Cytotoxic Selectivity and Antioxidant Activity of Aloe Arborescens Constituents

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ABSTRACT

Aloe arborescens plant (Family Asphodelaceae), is one of the main varieties of Aloe used worldwide. Plant filet and exudates extract was fractionated to yield a moderate polar fraction (MPF) and polar fraction (POF). The MPF proved to be most active fraction against HepG2, PC3 and MCF-7 cell lines rather than polar fraction (POF). Chromatographic fractionation of MPF fraction led to the isolation of fifteen compounds, their structures were characterized by comparison of their physical and spectral data MS, NMR (¹H NMR) to published data. Isolated compounds were assayed for their cytotoxicity toward the HepG2 cell line. Among these compounds, aloeresin, neoaloeresin, purpurin and aloenin showed potent cytotoxicity, having IC₅₀ values equal to or less than 12 µmole/ml. Since the majority of anticancer compounds is toxic to normal cells, their application in medicine is highly restricted. The effect of bioactive compounds in normal epithelial cells was evaluated at concentrations (100 µg/ml). Aloeresin E, nealoeresin and purpurin showed the least cytotoxic effect on WISH cells with a percentage of inhibition (0, 8.3, 0%) respectively. On the basis of the expanded understanding that oxidation is a crucial cause of tumor progression, the antioxidant activities of these compounds were determined by measuring 2,2-diphenyl-1-picrylhydrazyl DPPH photometric method. All the components showed dose-dependent increase in activity. In the light of these results, Aloe arborescens compounds may be considered as lead compounds for the treatment of cancer with a remarkable safety extent, which supports previous claims of the plant traditional use.
Keywords: Aloe arborescens, Chemopreventive, Antioxidant, Constituents, Quinones.

INTRODUCTION

Nowadays cancer is the leading cause of death in economically developed countries and the second leading cause of death in developing countries (Jemal et al; 2011). Plant derived compounds have played an important role in the development of several clinically effective anti-cancer agents and it is significant that over 60% of currently used anti-cancer agents are derived in one way or another from natural sources, including plants, marine organisms and micro-organisms (Newman et al; 2003; Cragg et al; 2011).

The anticancer agents are mainly related to their curative role in a damaged system. Under normal conditions, the cells in which the DNA or other components are irreversibly damaged by various causes undergo apoptotic cell death, which is a self-destructive metabolism according to the genetically encoded cell death-signal (Korsmeyer, 1995; Wyllie, et al; 1999). However, cancer cells, which are already irreversibly developed, obtain the capability to evade apoptosis by various ways. The aim of anticancer agents is to trigger the apoptosis signalling system in these cancer cells whilst disturbing their proliferation (Bold et al; 1997). The incidence of tumor relapse in traditional therapies such as ionizing radiation and chemotherapy is a significant problem that results from the development of drug resistance mechanisms in a portion of the tumor cells (Fahn et al; 1994; Giardi et al; 2013). Furthermore, chemotherapy alone does not achieve a satisfactory therapeutic outcome in terms of complete tumor remission and the prevention of metastasis (Mushiake et al; 2005; Prakash and Gupta, 2004). Therefore, there is a worldwide trend to go back to natural resources; hence, additional therapeutic approaches to eliminate these resistant tumor cells must be established (Siegel, 1985).

The use of natural products with therapeutic properties is as ancient as human civilization. For a long time, plants and animal products were the main sources of drugs (Pasquale, 1984). According to the World Health Organization, medicinal plants would be the best source for obtaining a variety of drugs (Rates, 2001). Many plant extracts and components have been examined to identify new and effective antioxidant and anticancer compounds, as well as to elucidate the mechanisms of cancer prevention and apoptosis (Pietta et al; 1998; Kim et al; 1998; Swamy and Tan 2000).

The public interest in Aloe species has quickly grown and a considerable reports and researches about the various components of Aloe is being conducted to find out more about their properties (Li, 2009). Aloe arborescens, belonging to the genus Aloe (Family Asphodelaceae), is one of the main varieties of Aloe used worldwide. It is native to South Africa; it has been imported
from many countries in the tropics and subtropics as an ornamental and medicinal plant. Growing of the plant for commercial uses has started recently in Israel and China (Grace et al; 2008).

Medicinally, the gel and dried leaf exudates of Aloe species have been used since ancient civilizations of the Egyptians, Greeks and Mediterranean peoples and also for its cosmetic uses (Evans, 2009). Several folk uses of A. arborescens were investigated; the split or crushed fresh leaves are widely used to treat burns and wounds. In South Africa, a leaf decoction is given to women to ease childbirth. In Japan, the leaves are used as a vegetable and to ease constipation. Preparations are sold as over-the-counter drugs for acceleration of gastric secretion, as a purgative and for dermatological use (Stewart, 2007, Smith et al; 2012). It is a traditionally valued herbal medicine as food supplement in case of cancer in Egypt and different countries
medicine/aloe-arborescens-protocol/. The biological activity of plant extracts, there is scant research about its chemical constituents. Few compounds were isolated such as; anthraquinones; aloe-emodin, elgonica-dimers. anthrone; aloins A and B, pyrones; aloenin, 4-methoxy-6-(4′-hydroxy-6' methylphenyl)-2-pyrene, chromones; aloesin, 2′-O-p-coumaroylaloesin, 2′-O-feruloylaloesin, 7-hydroxy-2,5-dimethylchromone, coumarin; umbelliferone, esculetin, B- sitosterol, veratric acids, succinic acid and vanillic acid (Dagne et al; 2000).

Currently, a number of researchers are focusing on the anti-cancer properties of compounds from natural resources. Additionally; pharmacological research has confirmed that quinone may be a significant antineoplastic drug. Quinone antitumor agents with a wide spectrum of activity have been extensively used in different forms of human cancers (Saify et al; 1999). This class of compounds has been widely investigated for medical purposes, in particular for diagnostics and therapy (Preobrazhenskaya et al; 2006).

Since various biological antitumor activities of A. arborescens extract have been previously demonstrated in-vivo, there were no reports concerning the effect of various phytochemical classes on tumor cells neither in-vivo nor in-vitro. Although the antitumor activity of quinones was previously reported in other Aloe Various reports investigated the activity of different plant extracts on the inhibition of various cancer types (Singab et al; 2015); such as intestinal tumor (Shimpo et al; 2006), colon carcinogenesis and duodenal cancer (Shimpo et al; 2001, 2003). In addition; in vivo effect of plant preparations as tumor angiogenesis inhibitors was demonstrated (Skopiński et al; 2013). Considerable significant clinical evaluation has been done to verify the therapeutic effects of the leaves extract plus chemotherapy in patients with metastatic cancer and revealed significant results (Lissoni et al; 2009). Although there are many reports about species (Faig et al; 2001; Badria and S. Ibrahim 2013), where that of A. arborescens was not studied. So, it deemed interested to study the effect of plant extracts, fractions and corresponding components on human cancer cell lines.
MATERIALS AND METHODS

General experimental procedures

Pre-coated silica gel 60 on aluminum sheets, 0.2 mm layer thickness; 20×20 cm (E-Merck), (Darmstadt, Germany). Silica gel 60, particle size (0.063-0.2), surface area (70-230 mesh ASTM), Sigma Chemical Co; (St. Louis, MO, USA). Sephadex LH-20 (25- 100 µm), Sigma Chemical Co; (St. Louis, MO, USA). All solvents used were of chromatography grade, the best resolution was achieved with the following solvent systems: dimethylsulphoxide (DMSO-d$_6$), acetone CD$_3$COCD$_3$-d$_6$, and methylene-chloride (CDCl$_3$-d$_6$). Human prostate cancer cells PC3, human hepatocellular carcinoma cells HepG2, human breast cancer MCF-7 cells, and WISH (normal epithelial cells were purchased from Vacsera, (Giza, Egypt) and then maintained in the tissue culture facility (Faculty of Pharmacy, Ain Shams University, Cairo, Egypt). Trypsin-EDTA and Sulforhodamine B (SRB) were obtained from Sigma-Aldrich (St. Louis, MO, USA). DPPH from Sigma (Germany).

Physical and spectral data recorded for each of the above isolates using the following apparatus or instruments: Mass spectrometer SSQ 7000 produced by Finnigan. Nuclear magnetic resonance spectrometers Jeol JNM ECA instrument ($^1$H-NMR, 400 MHz, Ain Shams University, Egypt). The NMR spectra were recorded in different suitable solvents. TMS was used as internal standard and chemical shift values were recorded in ppm. $^1$H-NMR spectral data are represented as follows: (Chemical shift, number of protons, multiplicity, coupling constants in Hertz), ESI-MS Water, Germany. IR-8400S, Shimadzu.

Plant material

Samples of Aloe arborescens were collected from East Deserts of Egypt in March. A voucher specimen (No. 32012) was identified by a botanist senior researcher, at the flora and taxonomy research department, Agricultural Museum, Giza, Egypt. The specimen was deposited in the department of Pharmacognosy, Faculty of Pharmacy, Ain shams University.

Extraction of quinones

A. Arborescens leaves (30 kg) were used, skin removed and then gel, juice and exudate homogenized in ultraturrrex with 60% ethyl alcohol in 10% acetic acid, percolated and concentrated to (1/10). The extract was kept for further fractionation (using methylene chloride to yield moderate polar fraction (MPF) and remaining polar fraction (POF)) and isolation process. Extraction and separation steps (Su and Ferguson, 1973) were shown in (Figure 1).
A. Quinones - Schematic extraction

31 kg Aloe leaves ----> 12 kg Aloe homogenate (gel + exudate)
homogenized in ultraturrex with 60% alcohol

Extract keep in refrigerator & conc. to (1/10)

percolation with 60% ale + 10% acetic acid

DCM

Moderate polar quinones (2.8 gm)

Marc (air dried for 2 days)

Remaining alcoholic extract

Alkalize with 5% KOH
filtration

Residue

Filterate

Anthraquinone salt

Suspension with alcohol & neutralize with glacial acetic acid

Filter while hot

Residue

Filterate (free glycosides)

Evaporate to dryness in vacuo
recrystallize by isopropyl alcohol

Polar quinone (30 gm)

Figure 1: Schematic diagram for extraction and separation of quinones (Su and Ferguson 1973).

Evaluation of cytotoxic activity

Human prostate cancer cells PC3, human hepatocellular carcinoma cells HepG2 and human breast cancer cells MCF-7 were grown in RPMI-1640 medium, supplemented with 10% heat inactivated FBS, 50 units/mL of penicillin and 50 mg/mL of streptomycin and maintained at 37°C in a humidified atmosphere (containing 5% CO₂). The cells were maintained as “monolayer culture” by serial subculturing. Cytotoxicity was determined using the SRB method as previously described by Skehan et al; (Skehan et al; 1990). Exponentially growing cells were collected using 0.25% Trypsin-EDTA and seeded in 96-well plates at 1000-2000 cells/well in RPMI-1640 supplemented medium.

After 24 hrs, cells were incubated for 72 hrs with various concentrations of the tested polysaccharides. Following 72 hrs treatment, the cells were fixed with 10% trichloroacetic acid for 1 hr. at 4 °C. Cells were stained for 10 min at room temperature with 0.4% SRB dissolved in 1% acetic acid. The plates were air dried for 24 hour and the dye was solubilized with Tris-HCl for 5 minutes on a shaker at 1600 rpm. The optical density (OD) of each well was measured.
spectrophotometrically at 564 nm with an ELISA microplate reader. The IC$_{50}$ values were calculated according to the equation for Boltzman sigmoidal concentration–response curve using the nonlinear regression fitting models (Graph Pad, Prism Version 5). The cytotoxic effects of extracts were tested on three cell lines (PC3, HepG2 and MCF-7) using different concentrations of 0.1, 1.0, 10.0, 100 and 1000 µg/ml. The IC$_{50}$ values were calculated according to the equation for Boltzman sigmoidal concentration–response curve using the nonlinear regression fitting models (Graph Pad, Prism Version 5).

**Chromatographic separation of bioactive fraction**

The moderate-polar fraction (MPF) (2.5 g) fractionated over a silica column (120 g, 1.5 x 100 cm). The column was eluted using increasing concentrations by10% of n-hexane, ethyl acetate and methanol. Twenty one fractions were collected based on their TLC profile. Twelve subfractions were subjected to further chromatographic separation by application on vacuum liquid chromatography (VLC) (1.5x2.5 cm) (using different gradient eluent; hexane, dichloromethane, ethyl acetate and methanol) followed by preparative thin layer chromatography (TLC).

Fraction FA8 eluted with (n-hexane: EtOAC 8:2). FA8$_2$ subfraction (DCM, 10.6 mg) was further chromatographed by preparative TLC using a solvent system (Pet.ether: EtAOC: formic acid 100:25:1) to afford compound 1 (4.9 mg). Fraction FB9 (n-hexane: EtOAC 7:3) was further purified over VLC. FB$_9_1$ (hexane) purified by preparative TLC (DCM: MeOH 5.5:4.5), where compound 2 was isolated (3.8 mg). Fraction FC14 (n-hexane: EtOAC 7:3) was fractionated over VLC silica column, FC14$_2$ subfraction (DCM) was purified by preparative TLC (DCM: MeOH 1:1), then it crystallized by MeOH, to yield compound 3 (5.8 mg). Fraction FD21 (n-hexane: EtOAC 2:8) purified over VLC silica column, FD21$_1$ (MeOH) applied to preparative TLC (toluene: EtOAc: MeOH: NH$_3$ 40:35:20:5), then washed and crystallized by DCM, to yield compound 4 (3.3 mg).

Fraction FE31 (EtOAC: MeOH 9:1) chromatographed by VLC silica column. The MeOH subfraction FE31$_1$ purified by preparative TLC (toluene: EtOAc: MeOH: NH$_3$ 40:35:20:5), then washed and crystallized by DCM yielding compound 5 (5.5 mg). Fraction FF32 (EtOAC: MeOH 8:2) chromatographed by VLC silica column, FF32$_1$ MeOH subfraction further purified by preparative TLC (DCM: EtOAc: MeOH: H$_2$O: Formic 28:32:36:1:0.2), washed with acetone to afford two compounds; compound 6 (11.1 mg) from acetone wash, and compound 7 (7.2 mg) from the acetone insoluble part. Fraction FG45 (EtOAC: MeOH 7:3) was further fractionated over VLC silica column. FG45$_2$ DCM subfraction washed with MeOH to give compound 8 (7.2 mg), while FG45$_4$ MeOH subfraction recrystallized with DCM and afforded compound 9 (7.1 mg).

Fraction FH46 (EtOAC: MeOH 2:8), FH46$_3$ EtOAC subfraction purified by preparative TLC (EtOAc: MeOH: H$_2$O 100:17:13) and crystallized by DCM, compound 10 (7.6 mg) was obtained. Fraction FI48 (EtOAC: MeOH 1:9), after fractionation over VLC column, FI48$_4$ eluted with MeOH, processed for further analysis by preparative TLC (EtOAc: MeOH: H$_2$O 100:17:13), and crystallized by DCM to afford compound 11 (19.4 mg). Fraction FJ56 (MeOH) further fractionated over VLC silica column, FJ56$_2$ DCM subfraction (VLC), washed with MeOH to afford
compound 12 (7.8 mg), on the other hand FJ56, MeOH subfraction was washed with acetone to yield compound 13 (5.6 mg). Fraction FL57, FM59 (MeOH) fractionated on VLC silica column, FL573 subfraction (EtOAC), after washing with DCM compound 14 (11.6 mg) was obtained. The FM594 MeOH subfraction recrystallized with DCM and compound 15 (16.7 mg) was obtained.

**Structural characterization**

All compounds isolated exhibited closely comparable $^1$H NMR and mass fragmentation spectral data to published data, results shown in Table 1 and Figure 2 respectively. The structures are presented in Figure 3.

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**Table 1.** $^1$H NMR Spectral Data Of Different Isolated Compounds (400 MHz).

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<th>Anthraquinone</th>
<th>Pyran</th>
<th>Coumarin</th>
<th>Anthrone</th>
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For Compound (6, 9, 10, 14, 8 and 11) analysis; solvent was (DMSO-d6), for Compounds (16, 7 and 15) solvent was (CD3COCD3-d6), compounds (1 and 2) solvent was (CDCl3).

Figure 2: Fragmentation pattern of isolated quinones by ESI-MS spectroscopy.

Aloenin-aglycone (1). Orange red amorphous solid, EIMS spectrum (m/z, rel. int.): 248 [C_{13}H_{12}O_{5}], 85 [M-C_{10}H_{11}O_{2}], 57 [M-C_{11}H_{11}O_{3}].

Methyl p-coumarate (2). White amorphous, EIMS spectrum (m/z, rel. int.): 178, 149 [C_{9}H_{8}O_{2}]^{+} [M-OCH_{3}], 71 [M-C_{7}H_{2}O], 57 [M-C_{8}H_{2}O].
(3R)-6, 8-dihydroxy-3-(6-oxoundecyl)-3, 4-dihydroisocoumarin (3). White powder, EIMS spectrum (m/z, rel. int.): 347 [C20H26O5] +, 321 [M-CO] +, 307 [M-C2H5O] +, 289 [M-C2H3O2], 167 [M-C3H7O4], 149 [M-C1H19O3], 141 [M-C11H11O4], 85 [M-C13H19O4], 57 [M-C17H23O4].

Dihydrocoumarin (4). White powder, EIMS spectrum (m/z): 149 [C9H8O2]+, 123 [M-C2H2], 108 [M-C2HO], 93 [M-C3H2O], 81 [M-C4H2O], 73 [M-C6H3], 58 [M-C7H6], 57 [M-C7H7].


Decarboxylated Lacciac acid E (6). Dark red amorphous solid, ESI-MS spectrum- positive mode (m/z): found a [M+2H] at m/z 469 C22H13NO6 (OH)5, 451 [M-H2O], 426 [M-C2H2O], 408 [M-C2O], 366 [C20H16O8], 323 [M-C2H2O], 171 [M+H-C16H18O6], 141 [M+H-C14H14O6], 94 [M+H-C14H8O7], 77 [M+H-C14H10O8], 66 [M+H-C17H11O7], 42 [M+H-C18H13O7].

Lacciac acid C (7). Dark red amorphous solid, ESI-MS spectrum- positive mode (m/z): found a [M+2H] at m/z C21H13NO5 (OH)4, 525 [M-H2O]+, 511 [M-CO], 497 [M-C2H2O], 481 [M-C2H2O2], 426 [M-C3H7NO2], 280 [M-C12H2O2], 237 [M-C15H14NO6], 156 [M-C19H13NO8], 67 [M-C21H14NO12], 44 [M-C24H15O12]. IR spectrum showed peaks at 3414.0, 2854.6 and 1600.9 cm⁻¹.

7-hydroxy-2,5- dimethylchromone (8). Pale yellow powder, MS spectrum (m/z): 190 [C11H10O3], 173 [M-H2O], 167 [M- OCH3], 149 [M-C2H2O]+, 71 [M-C3H3O], 57 [M-C3H2O].

Neoaloeresin A (9). Brownish red amorphous powder, EI-MS calculated for C19H22O9 394.12; found: 396 [M+2H], 365 [M+H-CH2O], 354 [M-C6H4], 277 [M+H-C8H3O3], 248 [M+H-C8H3O3], 162 [M-C12H2O4], 127 [M+H-C13H15O6], 120 [M+H-C13H14O5], 106 [M+H-C15H13O6].


Purpurin (11). Dark red powder, ESI-positive mode found [M+H] at 257 m/z of C13H6O5, 140 [M+H-C3H2O], 95 [M+H-C3H2O3], 80 [M-C10H3O3], 52 [M+H-C10H2O3], 40 [M+H-C11H3O3].

Tetrahydrofuran-3-yl methanol (12). White powder, EIMS spectrum (m/z): 103 [M+1] [C3H10O2], 84 [M-CH2O], 66 [C3H6]+, 57 [M+H-C2H6O].

Aloeresin B (13). Brownish yellow amorphous powder, ESI-MS spectrum- negative mode (m/z): found a [M-H] at m/z 393 represent C19H22O9, 321 [M+H-C3H6O2], 247 [M+H-C8H3O3], 193 [M-C8H3O3], 165 [M+H-C13H13O4], 126 [M-C13H16O6].
Aloenin (14). Orange red amorphous powder, ESI-MS spectrum – positive mode (m/z): found [M+2H] 412 m/z of C_{19}H_{22}O_{10}, 378 [M-CH_{3}O], 366 [M-C_{2}H_{4}O], 354 [M-C_{3}H_{6}O], 338 [M+H-C_{3}H_{6}O_{2}], 164 [M+H-C_{13}H_{13}O_{5}], 86 [M+H-C_{16}H_{21}O_{7}], 71 [M+H-C_{16}H_{20}O_{8}].

Aloeresin A (15). Yellowish brown amorphous powder, ESI-MS spectrum- positive mode (m/z): found [M+1] 541 m/z of C_{28}H_{28}O_{11}, 395 [M-C_{9}H_{5}O_{2}], 309 [M+H-C_{13}H_{12}O_{4}], 191 [M+H-C_{17}H_{18}O_{8}], 145 [M-C_{19}H_{23}O_{3}], 84 [M+H-C_{24}H_{25}O_{3}], 44 [M+H-C_{26}H_{27}O_{10}].
7: Lacciac acid C

8: 7-hydroxy-2,5- dimethylchromone

9: Nealoeresin A

10: Aloe- emodin--anthrone.

11: Purpurin

12: Tetrahydrofuran-3-yl methanol
Cytotoxic selectivity of isolated components

*In-vitro* cytotoxic screening of different isolated components on HepG2 carcinoma cell lines. Since the majority of anticancer compounds is toxic to normal cells, their application in medicine is highly restricted. In the light of this drawback of antitumor agent, the most potent cytotoxic compounds were estimated its toxicity on normal human epithelial cell line (WISH) at concentrations (100 µg/ml). Cell viability and growth were observed.
Free radical scavenging activity

Using diphenyl-1-picryl-hydrazil (DPPH) free radical scavenging assay. The antioxidant activity of the compounds was assessed based on the radical scavenging effect of the stable DPPH free radical based on the method of (Saxena and Patel, 2010) and using 300 μM DPPH in MeOH. The compounds were dissolved in MeOH, and each extract solution (10 μL) was allowed to react with 200 μL DPPH at 37 °C for 30 min in a 96-well microtiter plate. After incubation, the decrease in absorbance (optical density, OD) of each solution was measured at 490 nm using a microplate reader. Ascorbic acid was used as the positive control. For each sample concentration tested, the percentage of DPPH was calculated using the following formula:
Antioxidant activity (%) = (OD control – OD sample) / OD control × 100%

* OD sample is the OD of the samples or positive control, and OD control is the negative control OD.

RESULTS AND DISCUSSION

Chromatographic separation afforded 15 isolates including, three anthraquinone; lacciac acid E decarboxylated derivatives, lacciac acid C, purpurin. Five chromones; aloeresin E, 7-hydroxy-2,5-dimethylchromone, nealoeresin A, aloeresin B, aloeresin A. One anthrone; aloe emodin anthrone. Two pyran; aloenin-aglycone, aloenin. Two coumarin; (3R)-6,8-dihydroxy-3-(6-oxoundecyl)-3,4-dihydroisocoumarin, dihydrocoumarin. In addition; a benzene and a furan derivative; methyl p-coumarate, tetrahydro-3-furanmethanol. All isolated compounds were previously isolated from different Aloe species except; lacciac acid E decarboxylated derivatives, lacciac acid C and purpurin. While, aloenin and aloeresin A and B were previously identified from A. arborescens. Otherwise, all other compounds were isolated for the first time from the plant.

Isolated chromones compounds

Compound 8 was obtained as pale yellow powder, chromatographic investigation revealed violet spot under UV light. The 1H-NMR spectrum showed one olefinic (δ 3.4, 1H, br s) and two aromatic (δ 6.5, 1H, br d, J = 2.4 Hz and δ 6.7, 1H, d, J = 2.4 Hz) proton signals, together with methyl signals (δ 2.5, 3H, br s). EIMS data exhibited an (M+) peak at m/z 191 consistent with molecular formula C11H10O3. Therefore, this compound was identified to be 7-hydroxy-2,5-dimethylchromone, which was previously isolated from Cape Aloe (Dagne et al.; 2000), Rhubarb and Polygonum cuspidatum (Ametani et al.; 2007).

Compound 9 was isolated as brownish orange solid; it appears as yellow florescence under UV. The 1H-NMR spectrum showed signals being characteristic of a 5- methylchromone moiety, the corresponding signals were in good agreement with literature data for aloeresin A (Gramatica et al.; 1982 and Speranw et al.; 1988). Where, both showed some similar patterns, except for a difference in the patterns of proton peaks of the sugar moiety. In addition, sugar signal of 2'-4’ is more downfield than alosin which explained pentose form. In ESI-MS it was found abundant ion at m/z 231 corresponding to a loss of 162 u point to a feruloyl moiety. The compound elucidated as
Neoaloeresin A that previously isolated from *Aloe barbadensis* (Park *et al*; 1996).

Compound 13 obtained as brownish yellow, chromatographic investigation revealed orange spot under UV light. The $^1$H-NMR spectrum of compound 18 showed signals being characteristic of a 5- methylchromone moiety (Okamura *et al*; 1996b; Holzapfel *et al*; 1997). The corresponding signals were in good agreement with literature data for aloeresin B (Speranw *et al*; 1988). Furthermore, the proton signal at 2.5 ppm indicates a methoxyl attached to the aromatic ring at C-7 (6). Loss of an element 90 m/e was due to cross-ring cleavage in the hexosidic part involved in the formation of ion at m/z 247. The characteristic fragment of chromones 191 m/z is also detected. Compound 18 identified as Aloeresin B (aloesin), this compound was obtained after hydrolysis of 2"-O-p-coumaroylaloesin (aloesin A) from *A. arborescens* species (Makino *et al*; 1973, Wang *et al*; 2003).

Compound 5 was obtained as a brownish yellow solid, chromatographic investigation revealed yellow spot under UV light. The $^1$H and ESIMS data of the key structural features of aloeresin E; the acetonyl, pyrone, 5-Me, 7-0H and 8-C-glucoside, were in close agreement with those reported for aloesin (Holdsworth and Hill, 1970). Mass fragment ion at 148 m/z confirm presence of cinnamoyl ester, the chemical shift of this signal is characteristic of a proton influenced by the anisotropic effect of an ester carbonyl; the cinnamoyl group was, therefore, located at C-2 of the carbohydrate moiety. Furthermore; the 84-mass unit fragment is generated by retro Diels-Alder fragment that includes the hydroxypropyl side chain) represented as M; 148-232 m/z. Therefore, compound 10 was identified as Aloeresin E [8-C-β-D-[2' -O-(E)-cinnamoyl] glucopyranosyl-2-[ (S)-2-hydroxy] propyl-7-methoxy-5-methylchromone]. This compound has been isolated from *Aloe vera* species (Okamura *et al*; 1996a; Fanie *et al*; 2006).

Compound 15 is O-p-coumaroyl derivative of aloesin (13) with a molecular weight 540 m/z, according to the ESI mass spectrum. This compound exhibited blue color under UV, the $^1$H-NMR spectrum showed signals being characteristic of a 5- methylchromone moiety (Gutterman and Volfson 2007) concerning the glucose unit. Additionally; characteristic signal at δ 3.7 ppm (methoxylated group) in coumaroyloxy group located in C (2’) was detected. The EI-MS displays the complementary key ions of (m/z 395) and (m/z 146, coumaroyl ion) as deduced from high-resolution mass data, at m/z 193; characteristic signal of chromone skeleton. The corresponding signals of the compound were in good agreement with literature data for aloeresin A, that previously reported from *A. arborescens* (Gramatica *et al*; 1982).

Isolated anthraquinone compounds

Compound 6 found as deep red solid powder, has orange color under UV light, the UV spectrum in basic solution converted its color to yellow which confirm the acidic character of compound. In ESI-MS the signal of pseudomolecular ion [M+2H] was registered at m/z 469 and fragments with lost water and carbon dioxide neutral molecules at m/z 451 and 408, respectively. The $^1$H NMR
spectral data showed signals at 2.1 with high intensity (4H) assigned to an alkane protons that attached to amine group, the proton of amine appears as a triplet at δ 4.2 ppm. The compound tentatively identified as decarboxylated derivatives of lacciac acid E, a lac dye related to hydroxyanthraquinoid pigments, and its main natural sources is found in families like Rubiaceae, Polygonaceae, Fabaceae and Liliaceae, in lichens and in the animal kingdom (insects). Laccacic acid D methyl ester was previously isolated from Aloe saponaria (Dagne et al; 2000; Mohamed 2005).

Compound 7 found as deep red solid powder, it has orange color under UV light. In ESI-MS; pseudomolecular ion [M+2H] at m/z 541 accompanied by related peaks at m/z 525 [M-H2O]+ and 466 [M-H- CO2]+ revealed the presence of Laccacic acid C. Lac dye is a red coloured natural dye, which is present mainly in the body fluid of lac insect, Kerria lacca (Kerr) as the alkali salt (Ferreira et al; 2004; Rosenberg 2008). This compound is related to compound 8, as a hydroxyanthraquinoid pigments, different in presence of amide signal other than amine and the proton of amine not detected until δ 10 ppm, also alkane proton attached to NH2 appear more downfield because of the presence of carboxylic group. The FT-IR spectrum was used to identify the functional groups of the active components based on the peak value in the region of infrared radiation. IR analysis revealed that the presence of different functional groups ranging from O-H stretching, hydroxyl (3414.0 cm⁻¹), C-H stretching, carbonyl (2854.6 cm⁻¹), H2N-R stretching amine (3300.9 cm⁻¹). These natural colorants are usually applied in several industrial food processes for the same reasons as the synthetic counterparts (Aro et al; 2012).

Isolated pyran compounds

Compound 1 revealed as blue spot under UV light. The UV absorption of the methanol solution at 337 nm (0.03), 225 (0.56), 208 (0.5) suggested the presence of a long conjugated system. The EIMS showed an M⁺ analyzing for C12H10O5 with a loss of characteristic CO₂. The 1H NMR spectrum showed the presence of an aromatic methyl (δ 2.3 s), a methoxyl (4.2 s), and two aromatic protons (7.5, 7.7 ppm), phenolic protons appear at 12 ppm. Therefore this compound was identified as Aloenin-aglycone [6- (2', 4'-bihydroxy-6'-methyl) phenyl-4-methoxy- 2-pyrene]. This aglycone was previously separated from Aloe nyeriensis (Conner et al; 1987), and its corresponding glycosides was isolated from different aloe species such as Kidachi-rokai in Japanese, Kenya aloe (Fanie et al; 2006). Moreover; aloenin acetal has been reported from A. arborescens (Suga, 1978).

Compound 14 isolated as orange red color, chromatographic investigation revealed red purple zone under UV light. Fragments at m/z 164 and 248 in ESI-MS suggested the presence of glucose moiety attached to chromone residue, which was confirmed by the 1H-NMR spectrum. The proton spectra showed the presence of an aromatic methyl (δ 2.0 ppm), methoxy proton (δ 4.2 ppm), two olefinic protons at (δ 5.4 ppm) and two aromatic protons at (δ 7.6 and 7.7 ppm appears as singlet signal). Therefore, compound 19 identifies as Aloenin [6-(2'-β-D-glucopyranosylxy-4'-hydroxy-6'-methyl) phenyl-4-methoxy-2-pyrene]. It was previously identified and reported from different Aloe species and isolated from A. arborescense (Makino et al; 1973; Hirata and Suga 1976; Yamamoto et al; 1991; Gao et al; 2006; Olennikov et al; 2009). Moreover; aloenin is a
biologically active phenyl pyrone, identified in 16 Aloe species with a greater chemotaxonomic value (Fanie et al; 2006).

**Isolated coumarin compounds**

Compound 3 obtained as white powder, chromatographic investigation revealed yellow spot under UV light. The $^1$H NMR shows signals of 6, 8-disubstituted aromatic ring (δ 7.5, 7.7). There is also an aliphatic chain (δ 1.3 [H-2’-4’], 6 H, br s, δ 2.3 [H-5’, 7’]), where terminal methyl are linked (δ 0.9 ppm as br s). All these data allow us to propose for the structure of dihydroxyisocoumarin, which is in agreement with its EIMS spectroscopic features, the mass spectrum of showed a [M]$^+$ at m/z 167, in agreement with the formula C$_8$H$_7$O$_4$ resulted from fragmentation of the side chain. Furthermore, the presence of peak at m/z 321 [M-CO]$^+$ which indicate the presence of quinone structure. Dihyroisocoumarin glucoside was previously isolated from Aloe hildebrandtii (Braca et al; 2012). So the compound identified as (3R)-6, 8-dihydroxy-3-(6- oxoundecyl)-3, 4-dihydroisocoumarin.

**Isolated anthrone compounds**

Compound 10 was isolated as an orange red powder, chromatographic investigation revealed yellow spot under UV light. The $^1$H NMR spectrum revealed three mutually coupled aromatic protons at δ H 7.6 (1H, dd, J = 9.0, 1.1 Hz, H-7), 7.7 (1H, dd, J = 7.8 Hz, H-6, H-5) of ring C. In ring A, two broad singlet aromatic protons at δ H 7.67 and 7.66 were assigned to H-2 and H-4, respectively, the oxymethylene protons (δ H 3.0) supposed to be located at C-3, therefore; this compound was identified as Aloe-emodin-9-anthrone. The compound has been reported from some Aloe species (Knut and Dagfinn 1992; Hill and Sung 1998; Fanie et al; 2006).

Compound 11 obtained as dark red powder, chromatographic investigation revealed orange red under UV light and pink color after spraying with alcoholic KOH. The EIMS spectrum showed an M$^+$ analysing for C$_5$H$_{10}$O$_2$ with a loss of 2CO characteristic of anthraquinones; where, [M+H-44]$^+ ;$[139-95 m/z] and [M+H-28]$^+ ;$ [80-52 m/z], which represent fragmentation pathways characterize anthraquinoids, and corresponding to decarboxylation of the carboxylic acid group (Nowik et al; 2008). Therefore compound identified as Purpurin [1, 2, 4-Trihydroxyanthraquinone], and this is in agreement with chemotaxonomic criteria of Liliaceae family that usually identified by anthracenes. About 90% of these compounds occur as derivatives of 9, 10-anthracenedione (anthraquinones) with several hydroxy and other functional groups. Purpurin was previously reported in Rubia tinctorum and Relbunium species (family Rubiaceae) (Nowik et al; 2008; Rosenberg 2008).

Compound 2 obtained as white crystal, chromatographic investigation revealed faint blue spot under UV light. $^1$H-NMR revealed the presence of methoxy group as singlet at δ 4.2 ppm & in the same region H-2’ olefinic protons appears at δ 6.5 ppm as doublet peak. While, the aromatic protons were detected at δ 7.5 and 7.7 ppm as doublet of doublet. The EIMS signal at 149 indicates
fragmentation of methoxy group. This compound elucidated as Methyl p-coumarate that was previously isolated from *Aloe ferox* (Speranza *et al*; 1988; Dagne *et al*; 2000).

Compound 4 was isolated as white powder, its chromatographic investigation revealed yellow spot under UV light. EI-MS spectrum showed as molecular weight 149 m/z \([\text{C}_9\text{H}_3\text{O}_2]\) of \([\text{M}+1]\), the peak at 123 m/z was in agreement with a formula \([\text{M-C}_2\text{H}_2]\) resulted from fragmentation of benzene ring, while peak at 109 m/z corresponding to formula \([\text{M-C}_2\text{H}_2\text{O}]\) represent the fragmentation of coumarin ring. Therefore, this compound was identified as dihydrocoumarin and its glucoside was previously reported from *Aloe vera* and *Aloe hildebrandtii* (Braca *et al*; 2012). Moreover; immunomodulatory and antioxidant effect of corresponding derivatives were previously reported (Zhang *et al*; 2006).

Compound 12 isolated as white powder, chromatographic investigation revealed yellow spot under UV light. \(^1\text{H}-\text{NMR}\) indicate three different environments of olefinic protons the first at \(\delta 2.51\) ppm of H-3, while the protons at C2, and C5 appears more downfielded at \(\delta 3.11\) and 3.22 ppm respectively. On the other hand the signal at C4 and C6 appears as \(\delta 3.42\) ppm with more than half integration to the signal at 3.11 ppm (Wu *et al*; 2013). This result was with agreement of EIMS spectrum with a molecular weight 103 m/z \([\text{M}+1]\) characteristic signal of cyclopentene was present at 66 m/z (Pretsch *et al*; 2009). Moreover the peak at 84 m/z corresponding to M-CH\(_6\)O indicates fragmentation of water molecules. Therefore, this compound identified tentatively as Tetrahydrofuran-3-yl methanol that is saturated formula of previously isolated 3-furanmethanol from *A. arborescens* (Diaz-corks *et al*; 1997; Dagne *et al*; 2000).

**Biological Activity Screening**

**Cytotoxic effect**

The cytotoxic assay revealed that the moderate polar fraction (MPF) of *A. arborescens* possessed a potent effect in comparison with a polar one (POF). The IC\(_{50}\) values were calculated and the results are recorded in Tables 2. A through phytochemical investigation of quinones deemed, therefore, necessarily aim to isolate and identify the constituents responsible for the significant cytotoxic effect. The results showed that MPF-fraction possessed the most cytotoxic activity on the liver carcinoma (HepG2) cell lines in a concentration-dependent manner at the recommended NCI (USA) doses. Also MPF fraction showed high activity against prostate cancer cell (PC3) and breast cells (MCF-7).

<table>
<thead>
<tr>
<th>Extract</th>
<th>PC3</th>
<th>HepG2</th>
<th>MCF-7</th>
</tr>
</thead>
<tbody>
<tr>
<td>MPF (\mu\text{g/ml})</td>
<td>31.55± 0.5</td>
<td>40.33± 0.8</td>
<td>40.03± 0.7</td>
</tr>
</tbody>
</table>

**Table 2:** Cytotoxic Effect of Different Extracts (NPF, POF) against PC3, HepG2 and MCF-7 Cell Lines.
Data are presented as mean ± S.D. a: statistically significant for the control (p < 0.05). Statistically analysis was carried out using one way ANOVA followed by Tukey’s post-hoc test. *MPF (moderate polar fraction), POF (polar fraction).

Cytotoxicity assay was determined using the SRB method for all isolated constituents. Compounds (1, 2, 4, 8, 10 and 12) possess weak activity, where corresponding IC50 were above 30 µM, while other compounds showed a potent cytotoxic effect on HepG2 cell line (IC50 less than 30 µM). The safety assay of bioactive chemopreventive components was investigated on WISH (normal epithelial cells). All components showed a low percentage of cell inhibition from (8-16%) at 100 ppm except lacciac acid E showed about 35% cell inhibition. On the other hand; aloeresin A, emodin anthrone didn’t inhibit the growth of any normal cells (0%) (Table 3).

Table 3: Chemopreventive Effect of Different Isolated Quinone Compounds HepG2 Cancer Cell Line.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Chemical class</th>
<th>M.wt m/z</th>
<th>Formula</th>
<th>IC50 on HepG2</th>
<th>Wish cell % inhibition (100 µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aloenin-aglycone (1)</td>
<td>Pyran</td>
<td>248</td>
<td>C13H12O4</td>
<td>40.4</td>
<td>-</td>
</tr>
<tr>
<td>Methyl p-coumarate (2)</td>
<td>Benzen derivatives</td>
<td>178</td>
<td>C10H10O3</td>
<td>92.8</td>
<td>-</td>
</tr>
<tr>
<td>(3R)-6, 8-dihydroxy-3-(6-oxoundecyl)-3, 4-dihydroisocoumarin (3)</td>
<td>Coumarins</td>
<td>346</td>
<td>C20H25O5</td>
<td>60.6</td>
<td>-</td>
</tr>
<tr>
<td>Dihydropydranarin (4)</td>
<td>Coumarins</td>
<td>148</td>
<td>C6H8O2</td>
<td>176.1</td>
<td>-</td>
</tr>
<tr>
<td>Aloeresin E (5)</td>
<td></td>
<td>540</td>
<td>C22H32O13</td>
<td>25.3</td>
<td>0%</td>
</tr>
<tr>
<td>Decarboxylated Lacciac acid E (6)</td>
<td>Anthraquinones</td>
<td>467</td>
<td>C25H31NO6(OH)3</td>
<td>21.8</td>
<td>16.1%</td>
</tr>
<tr>
<td>Lacciac acid C (7)</td>
<td>Anthraquinones</td>
<td>539</td>
<td>C25H31NO9(OH)4</td>
<td>21.5</td>
<td>10.4%</td>
</tr>
<tr>
<td>7-hydroxy-2,5 dimethylchromone (8)</td>
<td>Chromones</td>
<td>190</td>
<td>C11H10O3</td>
<td>72.1</td>
<td>-</td>
</tr>
<tr>
<td>Nealoeresin A (9)</td>
<td>Chromones</td>
<td>394</td>
<td>C19H22O9</td>
<td>12.2</td>
<td>8.3%</td>
</tr>
<tr>
<td>Aloe-evin--anthrone (10)</td>
<td>Anthrone</td>
<td>256</td>
<td>C15H13O4</td>
<td>159.4</td>
<td>-</td>
</tr>
<tr>
<td>Purpurin (11)</td>
<td>Anthraquinones</td>
<td>256</td>
<td>C14H16O2</td>
<td>4.9</td>
<td>0%</td>
</tr>
<tr>
<td>Tetrahydrofuran-3-yl methanol (12)</td>
<td>Furan derivatives</td>
<td>102</td>
<td>C3H4O2</td>
<td>77.0</td>
<td>-</td>
</tr>
<tr>
<td>Aloeresin B (13)</td>
<td>Chromones</td>
<td>394</td>
<td>C19H22O9</td>
<td>26.2</td>
<td>15.4%</td>
</tr>
<tr>
<td>Aloenin (14)</td>
<td>Pyran</td>
<td>410</td>
<td>C19H22O10</td>
<td>5.3</td>
<td>35.1%</td>
</tr>
<tr>
<td>Aloeresin A (15)</td>
<td>Chromones</td>
<td>540</td>
<td>C28H33O11</td>
<td>5.1</td>
<td>13.2%</td>
</tr>
</tbody>
</table>

Data are presented as mean ± S.D. a: statistically significant for the control (p < 0.05). Statistically analysis was carried out using one way ANOVA followed by Tukey’s post hoc test.
Hydroxyanthraquinone derivatives have been evaluated previously using cancer cell lines and the pharmacological effect was explained by their antioxidant activity and their inhibition of certain enzymes. Two kinds of mechanism were reported; H-atom transfer and one-electron transfer, by which antioxidants can play their role (Liu, 2010). This elucidation can explain the chemopreventive activity of certain isolated compounds such as (lacciac acid E & C and purpurin). Chromone derivatives have been found to exhibit a broad range of biological activities, including antifungal, antiviral, antiallergenic, antitubulin and antitumor activity (Kawase et al; 2007; Khadem et al; 2011). The current study is in accordance to (Kawase et al; 2007) investigation which described chromones as an attractive source of medicinally interesting compounds due to their low toxicity. The results showed that bioactive chromones (aloeresin A, B, E and neoaloeresin A) possess 0-15% cytotoxicity on normal healthy epithelial cell.

**Antioxidant effect**

Antioxidant activity was investigated using DPPH radical scavenging assay for the most potent bioactive chemopreventive compounds. It was observed that most compounds had moderate antioxidant activity that also related to the total polyphenol contents. Laccaic acid E, purpurin, showed the highest activities while neoeloeresin A demonstrated the lowest activity antioxidant activity. The results were found in order to: neoeloeresin A, lacciac acid C, aloeresin B, aloenin, aloeresin E, aloeresin A, laccaic acid E, purpurin as following ( 9.7, 10.0, 10.8, 12.0, 11.1, 22.1, 33.1, 39.3 %) respectively at concentration 150 µg (Table 4).

**Table 4:** Free Radical Scavenging Activity of Different Potent Cytotoxic Quinone Compounds Using DPPH Photometric Method.

<table>
<thead>
<tr>
<th>Cpd. No</th>
<th>Name</th>
<th>50 µg</th>
<th>100 µg</th>
<th>150 µg</th>
</tr>
</thead>
<tbody>
<tr>
<td>C5</td>
<td>Aloeresin E</td>
<td>9.66±0.002</td>
<td>10.27±0.003</td>
<td>11.17±0.003</td>
</tr>
<tr>
<td>C6</td>
<td>Lacciac acid E</td>
<td>19.23±0.004</td>
<td>24.16±0.003</td>
<td>33.13±0.002</td>
</tr>
<tr>
<td>C7</td>
<td>Lacciac acid C</td>
<td>7.05±0.003</td>
<td>8.96±0.003</td>
<td>10.07±0.004</td>
</tr>
<tr>
<td>C9</td>
<td>Neoaloeresin A</td>
<td>2.11±0.005</td>
<td>8.55±0.004</td>
<td>9.76±0.003</td>
</tr>
<tr>
<td>C11</td>
<td>Purpurin</td>
<td>18.12±0.001</td>
<td>24.26±0.001</td>
<td>39.37±0.002</td>
</tr>
<tr>
<td>C13</td>
<td>Aloeresin B</td>
<td>9.06 ±0.002</td>
<td>9.26 ±0.003</td>
<td>10.88 ±0.001</td>
</tr>
<tr>
<td>C14</td>
<td>Aloenin</td>
<td>2.11±0.004</td>
<td>10.17±0.003</td>
<td>12.08±0.003</td>
</tr>
</tbody>
</table>
Antioxidants are added to a variety of foods to prevent or deter free radical-induced lipid oxidation, which is responsible for the development of off-flavors and the undesirable chemical compounds in food (Angelo et al; 1996). The free radicals can also be generated in biological systems in the form of reactive oxygen species (ROS), such as superoxide anion radicals (O$_2$•$^-$), hydrogen peroxide (H$_2$O$_2$), hydroxyl radicals (OH•), and the singlet oxygen (1O$_2$) (Halliwell et al; 1995). These reactive ROS cause destructive and irreversible damage to the components of a cell, such as lipids, proteins and DNA (Lopaczynski and Zeisel, 2001).

Although normal cells possess antioxidant defense systems against ROS, the continuous accumulation of damage to the cells induces diseases such as cancer and aging (Matés and Sánchez-Jiménez, 2000). The continuous antioxidant dose also plays a preventive role against these diseases by removing the ROS in biological systems (Sgambato et al; 2001). This study aimed to investigate the relation between the chemopreventive activity of bioactive isolated components and its corresponding antioxidant scavenging effect, in order to elucidate the mechanisms of their cancer prevention.

Phenolic compounds constitute one of the most numerous and ubiquitous group of plant metabolites, and are an integral part of the human diet. It was found that in addition to their primary antioxidant activity, this group of compounds displays a wide variety of biological functions which are mainly related to modulation of carcinogenesis (Dai and Mumper, 2010). It possess ideal structure chemistry for free radical scavenging activities because they have: (a) phenolic hydroxyl groups that are prone to donate a hydrogen atom or an electron to a free radical; (b) extended conjugated aromatic system to delocalize an unpaired electron.

Several relationships between structure and reduction potential have been established as; phenolic acids and their esters, the reduced activity depends on the number of free hydroxyl groups in the molecule, which would be strengthened by steric hindrance (Dziedzic and Hudson 1983; Rice-Evans and Paganga 1996). This principle is inappropriate to the result detected; where lacciac acid E and purpurin found to be the most potent compounds.

**CONCLUSION**

The moderate polar fraction of *A. arborescense* possess more chemopreventive effect than polar one against different cancer cell line PC3, HepG2 and MCF-7. Fifteen components were isolated; lacciac acid (B and C), aloeresin (A, B and E), neoaloeresin A, purpurin and aloenin showed the most potent cytotoxic activity against HepG2 carcinoma cell line with almost (0-15%) on normal...
epithelial cells except aloenin, which inhibit about 35% of normal cells. All isolated compounds were previously isolated from different Aloe species except; lacciac acid E, lacciac acid C and purpurin. Otherwise, all other compounds were isolated for the first time. Chemopreventive activity of bioactive isolated components and its corresponding antioxidant scavenging effect can elucidate the mechanisms of their cancer prevention. The study provides robust evidence for a new postulated natural compounds with antitumor activity from A. arborescens.

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