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Original Research Article

Cytotoxicity of 11 naturally occurring phenolics and terpenoids from Kenyan flora towards human carcinoma cells

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ABSTRACT

Background: Cancer constitutes a major hurdle worldwide and its treatment mainly relies on chemotherapy.
Objectives: The present study was designed to evaluate the cytotoxicity of 11 naturally occurring compounds including six phenolics amongst them were 4 chalcones and 2 flavanones as well as 5 terpenoids (3 clerodane and 2 trachylobane diterpenoids) against 6 human carcinoma cell lines and normal CRL2120 fibroblasts.
Materials and methods: The neutral red uptake (NR) assay was used to evaluate the cytotoxicity of the compounds, whilst caspase-Glo assay was used to detect caspase activation. Cell cycle and mitochondrial membrane potential (MMP) were all analyzed *via* flow cytometry meanwhile levels of reactive oxygen species (ROS) was measured by spectrophotometry.
Results: Chalcones: 2′,4′-dihydroxy-6′-methoxychalcone (**1**); 4′,6′-dihydroxy-2′,5′-dimethoxychalcone (**2**); 2′,4′,6′-trihydroxy-5′-methoxychalcone (**3**); 2′,6′-diacetate-4′-methoxychalcone (**4**), trachylobane diterpenoids: 2,6,19-trachylobanetriol; (ent-2α,6α)-form (**10**) and 2,18,19-trachylobanetriol; (ent-2α)-form (**11**) as well as doxorubicin displayed IC_{50} values below 110 μM in the six tested cancer cell lines. The IC_{50} values of the most active compounds were between 6.30 μM and 46.23 μM for compound **1** respectively towards breast adenocarcinoma MCF-7 cells and small lung cancer A549 cells and between 0.07 μM and 1.01 μM for doxorubicin respectively against SPC212 cells and A549 cells. Compounds **1** induced apoptosis in MCF-7 cells mediated by increasing ROS production and MMP loss.
Conclusion: Chalcones **1**–**3** are potential cytotoxic phytochemicals that deserve more investigations to develop novel anticancer drugs against human carcinoma.

1. Introduction

Cancer is a huge global burden with an increasing incidence not only due to the growth and aging of the population, but also to the increased prevalence of risk factors such as smoking, obesity, sedentary lifestyle and changing reproduction patterns related to urbanization and economic development [1]. About 14.1 million new cancer cases and 8.2 million deaths occurred in 2012 worldwide [2,3]. In both developed and less developed countries, lung cancer is the leading cause of cancer death in men, surpassing breast cancer as the leading cause of cancer death among women in more developed countries. However, breast cancer remains the leading cause of cancer death among women in less developed countries [1]. Other leading causes of cancer death in developed countries include colon cancer in men and women and prostate cancer in men. In developing countries, the liver and stomach cancers in men and the cervix in women are also the main causes of cancer death [1]. Chemotherapy remains the major mode of the treatment of various neoplastic diseases. Africa has a rich flora with high potential to fight against malignant diseases [4,5]. In the past, phytochemicals with interesting cytotoxic activities were isolated

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in several medicinal plants throughout the continent. Phenolic compounds or phenolics, are a class of chemical compounds consisting of a hydroxyl group bonded directly to an aromatic hydrocarbon group. Terpenoids are a class of naturally occurring organic chemicals derived from five-carbon isoprene units assembled and modified in thousands of ways [6]. Diterpenoids are terpenoids having two terpene units (C20). Phenolic compounds such as chalcone, flavanones as well as diterpenoids including clerodane and trachylobane types possess various pharmacological properties including cytotoxic effects in various cancer cell lines [7–11]. In our continuous search of antiproliferative molecules to fight cancers, the present study was undertook to investigate the cytotoxicity of eleven compounds including six phenolics amongst which were four chalcones and two flavanones as well as five terpenoids including three clerodane type and two trachylobane type diterpenoids previously isolated from Kenyan medicinal plants. The study was extended to the assessment of the mode of action of the most active compound, 2′,4′-dihydroxy-6′-methoxychalcone (**1**).

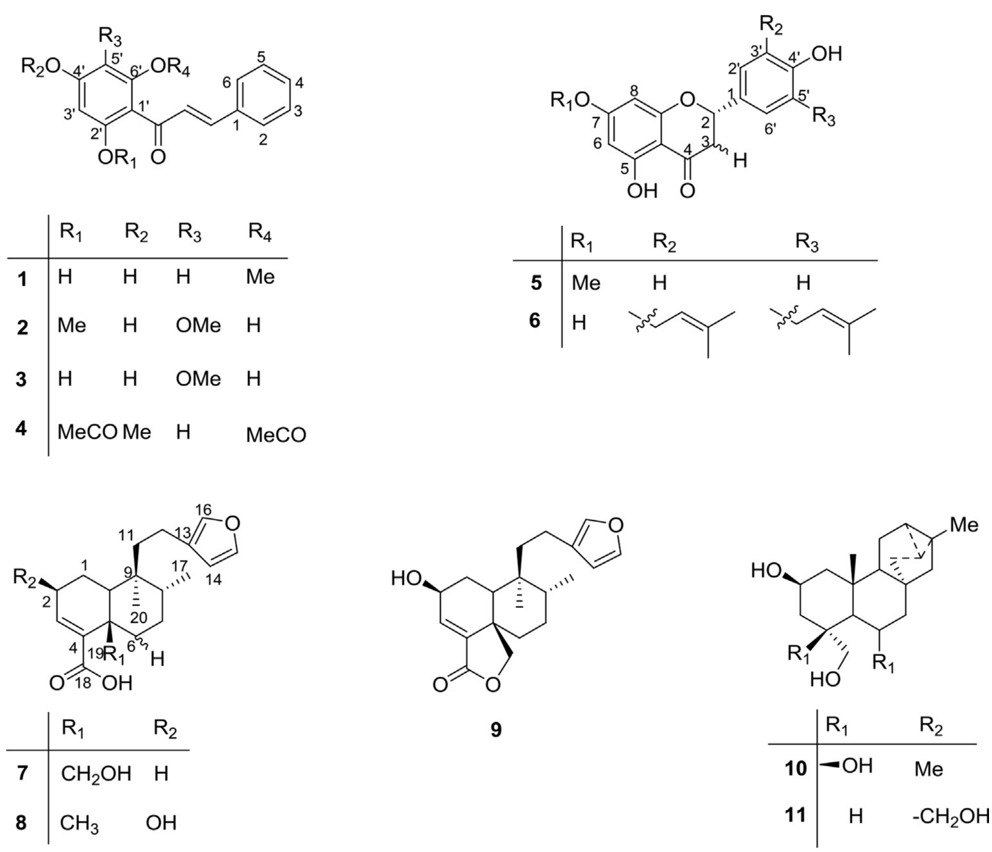
2. Materials and methods

2.1. Chemicals

The reference molecule, Doxorubicin 98.0% was purchased from Sigma–Aldrich (Munich, Germany) and used as positive control. Phytochemicals reported herein (Fig. 1) were obtained from the chemical bank of the natural products research laboratory of the Chemistry Department, University of Nairobi, Kenya. They were isolated from various Kenya flora: *Erythrina abyssinica, Dodonaea angustifolia, Polygonum senegalense, Psiadia punctulata, and Senecio roseiflorus* [12]. They include six flavonoids including four chalcones: 2′,4′-dihydroxy-6′-methoxychalcone, $C_{16}H_{14}O_4$ (**1**; *m/z*: 270.0892), 4′,6′-dihydroxy-2′,5′-dimethoxychalcone, $C_{17}H_{16}O_5$ (**2**; *m/z*: 300.0998), 2′,4′,6′-trihydroxy-5′-methoxychalcone, $C_{16}H_{14}O_5$ (**3**; *m/z*: 286.0841), 2′,6′-diacetate-4′-methoxychalcone, $C_{20}H_{18}O_6$ (**4**; *m/z*: 354.1103), two flavanones: 5,4′-dihydroxy-7-methoxyflavanone, $C_{16}H_{14}O_5$ (**5**; *m/z*: 286.0841) and 5,7,4′-trihydroxy-3′,5′-diprenylflavanone, $C_{25}H_{28}O_5$ (**6**; *m/z*: 408.1937), six terpenoids including diterpenoids, three clerodane type: hautriwaic acid, $C_{20}H_{28}O_4$ (**7**; *m/z*: 332.1988), 2*β*-hydroxyhardwickiic acid, $C_{20}H_{28}O_4$ (**8**; *m/z*: 332.1988), hautriwaic acid lactone, $C_{20}H_{26}O_4$ (**9**; *m/z*: 330.1831) and two trachylobane type: 2,6,19-trachylobanetriol; (ent-2*α*,6*α*)-form, $C_{20}H_{32}O_3$ (**10**; *m/z*: 320.2351) and 2,6,19-trachylobanetriol; (ent-2*α*,6*α*)-form, $C_{20}H_{32}O_3$ (**11**; *m/z*: 320.2351) [12]. These compounds are available in the Chemical bank of the Department of Chemistry, University of Nairobi.

2.2. Cell lines and culture

Seven cell lines including six human carcinoma and one normal cell line were tested. They were: A549 human non-small cell lung cancer (NSCLC) cell line (obtained from the Institute for Fermentation, Osaka (IFO, Japan) and provided by Prof. Dr. Tansu Koparal; Anadolu University, Eskisehir, Turkey), SPC212 human mesothelioma cell line (provided by Dr. Asuman Demiroğlu Zergeroğlu; Gebze Technical University, Kocaeli, Turkey), Caco-2 colorectal adenocarcinoma cells (obtained from the ŞAP Institute of Turkey (Ankara)), DLD-1 colorectal adenocarcinoma cell lines, HepG2



	R_1	R_2	R_3	R_4
1	H	H	H	Me
2	Me	H	OMe	H
3	H	H	OMe	H
4	MeCO	Me	H	MeCO

	R_1	R_2	R_3
5	Me	H	H
6	H	prenyl	prenyl

	R_1	R_2
7	CH_2OH	H
8	CH_3	OH

9

	R_1	R_2
10	OH	Me
11	H	$-CH_2OH$

Fig. 1. 2′,4′-dihydroxy-6′-methoxychalcone (**1**); 4′,6′-dihydroxy-2′,5′-dimethoxychalcone (**2**); 2′,4′,6′-trihydroxy-5′-methoxychalcone (**3**); 2′,6′-diacetate-4′-methoxychalcone (**4**); 5,4′-dihydroxy-7-methoxyflavanone (**5**); 5,7,4′-trihydroxy-3′,5′-diprenylflavanone (**6**); hautriwaic acid (**7**); 2*β*-hydroxyhardwickiic acid (**8**); hautriwaic acid lactone (**9**); 2,6,19-trachylobanetriol; (ent-2*α*,6*α*)-form (**10**); 2,18,19-trachylobanetriol; (ent-2*α*) (**11**).

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hepatocarcinoma cells and MCF-7 breast adenocarcinoma cells (purchased from American Type Culture Collection (ATCC) and provided by Prof. Dr. Tansu Koparal (Anadolu University, Eskisehir, Turkey), and the normal CRL2120 human skin fibroblasts (obtained from ATCC). DMEM medium (Sigma-aldrich, Munich, Germany) was used to maintain as a monolayer and was supplemented with 10% fetal calf serum and 1% penicillin (100 U/ml)-streptomycin (100 μg/ml) in a humidified 5% CO_2 atmosphere at 37 °C.

2.3. Neutral red (NR) uptake assay

The cytotoxicity of compound **1** and doxorubicin (positive control) was performed by NR uptake assay as previously described [13]. NR uptake assay is cheaper and more sensitive than other cytotoxicity tests and is based on the ability of viable cells to incorporate and bind the supravital dye NR in the lysosomes [14]. Dimethylsufoxide (DMSO) at less than 0.1% final concentration was used to dilute the tested samples. DMSO at 0.1% was used as solvent control. Briefly, cells were seeded at 1×10^4 cells in each well of a 96-well cell culture plate; Samples were tested in a total volume of 200 μl. After 72 h incubation in humidified 5% CO_2 atmosphere at 37 °C, the medium was removed, followed by coloration with medium containing 50 μg/ml NR [13,14]. ELx 808 Ultra Microplate Reader (Biotek) equipped with a 540 nm filter was used to measure the absorbance. Each experiment was performed at three times, with three replicate each. The viability was evaluated based on a comparison with untreated cells. The IC_{50} values represent the sample's concentrations required to inhibit 50% of cell proliferation and were calculated from a calibration curve by linear regression using Microsoft Excel [15].

2.4. Flow cytometry for cell cycle analysis and detection of apoptotic cells

The effect of compound **1** in cell cycle distribution of MCF7 cells was performed by flow cytometry using BD cycle test™ Plus DNA Kit Assay (BD Biosciences, San Jose, USA) as previously described [16]. Briefly, MCF-7 cells (3 ml, 1×10^5 cells/ml) were seeded into each well of 6-well plates and allowed to attach for 24 h (humidified 5% CO_2 atmosphere at 37 °C). Cells treated with ¼ × IC_{50}, ½ × IC_{50} and IC_{50} concentrations of compound 1 and doxorubicin as well as untreated cells (control) were then grown in 6-well plates for 72 h. The BD FACS Aria I Cell Sorter Flow Cytometer (Becton–Dickinson, Germany) was then used for cell cycle analysis. For each sample 10^4 cells were counted. For PI excitation, an argon-ion laser emitting at 488 nm was used. Cytographs were analyzed using BD FACSDiva™ Flow Cytometry Software Version 6.1.2 (Becton–Dickinson).

2.5. Caspase assay

Caspase activity in MCF-7 cells was detected using Caspase-Glo 3/7 and Caspase-Glo 9 Assay kits (Promega, Mannheim, Germany) as previously reported [16–19]. Cells were treated for 6 h (humidified 5% CO_2 atmosphere at 37 °C) with compound **1** and doxorubicin at their 2 × IC_{50} and IC_{50} values with DMSO as solvent control. The BioTek Synergy™ HT multi-detection microplate reader was used to measure the luminescence and caspase activity was expressed as percentage of the untreated control.

2.6. Analysis of mitochondrial membrane potential (MMP)

The MCF7 cells were treated with compound **1**, and the integrity of MMP was analyzed using 5,5′,6,6′-tetrachloro-1,1′,3,3′-tetraethylbenzimidazolylcarbocyanine iodide (JC-1; Biomol, Hamburg, Germany) staining as previously reported [16–19]. Cells (3 mL, 1×10^5 cells/ml) treated for 72 h (humidified 5% CO_2 atmosphere at 37 °C) with different concentrations (¼ × IC_{50}, ½ × IC_{50} and IC_{50}) of compound **1**, and doxorubicin (drug control) or DMSO (solvent control) were incubated with JC-1 staining solution for 30 min according to the manufacturer's protocol [16]. Cells were then measured in a BD FACS Aria I Cell Sorter Flow Cytometer (Becton–Dickinson, Germany). The JC-1 signal was measured at an excitation of 561 nm (150 mW) and detected using a 586/15 nm band-pass filter. The signal was analyzed at 640 nm excitation (40 mW) and detected using a 730/45 nm bandpass filter. Cytographs were analyzed using BD FACSDiva™ Flow Cytometry Software Version 6.1.2 (Becton–Dickinson). All experiments were performed in triplicates.

2.7. Measurement of reactive oxygen species (ROS)

The MCF7 cells (3 ml, 1×10^4 cells/ml) treated for 24 h (humidified 5% CO_2 atmosphere at 37 °C) with different concentrations (¼ × IC_{50}, ½ × IC_{50} and IC_{50}) of compound **1**, and doxorubicin (drug control) or DMSO (solvent control)) were analyzed for ROS production with 2′,7′-dichlorodihydrofluorescein diacetate (H_2DCFH-DA) (Sigma–Aldrich) using OxiSelect™ Intracellular ROS Assay Kit (Green Fluorescence) as recommended by the manufacturer, Cell Biolabs Inc. (San Diego, USA) (Kuete et al., 2016). The fluorescence was measured using SpectraMax® M5 Microplate Reader (Molecular Devices, Biberach, Germany) at 480/530 nm. All experiments were performed in triplicates.

3. Results

3.1. Cytotoxicity

The cytotoxicity of the 11 studied compounds and doxorubicin was determined by the NR uptake assay and the recorded IC_{50} values are summarized in Table 1. The selectivity index was determined as the ratio of IC_{50} value in the CRL2120 normal fibroblast divided by the IC_{50} in the cancer cell line. Compounds **1–4, 10** and **11** as well as doxorubicin displayed IC_{50} values below 110 μM in the six tested cancer cell lines. Compounds **6–9** were not active and the IC_{50} values were respectively above 101.52 μM, 120.48 μM, 125.79 μM, 127.30 μM and 124.22 μM in all cancer cell lines meanwhile the recordable IC_{50} values was obtained in 4/6 tested cancer cell lines for **5**. Concerning the most active compounds, IC_{50} values ranged from 6.30 μM (towards breast adenocarcinoma MCF-7 cells) to 46.23 μM (against small lung cancer A549 cells) for **1**, from 6.11 μM (MCF-7 cells) to 44.59 μM (mesothelioma SPC212 cells) for **2**, from 9.90 μM (MCF-7 cells) to 58.67 μM (A549 cells) for **3**, from 15.21 μM (colon carcinoma Caco-2 cells) to 104.33 μM (SPC212 cells) for **4**, from 18.91 μM (SPC212 cells) to 86.19 μM (A549 cells) for **10**, and from 36.96 μM (SPC212 cells) to 108.73 μM (MCF-7 cells) for **11**. The three most active compounds (**1–3**) were generally less toxic towards normal CRL2120 fibroblast than carcinoma cells, and the obtained selectivity indexes were above 1.00 in the majority of the cases (Table 1). Chalcone **1** (having the lowest IC_{50} values in 4/6 cancer cell lines) as well as doxorubicin were further tested for the effects on cell cycle distribution, caspase activity, MMP loss and ROS production in MCF-7 cells.

3.2. Mechanistic studies

Chalcone **1** was analyzed for its ability to alter the distribution of the cell cycle of MCF-7 breast cancer cells (Fig. 2). It was observed that compound **1** induced concentration-dependent cell cycle

Table 1
Cytotoxicity of tested compounds and doxorubicin towards cancer cell lines and normal cells as determined by the neutral red assay.

Compounds	Cell lines, IC_{50} values in μM and selectivity index[a] (in bracket)						
	A549	SPC212	DLD-1	Caco-2	MCF-7	HepG2	CRL2120
1	46.23 ± 3.87 (0.84)	20.97 ± 1.77 (1.85)	19.00 ± 0.76 (2.04)	12.77 ± 0.97 (3.03)	**6.30 ± 0.61** (6.15)	14.87 ± 1.02 (2.61)	38.73 ± 4.01
2	37.81 ± 2.45 (1.00)	44.59 ± 3.97 (0.85)	25.37 ± 1.86 (1.50)	15.59 ± 1.24 (2.44)	**6.11 ± 0.71** (6.22)	21.78 ± 1.49 (1.74)	38.00 ± 3.11
3	58.67 ± 4.98 (0.77)	28.29 ± 2.11 (1.60)	31.40 ± 2.53 (1.44)	12.38 ± 0.77 (3.66)	**9.90 ± 0.64** (4.58)	28.53 ± 1.86 (1.59)	45.28 ± 3.77
4	84.27 ± 6.58 (0.71)	104.33 ± 8.22 (0.57)	59.51 ± 3.87 (1.00)	15.21 ± 1.08 (3.91)	23.51 ± 2.01 (2.53)	59.51 ± 3.82 (1.00)	59.45 ± 4.92
5	43.43 ± 3.19 (0.77)	111.89 ± 9.47 (0.30)	>139.86	>139.86	**5.98 ± 0.48** (5.63)	59.27 ± 4.77 (0.57)	33.64 ± 2.95
6	>101.52	>101.52	>101.52	>101.52	>101.52	>101.52	>101.52
7	>120.48	>120.48	>120.48	>120.48	>120.48	>120.48	>120.48
8	>125.79	>125.79	>125.79	>125.79	>125.79	>125.79	>125.79
9	>127.39	>127.39	>127.39	>127.39	>127.39	>127.39	>127.39
10	86.19 ± 6.12 (0.85)	18.91 ± 0.96 (3.85)	85.41 ± 5.72 (0.85)	64.38 ± 5.64 (1.13)	78.91 ± 4.39 (0.92)	85.41 ± 5.28 (0.85)	72.84 ± 6.91
11	99.74 ± 6.34 (0.58)	36.96 ± 1.77 (1.55)	104.08 ± 8.93 (0.55)	94.84 ± 5.39 (0.61)	108.73 ± 7.81 (0.53)	104.08 ± 9.62 (0.55)	57.45 ± 3.19
Doxorubicin	**1.1 ± 0.19** (0.58)	**0.07 ± 0.01** (8.43)	**0.37 ± 0.05** (1.59)	**0.72 ± 0.13** (0.82)	**0.35 ± 0.05** (1.69)	**0.18 ± 0.03** (3.28)	**0.59 ± 0.01**

[a] The selectivity index was determined as the ratio of IC_{50} value in the CRL2120 normal fibroblasts divided by the IC_{50} in the cancer cell lines. 2′,4′-dihydroxy-6′-methoxychalcone (1); 4′,6′-dihydroxy-2′,5′-dimethoxychalcone (2); 2′,4′,6′-trihydroxy-5′-methoxychalcone (3); 2′,6′-diacetate-4′-methoxychalcone (4); 5,4′-dihydroxy-7-methoxyflavanone (5); 5,7,4′-trihydroxy-3′,5′-diprenylflavanone (6); hautriwaic acid (7); 2β-hydroxyhardwickiic acid (8); hautriwaic acid lactone (9); 2,6,19-trachylobanetriol; (ent-2α,6α)-form (10); 2,18,19-trachylobanetriol; (ent-2α) (11) In bold: significant activity [5, 21, 22].

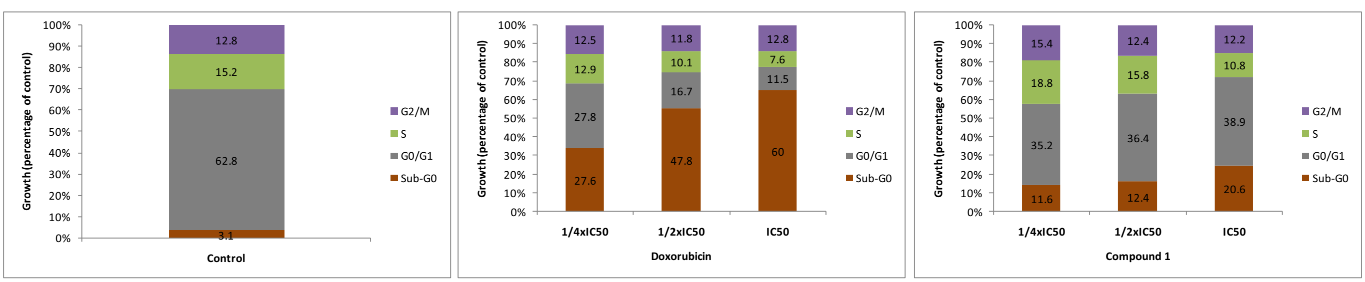

Fig. 2. Effects 2′,4′-dihydroxy-6′-methoxychalcone (**1**) and doxorubicin on cell cycle distribution in MCF-7 cells after 72 h.

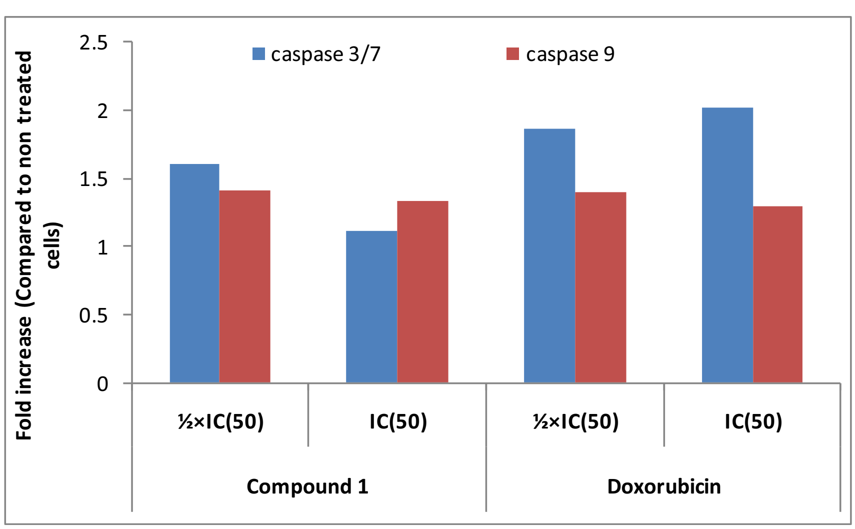

Fig. 3. Effects of 2′,4′-dihydroxy-6′-methoxychalcone (**1**) and doxorubicin on the activation caspases 3/7 and 9 in MCF-7 cells after 6 h.

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modifications with progressive increase of sub-G0/G1 phase cells where cell cycle arrest is between G0/G1 and S phases. MCF-7 cells treated with the phytochemical **1** progressively underwent apoptosis, with increase of sub-G0/G1 cells from 11.6% (¼ IC_{50}) to 20.6% (IC_{50}) compared with doxorubicin which caused up to 60% sub-Go/G1 phase with IC_{50} treatment while the non-treated cells showed only 3.1%.

Upon treatment of MCF-7 cells with compound **1** and doxorubicin with equivalent (eq.) to the ½ × IC_{50} and IC_{50} for 6 h, it was found that this molecule slightly (>one-fold) activate caspase 3/7 and caspase 9 (Fig. 3).

The integrity of the MMP was also investigated upon treatment of MCF-7 cells with chalcone **1** and doxorubicin. Treatment with compounds **1** at eq. to the 1/4 × IC_{50}, 1/2 × IC_{50} and IC_{50} values for 72 h induced concentration-dependent depletion of MMP (Fig. 4). The percentage loss of MMP ranged from 8.0% (1/4 × IC_{50}) to 24.0% (IC_{50}). In similar experimental condition, doxorubicin caused 26% loss of MMP meanwhile only 4.3% was observed with non-treated control.

After treatment of MCF-7 cells with compound **1** and doxorubicin at eq. to the 1/4 × IC_{50}, 1/2 × IC_{50} and IC_{50} values for 24 h, the production of ROS in cells was analyzed (Fig. 5). Flavonoid **1** induced increased ROS levels of more than 3-fold (at IC_{50}) as compared with non-treated cells meanwhile the increase was more than 2-fold after treatment with doxorubicin.

4. Discussion

Cancer is one of the leading causes of mortality worldwide [20]. In the present study, we investigated the ability of naturally occurring phenolics and terpenoids to prevent the proliferation of various carcinoma cell lines, including breast, colon, lung and liver cancers. These cancer types are amongst the leading cause of cancer death globally [1]. Phytochemicals having IC_{50} values around or below 4 μg/ml or 10 μM [5,21,22] have been recognized as potential cytotoxic substances. IC_{50} values below 10 μM were observed with chacones **1**–**3** as well as flavanone **5** in breast adenocarcinoma MCF-7 cells. These

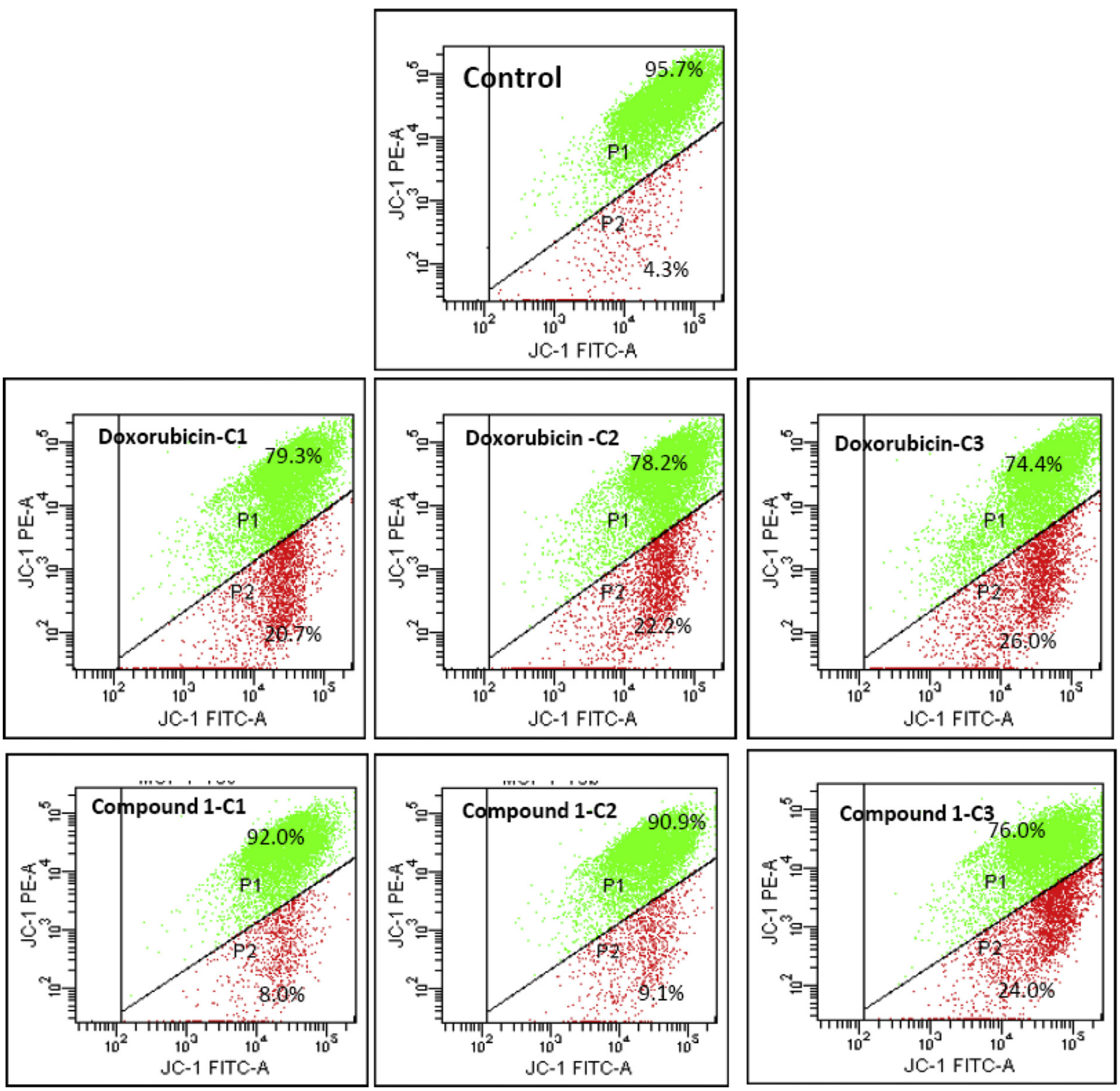

Fig. 4. Effects of 2′,4′-dihydroxy-6′-methoxychalcone (**1**) and doxorubicin on MMP in MCF-7 cells for 72 h. Cells were treated with ¼ × IC_{50} (C1), ½ × IC_{50} (C2) and IC_{50} (C3) of each compound.

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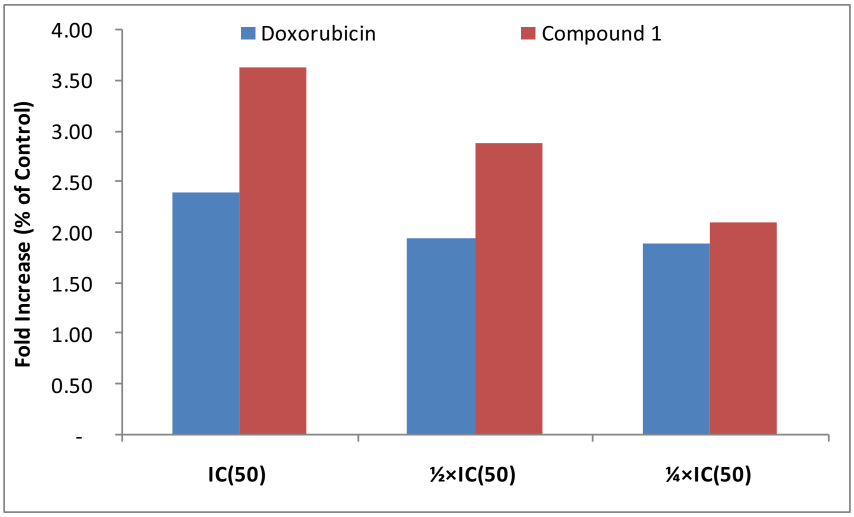

Fig. 5. Induction of ROS in MCF-7 cells after treatment with 2′,4′-dihydroxy-6′-methoxychalcone (**1**) and doxorubicin for 24 h.

data suggest that they can be useful in the management of breast cancer. Compound **1** also had IC_{50} values not far from the threshold of 10 μM against colon carcinoma Caco-2 cells (12.77 μM) and hepatocarcinoma HepG2 cells (14.87 μM), highlighting its good cytotoxic potential. Though none of the highly effective compounds (**1**–**3**) was as active as doxorubicin (Table 1), they generally had good selectivity index, compatible with their possible use in cancer chemotherapy. Hence, they were more toxic towards carcinoma cells than towards normal CRL2120 fibroblast (selectivity index > 1) in most of the cases, indicating their good selectivity. The good activity obtained with chalcones is in accordance with the previous studies. In fact, several chalcones previously isolated from African medicinal plant such as isobavachalcone [23], poinsettifolin B [24], 4′-hydroxy-2′,6′-dimethoxychalcone [25] and 2′,4′-dihydroxy-3′,6′-dimethoxychalcone [26] displayed good cytoxicity with IC_{50} values below 10 μM in various hematological and solid cancer cell lines. Though, flavanones **6** and **5** were not or moderately active, previous studies have demonstrated the good cytotoxicity of other flavanones in cancer cell lines. In fact, the flavanone dorsmanin F [24] previously displayed IC_{50} values below 10 μM towards leukemia CCRF-CEM cells and breast adenocarcinoma MDA-MB-231 cells; however, this compound also had IC_{50} values above 10 μM in several other cancer cell line when tested in similar experimental conditions [24]. The three clerodane type diterpenoids (**7**–**9**) were not active against the tested cancer cell lines within the tested concentration ranges; nevertheless, the cytoxicity of clerodane diterpenoids was demonstrated [7–10] where, caseariagraveolin isolated from *Casearia graveolens* showed strong cytotoxicity against oral cavity and breast cancer cell lines with IC_{50} values of 2.48 and 6.63 μM [27]. Other strong cytotoxic clerodane diterpene include casearin J [9] and caseagrewifolin B [8]. The cytotoxicity of trachylobane diterpenoids has also been reported and the reported effects of compounds **10** and **11** were in consistence with the previous studies [28] where, ent-trachyloban-3beta-ol, had cytotoxic activity against human cervix carcinoma cells, displaying IC_{50} value of 7.3 μM on HeLa cells [28]. In the present study, it was found that compounds **1** induced apoptosis in MCF-7 cells (Fig. 2). Consequently, further investigations of the mode of induction of apoptosis were performed. Caspases regulate apoptosis by cleaving cellular proteins at specific aspartate residues [29]. It was found that caspase-dependent cell death may not be the major pathways of induction of apoptosis by **1** as little changes were observed in the activity of caspases 3/7 and 9 in MCF-7 cells. Loss of MMP is also classical evidence for apoptosis, occuring during the early stage of apoptosis before the cell morphology changes. The depletion of MMP was suggested to be very strong at percentages above 50%, and strong between 20% and 50% [5]; Up to 24.0% MMP depletion was obtained, when MCF-7 cells were treated with IC_{50} concentrations of **1**, suggesting that MMP depletion is involved in apoptotic pathway induction by this compound. ROS levels between 20% and 50% are considered as high [5]; More than 3-fold increase in ROS production compared to non treated control was also obtained as results of treatment of MCF-7 with compound **1**. These data suggest that compound **1** induce apoptosis in MCF-7 cells mediated by MMP loss and increase in ROS production. This is in conformity with previous studies, as chalcones such as isobavachalcone [23] was found to induce apoptosis in CCRF-CEM leukemia cells, mediated by caspase activation and the disruption of MMP meanwhile poinsettifolin B [23] and 4′-hydroxy-2′,6′-dimethoxychalcone [25] induced MMP disruption and increased ROS production but with low activation of caspase enzymes.

Regarding the structure-activity relationship, it appears that chalcones (**1-4**) had the best cytotoxicity compared to flavanones (**5** and **6**) and diterpenoids (**7**–**11**). Within the tested chalcones, **1** was the most active compound when considering the six tested cancer cell lines; The *O*-methylation of **1** in C2 and C6 (to yield **2**) slightly reduced the cytotoxic activity. The shift of $-OCH_3$ group in C2 (**1**) to C3 (**3**) also moderately reduced the activity. Also the addition of $-CH_3CO$ groups to **1** to yield **4** also reduced the cytotoxicity. For the two tested flavanones (**5** and **6**), the prenylation of **5** to yield **6** significantly reduced the activity.

5. Conclusion

Finally, the cytotoxicity of naturally occuring phenolics and diterpenoids against human carcinoma cell lines has been demonstrated. Chalcones **1**–**4** as well as trachylobane diterpenoids **10** and **11** displayed cytotoxic effects on all tested cancer cell lines. Compounds **1** induced apoptosis in MCF-7 cells mediated by increase ROS production and MMP loss. The compounds undertaken

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for study especially chalcones **1**–**3** deserve more investigations to develop novel cytotoxic drugs against cancers.

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Conflict of interest

None.

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