess the statistical significance of differences between means and a value of $P<0.05$ was considered statistically significant.

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Author Contribution Statement

BJT and AAF designed the study; BJT conducted the chemistry experiments and analyses; TDP and AAF conducted the biological assays and statistical analyses; BJT, TDP, and AAF wrote the manuscript; FvRH and AAF supervised the chemistry and biology aspects of the work, respectively; AAF reviewed the final draft of the manuscript. All authors approved the manuscript for submission.

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GRAPHICAL ILLUSTRATION