ASIAN J NAT PROD BIOCHEM
Volume 20, Number 2, December 2022
Pages: 75-88

P-ISSN: 2775-4189
E-ISSN: 2775-4197
DOI: 10.13057/biofar/f200205

Phytochemical research of the anticancer potential of *Aloe turkanensis*

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Manuscript received: 22 August 2022. Revision accepted: 28 December 2022.

Abstract. *Adem FA, Yenessew A, Yusuf AO, Wanjohi JM. 2022. Phytochemical research of the anticancer potential of* Aloe turkanensis*. Asian J Nat Prod Biochem 20: 75-88.* The number of people diagnosed with cancer is rising worldwide, particularly in Sub-Saharan Africa. The search for new cancer treatments continues to benefit greatly from nature as a rich supply of promising chemicals. The anticancer effects of quinones have made them a popular medicinal class among natural chemicals (e.g., daunomycin and doxorubicin). *Aloe turkanensis* Christian, like other members of the genus *Aloe*, is a good place to get quinones. Dried and powdered *A. turkanensis* rhizomes and leaves were cold percolated in a mixture of dichloromethane and methanol (1:1). The crude extracts significantly decreased the viability of the human extrahepatic bile duct cancer cell line (TFK-1). Twelve chemicals were isolated from the crude extracts through chromatographic separations on silica gel, Sephadex LH-20, and preparative TLC. Spectroscopic techniques such as UV, ^{1}H, and ^{13}C NMR, COSY, NOESY, HMBC, and HSQC were used to determine the structures of the isolated compounds. Two naphthoquinones [3,5,8-trihydroxy-2-methylnaphthalene-1,4-dione (1) and 5,8-dihydroxy-3-methoxy-2-methylnaphthalene-1,4-dione (2)], seven anthraquinones [chrysophanol (3), aloesaponarin I (4), aloesaponarin II (5), laccaic acid D methyl ester (6) helminthosporin (8) aloe-emodin (10) and α-L-11-*O*-rhamnopyranosylaloe-emodin (11)], a pre-anthraquinone [aloesaponol I (7)] a pyrone derivative [feralolide (9)] and a benzoic acid derivative [3,4-dihydroxybenzoic acid (12)] were the chemicals that made up these substances. This study reported again that the *Aloe* genus produced the naphthoquinones [3,5,8-trihydroxy-2-methylnaphthalene-1,4-dione (1) and 5,8-dihydroxy-2-methoxy-2-methylnaphthalene-1,4-dione (2)]. In addition, this study made the first report of 3,5,8-trihydroxy-2-methylnaphthalene-1,4-dione (1) from the Asphodelaceae family. Human extrahepatic bile duct carcinoma (TFK-1) and liver cancer (HuH7) cell lines were used to test the extracted compounds for in-vitro anticancer activity. The anthraquinone aloe-emodin (10) and the naphthoquinone 5,8-dihydroxy-3-methoxy-2-methylnaphthalene-1,4-dione (2) showed significant inhibition against TFK-1 cell lines using the 3- [4,5-dimethylthiazol-2-yl]-2-,5-diphenyltetrazolium bromide (MTT) assay, with IC_{50} values of 6.0 and Aloesaponol I (7), a pre-anthraquinone, inhibited the development of TFK-1 cells with IC_{50} values of 10.0 µg/mL and HuH7 cells with 88.0 µg/mL. Both α-L-11-*O*- rhamnopyranosyl aloe-emodin (11) and aloesaponarin II (4), an anthraquinone, decreased TKF-1 cell line viability with IC_{50} values of 23.0 and 34.0 µg/mL, and HuH7 cell line viability with IC_{50} values of 47.0 and 55.0 µg/mL, respectively. IC_{50} values of 46.0 µg/mL for helminthosporin (8) showed considerable inhibition of TFK-1 cell growth, although it did not affect HuH7 cells at those concentrations. Extrahepatic bile duct (TFK-1) and liver (HuH7) cancer cell lines are sensitive to these chemicals identified here for the first time. Before these chemicals can be established as potentially effective anticancer medicines, more research on normal cell lines and their mechanism of action is required.

Keywords: *Aloe turkanensis,* anticancer potential, phytochemistry

INTRODUCTION

The human race has relied on natural remedies for thousands of years to cure and prevent disease. At least a thousand years of written evidence of ethnomedical science exist in countries like China (Chang and But 1986; Liu et al. 2014; Shen et al. 2014; Liu et al. 2015; Huang et al. 2016; Li and Xing 2016; Han et al. 2017; Gao et al. 2018; 2019) and India (Kapoor 1990; Baruah et al. 2013; Taram et al. 2020). Old plant cures are being used by many people today, especially in developing countries. Conventional medicines are either prohibitively expensive or in short supply in low-income nations. As a result, nearly 80% of the population in developing countries may use traditional medicine as their primary source of healthcare (WHO 2002).

Scientists have been refining the active ingredients responsible for curing many ailments based on ancient traditional procedures. *Artemisia annua*, from which the antimalarial ingredient artemisinin (13) was extracted, is an example of traditional Chinese medicine used to treat malaria (Dewick 2002). Moreover, thanks to developments in pharmacy and chemistry, aspirin (14), one of the first entirely synthetic medications, was created by acetylating salicylic acid (15), the pain-relieving active element in *Salix alba* (Samuelson 2004). Taxol (16), extracted from the Indian herb *Taxus brevifolia*, was the first substance developed for cancer chemotherapy. It has been demonstrated that this is useful for cancer therapy (Wani et al. 1971).

Cancer develops when the body's natural processes for dividing and eliminating cells from the population become unbalanced. The body's normal cells have a predictable life cycle of growth, division, and eventual death. Apoptosis refers to the processes of programmed cell death, the failure of which can lead to cancer development. Cancer cells, in contrast to healthy cells, do not die off naturally but keep dividing and expanding. As a result, it causes an

accumulation of aberrant cells that can spread to other body parts during their unchecked proliferation (Bright and Khar 1994). Cancer risk factors include illness, exposure to chemicals and radiation at work, and environmental variables, but one's personal lifestyle choices are by far the most influential (Buell and Dunn 1965; Kolonel and Wilkens 2006).

Natural chemicals with therapeutic potential are a constantly expanding field of scientific inquiry. The process of isolating and characterizing active ingredients from medicinal plants is ongoing. For example, Vinca alkaloids, such as vinblastin (17) and vincristine (18) from the Madagascar Catharanthus roseus (Apocynaceae), are effective cancer chemotherapeutic drugs (Stefania et al. 2009). Recently, a bi-cyclic peptide named romidepsin (19) was identified from the bacteria Chromo bacterium violaceum strain 968 and found to be cytotoxic against multiple human cancer cell lines. Currently, it is being tested in clinical studies for cancer treatment (Haigentz et al. 2012).

Among naturally occurring chemicals, those containing the quinone moiety are among the most well-known for their cancer-fighting effects. For example, streptomyces peucetius var. caesius was the source for daunomycin (20), and its derivative doxorubicin (21) is a quinone moiety drug used to treat various solid tumors and acute myeloid leukemia (Octavia et al. 2012).

The availability of molecules containing the quinone moiety suggests that the genus *Aloe*, which includes *Aloe turkanensis* Christian, may prospectively present in the search for new anticancer medicinal medicines.

Cancer is a leading cause of death worldwide, accounting for over 13% of all fatalities from the disease. An estimated 12.2 million people were diagnosed with cancer in 2008. In addition, there was an uptick in cancer incidence in developing nations (Ferlay et al. 2013). Furthermore, 80% of the world's population relies on medicinal plants whose safety and efficacy are not well recognized or documented and where cancer rates are predicted to rise by 2020 (Murray and López 1996). Therefore, research into new anticancer medications and plant preparations that are both effective and reasonably priced is essential.

MATERIALS AND METHODS

General

Instrumentation

The NMR spectra were acquired by the Department of Pharmaceutical Biology at Saarland University, Germany, using a Bruker Avance (500 MHz) spectrometer and a reference of residual solvent signals. Standard Bruker software was used to acquire data in the fields of homonuclear Correlation Spectroscopy (COSY), Heteronuclear Single Quantum Correlation (HSQC), and Heteronuclear Multiple Bond Correlation (HMBC) (Top spin 3.0 pl 3). In addition, Saarland University in Germany used TECAN sunrise software XFluor4 to measure the absorbance of a purple formazan solution in living cells at 550 nm and 690 nm. Furthermore, silica gel (70-23 mesh) and Sephadex LH-20 were employed in column chromatography (CC) for chemical purification. In addition, pre-coated silica gel 60 F254 plates are used in analytical thin-layer chromatography (Merck).

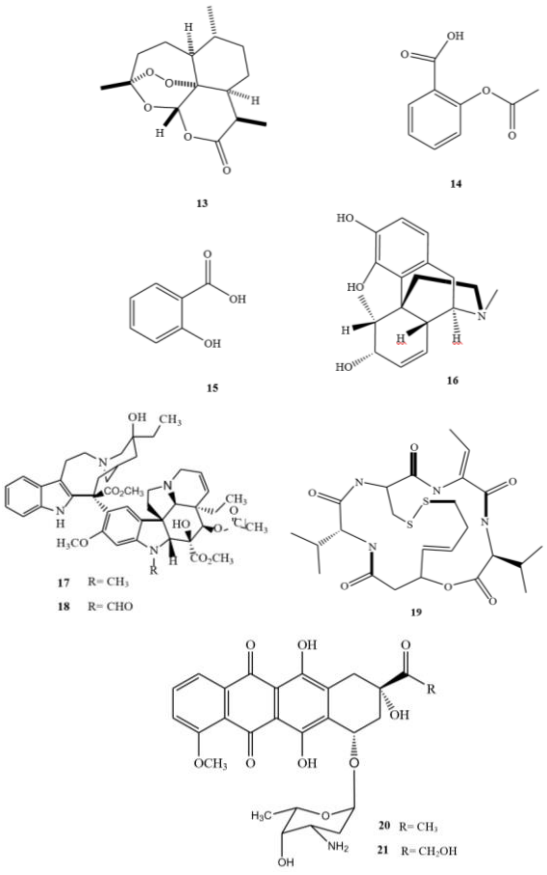
Plant material

Marigat in Indao, Kenya, provided the *A. turkanensis* rhizome and leaves for this study in June 2012. Mr. Simon Mathenge of the University of Nairobi's Botany Department School of Biological Science positively recognized this plant. Therefore, a voucher specimen was deposited with the deposit number FAA 2012/001.

Extraction and isolation

Extraction and isolation from rhizomes of Aloe turkanensis

The *A. turkanensis* rhizomes were sun-dried, then ground into a powder. Exhaustive cold percolation with a 1:1 mixture of CH_2Cl_2 and MeOH was used to extract 2 kilograms of powdered plant material. After combining the extract, it was filtered and concentrated under low pressure to obtain 30 g of crude extract. Ethyl acetate and water were used to separate the extract. A rotary evaporator concentrated the ethyl acetate layer into a crude extract weighing 20 grams. Column chromatography on oxalic acid deactivated silica gel (400 g) eluting with n-hexane containing increasing levels of ethyl acetate yielded 250 fractions of ca. 250 mL each from a 15 g sample of the ethyl acetate extract. Based on their TLC profiles, these were merged to form 21 different fractions.



Using CC on Sephadex LH-20 (eluting with CH2Cl2/MeOH, 1:1), fraction 2 (1% ethyl acetate in n-hexane) was further separated to provide 3,5,8-trihydroxy-2-methyl naphthalene1,4-dione (2, 4.0 mg) and 5,8-dihydroxy-3-methoxy-2-methylnaphthalene-1,4-dione (2, 4.0 mg) (1, 3.0 mg). Chrysophanol (3, 6 mg) was obtained by collecting fraction 3 (3% ethyl acetate in n-hexane) as a yellow solution, then concentrating the resulting yellow precipitate, filtering, and washing with n-hexane. By filtering and washing with n-hexane, fraction 6 (10% ethyl acetate in n-hexane) was converted to aloesaponarin I (4.25 mg). The filtrate from fraction 6 was purified using Sephadex LH-20 (eluted with CH2Cl2/MeOH; 1:1) to yield aloesaponarin II (5.20 mg). 6.4 milligrams of laccaic acid D-methyl ester were isolated from a brown solution of fraction 12 (30% ethyl acetate in n-hexane) using CC on Sephadex LH 20 (eluting with CH2Cl2/MeOH; 1:1). Aloesaponol I was isolated from fraction 16 (50% ethyl acetate in n-hexane), which precipitated as a colorless solid after being filtered and washed with an n-hexane/acetone combination (7.20 mg).

Extraction and isolation from the leaves of Aloe turkanensis

The *A. turkanensis* leaf powder (2 kg) was extracted and concentrated as described above to get 31 grams of crude extract. After separating the extract into ethyl acetate and water, 25 g was obtained after evaporating the organic solvent. On oxalic acid-impregnated silica gel (400 g) eluting with n-hexane containing increasing quantities of ethyl acetate, CC was performed on 20 g of the ethyl acetate extract. The collected 250 mL was divided into 20 equal parts. Further purification by CC over Sephadex LH-20 (eluent: CH_2Cl_2/MeOH; 1:1) yielded helminthosporin (8.3 mg) and 3,5,8-trihydroxy-2-methylnaphthalene1,4-dione (14 mg). Fraction 2 was eluted with 1% ethyl acetate in n-hexane (1, 2.5 mg). Chrysophanol was produced using a similar purification process using fraction 3 (3 percent ethyl acetate in *n*-hexane) (3, 5 mg). Purification of fraction 6 (7% ethyl acetate in *n*-hexane) using Sephadex LH-20 (CH_2Cl_2/MeOH; 1:1) yielded aloesaponarin I (4, 15 mg) and aloesaponarin II (11, 30 mg) as dark green solutions (5, 10 mg). Two blue fluorescence spots were observed in fraction 8 (15% ethyl acetate in *n*-hexane) and were resolved by CC over Sephadex LH-20 (elution: CH_2Cl_2/MeOH; 1:1), yielding feralolide (9, 15 mg) and 3,4-dihydroxybenzoic acid (12, 12 mg).

From fraction 13 (40% ethyl acetate in *n*-hexane) and fraction 16 (60% ethyl acetate in *n*-hexane), crystals of aloe-emodin (10, 10 mg) and *α* -L-11-*O*-rhamnosyl aloe-emodin (11, 8.5 mg) were produced.

Physical and spectroscopic properties of isolated compounds

3,5,8-Trihydroxy-2-methyl naphthalene-1,4-dione (1)

Red amorphous solid. UV λ_{max} 300, 420, 480 nm. ^{1}H NMR ($CDCl_3$, 500 MHz): δ_H 12.81 (1H, *s*, 5-OH), 11.48 (1H, *s*, 8-OH), 7.29 (1H, *d*, *J* = 9.5 Hz, H-6), 7.18 (1H, *d*, *J* = 9.5 Hz, H-7), 2.11(3H, *s*, 2-CH_3). ^{13}C NMR ($CDCl_3$, 125 MHz): δ_C 188.8 (C-1), 182.0 (C-4), 157.6 (C-5), 157.1 (C-8), 153.8 (C-3), 134.1 (C-6), 127.4 (C-7), 121.6 (C-2), 110.7 (C-5a), 110.1 (C-8a), 8.3 (2-CH_3).

5,8-Dihydroxy-3-methoxy-2-methyl naphthalene-1,4-dione (2)

Red amorphous solid. UV λ_{max} at 300, 480 nm. EIMS *m/z* $[M]^+$ 234.9. ^{1}H NMR ($CDCl_3$, 500 MHz): δ_H 12.72 (1H, *s*, 8-OH), 12.32 (1H, *s*, 5-OH), 7.24 (1H, *d*, *J* = 10.0 Hz, H-7), 7.22 (1H, *d*, *J* = 10.0 Hz, H-6), 4.14 (1H, *s*, 3-OCH_3), 2.11 (3H, *s*, 2-CH_3). ^{13}C NMR ($CDCl_3$, 125 MHz): δ_C 188.4 (C-1), 183.5 (C-4), 158.3 (C-3), 158.3 (C-5), 157.5 (C-8), 133.2 (C-2), 129.9 (C-7), 128.6 (C-6), 111.5 (C-8a), 111.1 (C-5a), 61.4 (OCH_3), 9.0 (CH_3).

Chrysophanol (3)

Orange needles. UV λ_{max} 300, 420 nm. ^{1}H NMR ($CDCl_3$, 500 MHz): δ_H 12.11 (1H, *s*, H-8), 12.00 (1H, *s*, H-1), 7.82 (1H, *dd*, *J* = 1.0, 8.5 Hz, H-5), 7.68 (1H, *t*, *J* = 7.5 Hz, H-6), 7.64 (1H, *bs*, H-2), 7.29 (1H, *dd*, *J* = 1.0, 8.5 Hz, H-7), 7.09 (1H, *bs*, H-4), 2.46 (3H, *s*). ^{13}C NMR ($CDCl_3$, 125 MHz): δ_C 192.5 (C-10), 181.9 (C-9), 162.7 (C-8), 162.4 (C-1), 149.3 (C-3), 136.9 (C-6), 133.6 (C-5a), 133.2 (C-4a), 124.5 (C-7), 124.3 (C-4), 121.3 (C-2), 119.9 (C-5), 115.8 (C-8a), 113.7 (C-1a), 22.2 (CH_3).

Aloesaponarin II (4)

Orange crystals. UV λ_{max} 260, 290, 400 nm. EIMS *m/z* $[M]^+$ 254.58. ^{1}H NMR (DMSO-d_6, 500 MHz): δ_H 12.94 (1H, *s*, 1-OH), 7.68 (1H, *t*, *J* = 7.5 Hz, H-3), 7.58 (1H, *dd*, *J* = 7.5, 1.5 Hz, H-2), 7.41 (1H, *d*, *J* = 3.0 Hz, H-5), 7.29 (1H, *dd,* *J* = 8.5, 1.0 Hz, H-4), 6.99 (1H, *d*, *J* = 2.5 Hz, H-7), 2.66 (3-H, *s*, 8-CH_3). ^{13}C NMR (DMSO-d_6, 125 MHz): δ_C 189.2 (C-9), 182.1 (C-10), 162.2 (C-1), 161.4 (C-6), 145.2 (C-8), 136.7 (C-5a), 135.8 (C-3), 132.4 (C-4a), 116.3 (C-1a), 124.4 (C-7), 124.1 (C-2), 122.2 (C-8a), 118.1 (C-4), 111.9 (C-5), 23.5 (CH_3).

Aloesaponarin I (5)

Orange crystals. UV λ_{max} 260, 300, 410 nm.^{1}H NMR (acetone-d_6, 500 MHz): δ_H 12.87 (1H, *s*, 1-OH), 7.70 (1H, *dd*, *J* = 5.5, 2.5 Hz, H-4), 7.73 (1H, *s*, H-5), 7.68 (1H, *t*, *J* = 1.9, H-3), 7.29 (1H, *dd,* *J* = 7.5, 1.0 Hz, H-2), 3.95 (3H, *s*, OCH_3), 2.70 (3-H, *s,* 8-CH_3). ^{13}C NMR (acetone-d_6, 125 MHz): δ_C 190.4 (C-9), 182.3 (C-10), 167.9 (C=O), 162.9 (C-1), 159.5 (C-6), 142.9 (C-7), 136.9 (C-5), 133.8 (C-5a), 133.6 (C-4a), 130.8 (C-8), 125.2 (C-8a), 124.4 (C-2), 119.2 (C-3), 117.9 (C-1a), 113.0 (C-4), 52.7 (OCH_3), 20.3 (8-CH_3).

Laccaic acid D methyl ester (6)

Orange crystals. UV λ_{max} 300, 410 nm. EIMS *m/z* $[M]^+$ 328.71. ^{1}H NMR (acetone-d_6, 500 MHz): δ_H 13.16 (1H, *s*, 1-OH), 7.72 (1H, *s*, H-5), 7.18 (1H, *d*, *J* = 2.5 Hz, H-4), 6.65 (1H, *d*, *J* = 2.5 Hz,H-2), 3.93 (3H, *s*, OCH_3), 2.70 (3-H, s). ^{13}C NMR (acetone-d_6, 125 MHz): δ_C 189.3 (C-9), 182.7 (C-10), 168.1 (C=O), 166.2 (C-1), 165.1 (C-3), 159.3 (C-6), 142.3 (C-7), 138.0 (C-5a), 135.5 (C-4a), 130.9 (C-8), 109.2 (C-2), 108.1 (C-4), 113.1 (C-5), 111.7 (C-1a), 52.6 (OCH_3), 20.3 (CH_3).

Aloesaponol I (7)

Colorless solid. The TLC showed blue fluorescence under UV light (366 nm). UV λ_{max} 300, 380 nm. EIMS *m/z* $[M]^+$ 316.90. ^{1}H NMR (DMSO-d_6, 500 MHz): δ_H 15.27 (1H, *s*, 9-OH), 6.95 (1H, *s*, H-5), 6.92 (1H, *s*, H-10), 4.24 (1H, *m*, H-3), 3.83 (3H, *s*, OCH_3), 3.14 (1H, *dd*, *J* = 3.3,15.8 Hz, H-4), 2.96 (1H, *dd*, *J* = 3.3, 17.1 Hz, H-2), 2.90 (1H, *dd*, *J* = 6.8, 15.6 Hz, H-4), 2.70 (1H, *dd*, *J* = 1.8, 5.4 Hz, H-2), 2.70 (3H, *s*). ^{13}C NMR (DMSO-d_6, 125 MHz): δ_C 203.7 (C-1), 168.2 (C=O), 165.9 (C-9), 140.8 (C-7), 137.2 (C-8), 136.6 (9a), 155.1(C-6), 125.4 (C-8a), 116.6 (C-5), 107.5 (C-10), 110.2 (C-1 0a), 64.4 (C-3), 52.1 (OCH_3), 46.4 (C-4), 37.5 (C-2), 20.8 (CH_3).

Helminthosporin (8)

Red needle. UV λ_{max} at 500, 580 nm. ^{1}H NMR (DMSO-d_6, 500 MHz): δ_H 12.83 (1H, *s*, 5-OH), 12.03 (2H, *s*, 1-OH and 8-OH), 7.65 (1H, *brs*, H-4), 7.44 (1H, *d*, H-7), 7.44 (1H, *d,* H-6), 7.26 (1H, *brs*, H-2), 2.50 (3H, *s,*3-CH_3). ^{13}C NMR (DMSO-d_6, 125 MHz): δ_C 189.9 (C-9), 186.3 (C-10), 161.7 (C-1), 157.1 (C-5), 156.4 (C-8), 149.1 (C-3), 132.8 (C-4a), 129.6 (C-7), 129.4 (C-6), 113.8 (C-1a), 124.3 (C-2), 120.2 (C-4), 112.6 (C-8a), 112.5 (C-5a), 22.1 (CH_3).

Feralolide (9)

Brown solid. UV λ_{max} 310 nm. EIMS *m/z* $[M]^+$ 344.75. ^{1}H NMR ($CDCl_3$, 500 MHz): δ_H 11.21 (1H, *s*, OH-8), 6.42 (1H, *d*, *J* = 1.9 Hz, H-7'), 6.37 (1H, *d*, *J* = 2.5 Hz, H-5'), 6.31 (1H, *d*, *J* = 1.5, H-5), 6.26 (1H, *d, J* = 1.9, H-7), 4.80 (1H, *m*, H-3), 3.08 (1H, *dd*, *J* = 5.5,14Hz, H-1'), 3.22 (1H, *dd*, *J* = 6.9,13.5 H-1‘), 2.94 (1H, *dd*, H-4), 2.92 (1H, *dd*, H-4), 2.57 (1H, *s*, CH_3). ^{13}C NMR ($CDCl_3$, 125 MHz): δ_C 203.9 ($COCH_3$), 170.3 (C-1), 165.2 (C-8), 165.0 (C-6), 160.3 (C-6'), 160.1 (C-4'), 142.9 (C-4a), 139.4 (C-2'),121.0 (C-3'), 111.7 (C-7'), 107.6 (C-5), 102.5 (C-5'), 101.9 (C-7), 101.8 (C-8a), 80.5 (C-3), 39.5 (C-1'), 33.1 (CH_3), 32.8 (C-4).

Aloe-emodin (10)

Orange crystals. UV λ_{max} at 260, 300, 420 nm. ^{1}H NMR (DMSO-d_6, 500 MHz): δ_H 11.93 (2H, *s*, 1-OH, 8-OH), 7.81 (1H, *t*, *J* = 8.0 Hz, H-6), 7.71 (1H, *dd*, *J* = 7.5, 1.1 Hz, H-5), 7.68 (1H, *d*, *J* =1.6 Hz, H-4), 7.38 (1H, *dd*, *J* = 9.0, 1.1 Hz, H-7), 7.28 (1H, *d*, *J* = 1.6 Hz, H-2), 4.62 (2H, *s,*H-11). ^{13}C NMR (DMSO-d_6, 125 MHz): δ_C 191.6 (C-9), 181.4 (C-10), 161.6 (C-1), 161.3 (C-8), 153.7 (C-3), 137.3 (C-6), 133.3 (C-4a), 133.1 (C-5a), 124.4 (C-7), 120.6 (C-2), 119.3 (C-5), 115.9 (C-8a), 114.4 (C-1a), 107.1 (C-4), 62.07 (C-11).

α-L-11-O-Rhamnopyranosyl aloe-emodin (11)

Orange crystals. UV λ_{max} 260, 300, 430 nm. EIMS *m/z* $[M]^+$ 416. ^{1}H NMR (DMSO-d_6, 500 MHz): δ_H 7.82 (1H, *st*, *J* = 8.4 Hz, H-6), 7.73 (1H, *dd*, *J* = 7.5, 1.1 Hz, H-7), 7.68 (1H, *d*, *J* =1.6 Hz, H-4), 7.41 (1H, *dd*, *J* = 8.4, 1.1 Hz, H-5), 7.32 (1H, *d*, *J* = 1.6 Hz, H-2), 4.75 (1H, *d*, *J* = 14.0 Hz, H-11), 4.71 (1H, *d*, *J* = 1.6 Hz, H-1'), 4.61 (1H, *d*, *J* = 13.9 Hz, H-11), 3.73 (2H, *dd*, *J* = 3.4, 1.7 Hz, H-2'), 3.62-3.28 (33H, *m*, H-3'), 3.23 (2H, *s*, H-4'), 1.16 (3H, *d*, *J* = 6.2 Hz, CH_3). ^{13}C NMR (DMSO-d_6, 125 MHz): δ_C 191.6 (C-9), 181.4 (C-10), 161.4 (C-1), 161.3 (C-8), 148.8 (C-3), 137.4 (C-6), 133.4 (C-4a), 133.3 (C-5a), 124.4 (C-7), 121.8 (C-2), 119.3 (C-5), 117.7 (C-4), 115.9 (C-8a), 115.3 (C-1a), 99.9 (C-1'), 71.8 (C-4'), 70.4 (C-2'), 69.0 (C-3'), 66.9 (C-11), 70.7 (C-5'), 17.9 (CH_3).

3,4-Dihydroxybenzoic acid (12)

Brown solid. ^{1}H NMR ($CDCl_3$, 500 MHz): δ_H 7.53 (1H, *d*, *J* = 2.0 Hz, H-2), 7.47 (1H, *dd*, *J* = 6.5, 2.0 Hz, H-6), 6.89 (1H, *d*, *J* = 4.9 Hz, H-5). ^{13}C NMR ($CDCl_3$, 125 MHz): δ_C 168.7 (C=O), 167.5 (C-3), 150.6 (C-4), 146.2 (C-1), 123.6 (C-6), 117.4 (C-2), 115.6 (C-5).

Biological tests

In vitro anticancer activities

The cytotoxicity test was conducted in Germany at Saarland University's Department of Pharmaceutical Biology using the colorimetric assay, also known as the MTT assay, per the methods described by Heo et al. (1990).

Cell culture

DMEM (#42460-025, Gibco, Germany) supplemented with 20% Fetal Calf Serum (FCS) and 1% penicillin/streptomycin (10.000 units/mL/10mg/mL) was used to cultivate the human extrahepatic bile duct cancer cell line TFK-1. In addition, cultures of the human hepatocellular carcinoma cell line HuH7 were maintained in RPMI 1640 (#R8757, Sigma, Germany) supplemented with 20% fetal calf serum (FCS) and 1% penicillin/streptomycin (10.000 units/mL/10mg/mL). The cell line was grown in a humidified incubator at 37 ° C with 5% carbon dioxide (Heo et al. 1990).

MTT assay

The cells were tallied, and then 1 x 104 cells were plated into each well of a 96-well plate. Both cancer cell lines were exposed to isolated chemicals dissolved in DMSO and diluted with culture media (1-100 g/mL) for 48 hours. First, cell proliferation was measured using 3-[4, 5-dimethylthiazol-2- yl]-2,5-diphenyltetrazolium bromide (MTT) (0.5 mg/mL) in Phosphate-Buffered Saline (PBS) after incubation. Next, MTT solution (150 L/well) was added after incubating for an hour, and the incubation media containing the MTT was aspirated to dissolve the formazan crystals. Then 80 L of DMSO was added to each well. The formazan concentration was determined with the help of TECAN's dawn software XFluor4, using 550 and 690 nm as reference wavelengths (Figure 1).

Statistical analysis

Microsoft Office Excel was used for all data analysis. The data were shown as means ± SEM. The t-test for independent samples, two-sample-size-estimation, was used to calculate the differences' significance. The significance level for a difference was set at a p-value of less than 0.05 (* $p<0.05$; ** $p<0.01$; *** $p<0.001$).

RESULTS AND DISCUSSION

Preliminary test

The cell viability of the TFK-1 cell line was significantly decreased in preliminary tests using a crude

extract from the rhizomes and leaves of *A. turkanensis*. In addition, TLC detected colored spots that absorb UV light in the crude extracts (254 and 366 nm). Scientists believe these to be quinone derivatives as the yellow dots became crimson when exposed to ammonia vapor. Chromatographic separation was used to obtain the chemicals. Finally, the extracted compounds were put through anticancer testing using HuH7 hepatoma carcinoma and TFK-1 extrahepatic bile duct carcinoma cell lines. Compounds identified from A. turkanensis rhizomes and leaves are discussed, along with their pharmacological profiles and cancer-fighting properties below.

Characterization of compounds from the rhizomes of *Aloe turkanensis*

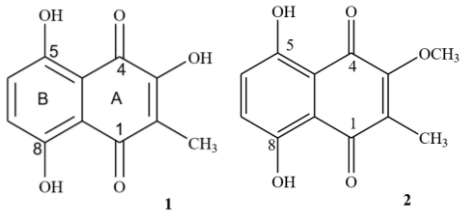
Following a cold percolation extraction with dichloromethane/ methanol (1:1), the rhizomes of A. turkanensis were partitioned between ethyl acetate and water. Two naphthoquinones, four anthraquinones, a pre-anthraquinone, a chromone derivative, and a benzoic acid derivative were separated chromatographically from the ethyl acetate extract. As will be seen in a moment, spectroscopic methods were used to determine the precise structures of the compounds.

3,5,8-Trihydroxy-2-methylnaphthalene-1,4-dione (1)

Isolated as a red amorphous solid, compound 1 possesses the UV spectral features typical of 3,5,8-trihydroxy substituted naphthoquinones (λ_{max} 300, 420, 480 nm) (Bringmann et al. 2011). The ^{13}C NMR spectra corroborated this finding by revealing three oxygenated carbon atoms at δ_C 153.8, 157.1, and 157.6 for C-3, C-8, and C-5, respectively.

Eleven carbon signals were found in the ^{13}C NMR spectrum (Table 1), both indicative of carbonyl (at δ_C 182.0 and 188.8, respectively, for C-4 and C-1). NMR spectra further revealed that the naphthoquinone structure contained a methyl carbon at δ_C 8.3 (δ_H 2.11) and two chelated hydroxyl substituents at δ_H 11.48 and 12.81. Additionally, two ortho-coupled aromatic protons were detected in ring B of 1's ^1H NMR spectra (Table 1), which are replaced at C-5 and C-8 with hydroxyl groups. The ^1H NMR spectra (Table 1) of 1 also showed the existence of two *ortho*-coupled aromatic protons in ring B, which are exchanged at C-5 and C-8 with hydroxyl groups [at δ_H 7.18 (J = 9.5 Hz, δ_C 127.4) and 7. 29 (J = 9.5 Hz, δ_C 131.4].

The aromatic proton at δ_H 7.18 in the HMBC spectrum was assigned to H-7 due to its Correlation with C-8 and C-8a, and the signal at δ_H 7.29 was assigned to H-6 due to its Correlation with C-5 and C-5a. The HMBC Correlation with C-1, C-2, and C-3 supports the position of the C-2 methyl group at δ_H 2.11.1. Therefore, the structure of 1 was identified as 3,5,8-trihydroxy-2-methyl-1,4-naphthoquinone, also known as 8- hydroxydroserone, a somewhat unimaginative term. It is the first report of compound 1 from the family Asphodelaceae, which has before been isolated from Droseraceae and Nepenthaceae families (Macbeth and Winzor 1935); it was also reported from lyophilized cell culture of *Triphyophyllum peltatum* (Bringmann et al. 2011).



5, 8-Dihydroxy-3-methoxy-2-methylnaphthalene-1,4-dione (2)

EIMS showed a molecular ion [M]+ peak at m/z 234.91, which corresponds to the molecular formula of $C_{12}H_{10}O_5$; this compound was separated as a red amorphous solid. The ultraviolet (UV) spectrum (maximum wavelengths of 300 and 480 nm) is indicative of a 1,4-naphthoquinone structure (Bringmann et al. 2008). The ^{13}C NMR spectra of 1,4-naphthoquinone matched these measurements, revealing two carbonyl signals at δ_C 183.5 and 188.4 for carbon atoms C-4 and C-1, respectively. It appears that 2 is a methyl ether derivative of 1 based on its ^1H and ^{13}C NMR spectra (Table 1), which are nearly identical to those of 1, except for the presence of a methoxy group at C-2 (δ_H 4.14, δ_C 61.4). As a result, twelve carbon signals, including methyl carbon at δ_C 9.01 (δ_H 2.11), were observed in the ^{13}C NMR spectrum (Table 1). There were also two chelated hydroxyl proton signals in the ^1H NMR spectra, at δ_H 12.32 and δ_H 12.72 for 5-OH and 8-OH, respectively. Assigned proton energies of δ_H 7.24 (δ_C 129.9) and δ_H 7.22 (δ_C 128.6) (J = 10.0 Hz) for the aromatic protons H-6 and H-7 in ring B, respectively. HMBC spectrum corroborated the substitution pattern in ring B (Table 1).

Accordingly, the chemical was 5,8-dihydroxy-3-methoxy-2-methylnaphthalene-1,4-dione (2), a recently described novel compound from the roots of *A. secundiflora* (Induli et al. 2012). On the contrary, it is the second instance of compound 2 found in *Aloe*.

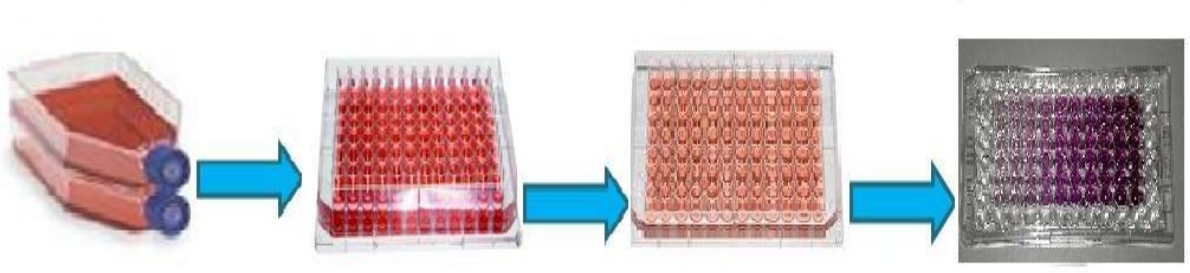


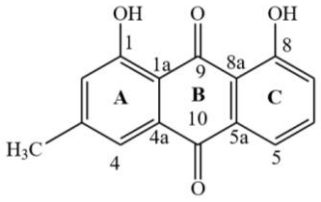
Figure 1. Picture of MTT assay

Chrysophanol (3)

Isolated in the form of orange needles, compound 3 exhibits the signature UV absorption of 1,8-dihydroxyanthraquinones at λ_{max} 300 and 420 nm (Dagne et al. 1994). Furthermore, two chelated hydroxyl protons, at δ_H 12.00 and 12.11 for 1-OH and 8-OH, were found in this molecule. In addition, one methyl, five methines, nine quaternary carbons (two of which are oxygenated, at (δ_C 162.4 and 162.7), and two carbonyls (δ_C 181.9 and 192.5) were detected in the ^{13}C NMR spectrum (Table 2).

On the ^1H NMR spectrum, the biogenetically predicted methyl group was located at C-3 (δ_H 2.46, δ_C 22.6). In addition, there were two broad singlet aromatic protons at δ_H 7.64 and 7.09, both ascribed to H-2 and H-4 of ring A. Protons in the ring C show an AMX pattern at 7.82 (1H, *dd*, *J* = 1.0, 8.5 Hz, H-5), 7.68 (1H, *t*, *J* = 8.5 Hz, H-6), and 7.29 (1H, *dd,* *J* = 1.0, 8.5 Hz, H-7).

Therefore, 1,8-hydroxy-3-methylanthraquinone (commonly known as chrysophanol) was determined to be the correct name for this substance (3). In addition, there is evidence that additional genera of the Asphodelaceae family also contain compound 3, which was originally isolated from Aloe roots (Yenesew et al. 1988).



3

Table 1. ^1H (500 MHz), ^{13}C (125 MHz), and HMBC spectral data of compounds 1 and 2 ($CDCl_3$)

Carbon no.	Compound 1			Compound 2		
	^1H δ_H (*m*, *J* in Hz)	^{13}C	HMBC	^1H δ_H (*m*, *J* in Hz)	^{13}C	HMBC
1	-	188.8		-	188.4	
2	-	121.6		-	133.2	
3	-	153.8		-	158.3	
4	-	182.0		-	183.5	
5	-	157.6		-	158.3	
5a	-	110.7		-	111.5	
6	7.29 (*d,* 9.5)	127.4	C-5, C-5a	7.22 (*d*,10.0)	128.6	C-5, C-5a, C-8
7	7.18 (*d*, 9.5)	131.4	C-8, C-8a	7.24 (*d*,10.0)	129.9	C-8, C-8a, C-5
8	-	157.1		-	157.5	
8a	-	110.1		-	111.1	
2-CH_3	2.11 (*s*)	8.2	C-1, C-2, C-3	2.11 (*s*)	9.0	C-1, C-2, C-3
3-OCH_3				4.14 (*s*)	61.4	C-3
5-OH	12.81(*s*)	-	C-5, C-5a	12.32 (*s*)	-	C-5a, C-6, C-5
8-OH	11.48 (*s*)	-	C-8, C-8a	12.72 (*s*)	-	C-8a, C-7, C-8

Table 2. ^1H (500 MHz) and ^{13}C (125 MHz) spectral data of compounds 3 and 4 (DMSO-d_6)

Carbon no.	Compound 3		Compound 4	
	^1H δ_H (*m*, *J* in Hz)	13C	^1H δ_H (*m*, *J* in Hz)	^{13}C
1	-	162.4	-	162.2
1a	-	113.7	-	116.3
2	7.64 (*bs*)	121.3	7.29 (*dd, J* = 8.5, 1.0 Hz)	124.1
3	-	149.3	7.68 (*t*, *J* = 7.5 Hz)	136.7
4	7.09 (*bs*)	124.3	7.58 (*dd*, *J* =7.5, 1.5 Hz)	118.1
4a	-	133.2	-	132.4
5	7.82 (*dd, J* = 8.5, 1.0 Hz)	119.9	7.41 (*d*, *J* = 3.0 Hz)	111.9
5a	-	133.6	-	136.7
6	7.68 (*dd*, *J* = 7.5, 1.5 Hz)	136.9	-	161.4
7	7.29 (*dd*, *J* = 7.5, 1.5 Hz)	124.5	6.99 (*d*, *J* = 2.5 Hz	124.4
8	-	162.7	-	145.2
8a	-	115.8	-	122.6
9	-	181.9	-	189.2
10	-	192.5	-	182.1
1-OH	12.00 (*s*)	-	12.94 (*s*)	-
8-CH_3	2.46 (s)	22.6	2.66 (*s*)	23.5

Aloesaponarin II (4)

Isolated in an orange crystal, compound 4 has UV absorption at λ_{max} 260, 290, and 400 nm, the characteristic of anthraquinones (Yagi et al. 1974). The molecular formula ($C_{15}H_{10}O_4$) equated to an ESI [M]+ value of 254.58. Fifteen carbon signals, including two carbonyl signals (at δ_C 189.2 and 182.1, designated for C-9 and C-10, respectively, of an anthraquinone), two oxygenated aromatic carbon signals (at δ_C 162.2 and 161.4), and a methyl signal at δ_C 23.5, were seen in the ^{13}C NMR spectrum (Table 2). This molecule is thought to be isomeric with 3 according to its ^1H NMR spectra, which displays only one chelated hydroxyl proton signal at δ_H 12.94 and a down-field shifted methyl proton at δ_H 2.66.

Protons H-7 and H-5 of ring C are *meta*-coupled, with their corresponding H values being 7.41 (1H, *d*, *J* = 3.0 Hz) and 6.99 (1H, *d*, *J* = 2.5 Hz). In addition, the ^1H NMR demonstrated the presence of an AMX spin system with three aromatic protons resonating at δ_H 7.58 (1H, *dd*, *J* = 7.5, 1.5 Hz), δ_H 7.68 (1H, *t*, *J* = 7.5 Hz), and δ_H 7.29 (1H, *dd*, *J* = 8.5, 1.0 Hz) for H-4, H-3, and H-2 of ring A, respectively. Since this component was previously only found in *A. saponaria* (Yagi et al. 1974), it was given the name aloesaponarin II (4). (Yenesew et al. 1993; Dagne et al. 1994). It has also been stated that compound 4 is a metabolite produced by bacteria (Cui et al. 2006; Bartel et al. 1990; Fotso et al. 2003).

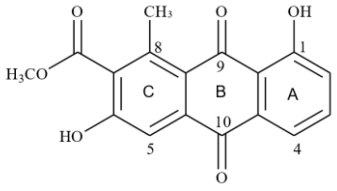
Aloesaponarin I (5)

The UV spectra of compound 5, isolated as an orange crystal, showed absorption at λmax 260, 300, and 410 nm, which is suggestive of an anthraquinone chromophore, similar to compound 4. Table 3 displays the ^1H and ^{13}C NMR spectra of compound 5, which are very similar to those of 4, with the addition of signals due to a methyl ester group (δ_H 3.95, δ_C 52.7 and δ_C 167.9) at C-7. Since there is only one aromatic singlet in the ^1H NMR spectrum of 5, it is determined to be at δ_H 7.73 and is ascribed to the H-5 on ring C. Ring A has three aromatic protons with identical H

values to ring 4; these protons are positioned at δ_H 7.70 (*dd*, *J* = 5.5, 2.5 Hz for H-4), 7.68 (*t*, *J* = 2.0 Hz, H-3), 7.29 (*dd,* *J* = 7.5, 1.0 Hz, H-2). There were 17 signals in the ^{13}C NMR spectra (Table 3). It has been determined through analysis that this substance is 1,6-dihydroxy-8-methylanthraquinone-7-carboxy methyl ester (trivial name aloesaponarin I). It has been observed in several species of *Aloe*, including *A. graminicola* (Yenesew et al. 1993; Dagne et al. 1994).

Laccaic acid D methyl ester (6)

Orange crystals of compound 6 were isolated, and its chemical formula, $C_{17}H_{12}O_7$, was determined by mass spectrometry (MS) from the presence of a molecular ion peak at *m/z* 328.71. The UV absorption spectra of compound 6 were compatible with a 9,10-anthraquinone chromophore at λ_{max} 300, 410 nm. Similarities between 5 and 6 were identified in ^1H and ^{13}C NMR (Tables 3 and 4), except that 6 has an extra hydroxyl group at C-3 (δ_C 165.1). In addition, two *meta*-coupled aromatic protons, at δ_H 6.65 (*d*, *J* = 2.5 Hz, H-2), 7.18 (*d*, *J* = 2.5 Hz, H-4), and a singlet aromatic proton, at 7.72 (*d, J* = 2.5 Hz, H-3), were detected in ring A of 6 by ^1H NMR spectroscopy (H-5). NMR spectra established the existence of a methyl ester (at C-6), methyl (at C-8), and three hydroxyl substituents (at C-1, C-3, and C-6) (Tables 3 and 4). Thus, laccaic acid D methyl ester (6) was determined to be the component in question; this chemical was previously found in some *Aloe* species (Yagi et al. 1974; Dagne et al. 1992; van Wyk et al. 1995).



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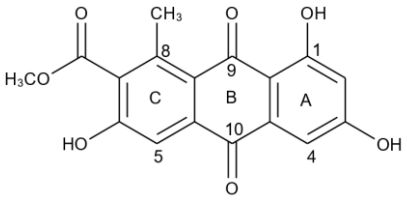


6

Table 3. ^1H (500 MHz, acetone-d_6) spectral data of compounds 5 and 6

Carbon no.	Compound	
	^1H δ_H (*m*, *J* in Hz)	
	5	**6**
2	7.29 (*dd*, 7.5, 1.0)	6.65 (*d*, 2.5)
3	7.68 (*t*, 1.9)	-
4	7.70 (*dd*, 5.5, 2.5)	7.18 (*d*, 2.5)
5	7.73 (*s*)	7.72 (*s*)
1-OH	12.8 (*s*)	13.16 (*s*)
8-CH_3	2.70 (*s*)	2.70 (*s*)
CO-OCH_3	3.95 (*s*)	3.93 (*s*)

Table 4. ^{13}C (125 MHz, acetone-d_6) spectral data of compounds 5 and 6

Carbon no.	δ_C	
	5	**6**
1	162.9	166.2
1a	125.3	111.7
2	124.4	109.2
3	119.2	165.1
4	113.0	108.1
4a	133.6	135.5
5	136.9	113.1
5a	133.8	138.0
6	159.5	159.3
7	142.9	142.3
8	130.8	130.9
8a	125.2	124.2
9	190.4	189.3
10	182.3	182.7
*C*O-OCH_3	167.9	168.1
CO-*OC*H_3	52.7	52.6
CH_3	20.3	20.3

Aloesaponol I (7)

The solid form of Compound 7 was developed, and it fluoresced blue when exposed to ultraviolet light (366 nm). Pre-anthraquinone chromophores, like the one displayed by this chemical, often absorb UV light at λ_{max} 300, 380 nm (Yagi et al. 1974). Peaking at *m/z* 316.90, ESIMS detected a molecular ion indicative of the formula $C_{17}H_{15}O_6$.

Table 6 shows that the ^1H and ^{13}C NMR spectra of 7 of the samples were consistent with a pre-anthraquinone skeleton. A deshielded methyl group (at δ_H 2.70) was located at C-8 in the ^1H NMR spectrum, in addition to two singlet aromatic proton signals (at δ_H 6.95 and 6.92, respectively, for H- 5 and H-10). In addition, a methyl ester at position 3.83 (C = 52.1) and a strongly chelated hydroxyl signal at position 15.27 (^1H NMR) indicate that this molecule is a precursor to aloesaponarin I. The aliphatic signals in compound 7 included a multiplet for oxymethine (δ_H 4.24) at C-3 and two methylene groups [δ_H 3.14 (*dd*, *J* = 3.3, 15.8 Hz); 2.96 (*dd*, *J* = 3.3, 17.1 Hz)]; and [2.90 (*dd*, *J* = 6.8, 15.6 Hz); 2.50 (*dd*, *J* = 1.8, 5.4 Hz)], corresponding to CH_2-2 and CH_2-4.

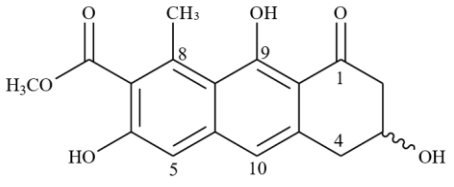
The ^{13}C NMR spectrum (Table 5) uncovered the presence of an oxymethine carbon at a position of δ_C 64.4 (C- 3), two oxygenated sp2 hybridized carbon atoms at δ_C 155.1 for C-9 and δ_C 165.9 for C-6, and a carbonyl signal at a position of δ_C 203.7 (C-1). In addition, an ester group was detected in the ^{13}C NMR spectra (carbonyl at δ_C 168.2, methoxy at δ_C 52.1). Further, an ester group was detected in the ^{13}C NMR spectra (carbonyl at δ_C 168.2 and methoxy at δ_C 52.1). Next, this logic compound 7 was found to be a methyl ester of 3,6,9-trihydroxy-8-methyl-1-oxo-5,6,7,8-tetrahydroanthracene-2-carboxylic acid (trivial name aloesaponol I). The precise structure of this molecule at carbon position 3 is not resolved here. On the other hand, an (R) configuration at C-3 has been described (Dagne et al. 1992; Yenesew et al. 1993). The chemical was initially isolated from the underground stem of *Aloe saponaria* (Yagi et al. 1974) and subsequently isolated from other species of *Aloe* (van Wyk et al. 1995; Dagne et al. 1994).

Characterization of compounds from the Leaves of *Aloe turkanensis*

Extractions with dichloromethane/methanol (1:1) were performed using cold percolation on air-dried, powdered leaves of A. turkanensis. First, the crude extract was separated into ethyl acetate and water. Following silica gel column chromatography of the ethyl acetate layer, a naphthoquinone, five anthraquinones, a pyrone derivative, and a benzoic acid derivative were isolated. Next, roots and leaves of A. turkanensis were used to isolate compounds 1, 4, and 5, with structural elucidation reported in section 4.2. Following is a discussion of the characterization of five more compounds that could only be extracted from the leaves.

Helminthosporin (8)

The red solid compound 8 was isolated, and its UV absorption maxima were observed at 230, 250, 500, and 580 nm, all the characteristics of 1,5,8-trihydroxyanthraquinone (Yagi et al. 1977). Table 6 of the ^{1}H NMR spectra confirm this to be the case by revealing the presence of three chelated hydroxyl protons at δ_H 12.03, 12.03, and 12.83 for 1-OH, 5-OH, and 8-OH, respectively. In addition, there were fifteen carbon signals detected in the ^{13}C NMR spectrum (Table 6), two of which correspond to carbonyl groups (at δ_C 189.9 and δ_C 186.3) and one to a methyl group (at δ_C 22.1; δ_H 2.50). In addition, two broad singlet aromatic protons, corresponding to H-2 and H-4 of ring A, were observed at δ_H 7.26 (δ_C 124.3) and δ_H 7.65 (δ_C 120.2) in the ^{1}H NMR spectrum (Table 6) of compound 8. A singlet integration of two protons at δ_H 7.44 was subsequently assigned to H-6 and H-7 of ring C. Compound 8 was thus found to be 1,5,8-trihydroxy-3-methyl-9,10- anthraquinone (trivial name helminthosporin). Some species of Aloe (Yagi et al. 1977; Yenesew et al. 1993; Dagne et al. 1994) and the plants *Drechslera holmii* and *Drechslera ravenelii* have been studied for the presence of this compound (van Eijk and Roeymans 1981).



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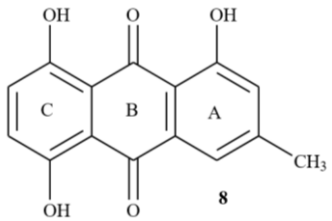


8

Table 5. ^{1}H (500 MHz) ^{13}C (125 MHz) data of compound **7** (DMSO-d_6)

Carbon no.	δ_H	δ_C
1	-	203.7
CH_2-2	2.70 (*dd*, *J* = 1.8, 5.4 Hz) 2.96 (*dd*, *J* = 3.3, 17.1 Hz)	46.4
3	4.24 (*m*)	64.4
CH_2-4	2.90 (*dd*, *J* = 6.8, 15.6 Hz) 3.14(*dd*, *J* = 3.3,15.8 Hz)	37.5
5	6.95 (*s*)	116.6
5a	-	-
6	-	155.1
7	-	140.8
8	-	137.2
8a	-	125.4
9	-	165.9
9a	-	136.6
10	6.92 (*s*)	107.5
10a	-	110.2
OCH_3	3.83 (*s*)	52.1
$COOCH_3$	-	168.2
CH_3	2.70 (*s*)	20.8
9-OH	15.27 (*s*)	-

Table 6. ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR data of compound **8** (DMSO-d_6)

Carbon no.	^{1}H δ_H (*m*)	^{13}C
1	-	161.7
1a	-	113.8
2	7.26 (*brs*)	124.3
3	-	149.1
4	7.65 (*brs*)	120.2
4a	-	132.8
5	-	157.1
5a	-	112.5
6	7.44 (*brs*)	129.4
7	7.44 (*brs*)	129.4
8	-	156.4
8a	-	112.6
9	-	189.9
10	-	186.3
CH_3	2.50 (s)	22.1
OH	12.83	-
OH	12.83	-
OH	12.03	-

Table 7. ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR spectral data of compound **9** (acetone-d_6)

Carbon No.	^{1}H δ_H	^{13}C δC
1	-	170.3
3	4.80 (*m*)	80.5
4	-	32.8
CH_2-4	2.92 (*dd*, *J* = 6.9 Hz) 2.94 (*dd*, *J* = 6.9 Hz)	32.8
4a	-	142.9
5	6.31 (*d*, *J* = 1.5 Hz)	107.6
6	-	165.0
7	6.26 (*d*, *J* = 1.9 Hz)	101.9
8	-	165.2
8a	-	101.8
1'	-	39.5
CH_2-1'	3.08 (*dd*, *J* = 5.5, 14 Hz) 3.22 (dd, *J* = 6.9, 13.5 Hz)	39.5
2'	-	139.4
3'	-	121.0
4'	-	160.1
5'	6.37 (*d*, *J* = 2.5 Hz)	102.5
6'	-	160.3
7'	6.42(*d*, *J* = 1.9 Hz)	111.7
COCH_3	-	203.9
CH_3	2.57 (*s*)	33.1
8-OH	11.21 (*s*)	-

Feralolide (9)

The compound obtained, designated 9 in this study, is a brown solid with blue fluorescence when exposed to ultraviolet light (366 nm). The maximum UV absorption of this chemical occurs at 310 nm. [M]+ was detected at *m/z* 344.75 by ESIMS, which corresponds to a chemical formula of $C_{18}H_{16}O_7$. Based on a comparison of spectroscopic data to published records, the compound was determined to be feralolide (9) (Speranza et al. 1993, Abd-Alla et al. 2009; Elhassan et al. 2012).

Two aromatic rings, each with a pair of *meta*-coupled protons (δ_H 6.31, 6.26 for H-5, H-7, and (δ_H 6.37, 6.42 for H- 5', H-7'), were detected in the ^{1}H NMR spectrum of 9 (Table 7). Additionally, at δ_H 11.21 (8-OH), a chelated hydroxyl proton was seen in ^{1}H NMR. The ^{13}C NMR spectrum showed that oxymethine carbon (δ_C 80.5, C-3) exists, and its corresponding proton appears as a multi-plate at δ_H 4.80. Additionally, due to CH_2-4 and CH_2-1' were seen in the ^{1}H NMR spectrum. In contrast, the 13C NMR spectra displayed two methylene carbon atoms (δ_C 32.8 and 39.5) with corresponding proton signals at δ_H 2.94, 2.92, and 3.22, 3.08. Additionally, a carbonyl signal for lactones (δ_C 170.3, C-1) and a carbonyl signal for ketones (δ_C 203.9) were seen in the ^{13}C NMR spectra. It led to the determination that the compound was feralolide (9). However, it does not settle what precisely the structure of C-3 is. Nonetheless, this molecule has been described with an (R)-configuration at C-3 (Speranza et al. 1993).

Aloe-emodin (10)

In its pure form, compound 10 appears as orange crystals and exhibits the characteristic UV absorption bands of 9,10-anthraquinones (max 260, 300, 420 nm). The ^{13}C NMR spectra (Table 9) corroborated this, revealing two carbonyl signals at δ_C 191.6 and δ_C 181.4 correspond to carbon atoms 9 and 10, respectively. Three mutually linked aromatic protons were seen at δ_H 7.38 (1H, *dd*, *J* = 9.0, 1.1 Hz, H-7), 7.81 (1H, *t*, *J* = 8.0 Hz, H-6), and 7.71 (1H, *dd*, *J* = 7.5, 1.1 Hz, H-5) of ring C in the ^{1}H NMR spectrum (Table 8 of 10). H-2 and H-4 in ring A have been assigned broad singlet aromatic protons at δ_H 7.28 (1H, *d*, *J* = 1.6 Hz) and 7.68 (1H, *d*, *J* = 1.6 Hz), and oxymethylene (δ_H 4.61, δ_C 62.1), which must have been generated via high oxidation of the methyl in compound 3, has been ascribed to C-3. Because of this, aloe-emodin has been established as the correct name for this compound (10). Some *Aloe* species have been found to contain the compound (Reynolds 1985; Conner et al. 1990; Elhassan et al. 2012).

α-L-11-O-Rhamnosyl aloe-emodin (11)

Based on its ESI mass spectra, compound 11 is confirmed aloe-emodin glycoside (10). These compounds have UV absorption maxima of 260, 300, and 430 nm. There are two mutually linked protons at δ_H 4.75 (1H, *d, J* = 14.0 Hz) and δ_H 4.61 (1H, *d, J* = 13.9 Hz) in the ^{1}H NMR spectrum, which are absent from the spectra of 10, indicating the presence of a sugar unit connected at the oxymethylene spot. NMR spectra (Table 8) confirmed that the sugar unit was L-rhamnose, with the most prominent signal coming from the methyl group on the sugar moiety at δ_H 1.16 (d, J= 6.2 Hz) (c 17.9). In addition, the anomeric proton was detected in the ^{1}H NMR spectrum at δ_H 4.71 (1H, *d, J* = 1.6 Hz), and the equivalent ^{13}C NMR signal was found at C 99.9, showing the sugar moiety was in the *α* -configuration (Elizabeth et al. 1998). The signal at 3.73 (2H, *dd, J* = 3.4, 1.7 Hz) for H-2' in the ^{1}H NMR spectrum proved the $^1C_4$ conformation of the sugar unit.

Two carbonyls, corresponding to carbon atoms 9 and 10, were detected in the ^{13}C NMR spectrum at δ_C 191.6 and δ_C 181.4. Based on the HMBC association of the anomeric proton with C-11, C-2 ', and C-3 ', the sugar moiety was determined to be attached at the oxymethylene position (Table 8). The component was found to be -L-11-O-Rhamnosyl Aloe-emodin, which has previously been described from *Aloe rabaiensis* exudates (Conner et al. 1989).

3, 4-Dihydroxybenzoic acid (12)

The brown solid identified as compound 12 was found to be isolates. The ^{1}H NMR spectrum of 1,3,4-trisubstituted benzene displays three aromatic proton signals, each having an AXY spin system at δ_H 7.53 (*d*, *J* = 2.0 Hz) δ_H 6.89 (*d*, *J* = 4.9 Hz) and δ_H 7.48 (*dd*, *J* = 6.5 Hz, 2.0 Hz) corresponding to H-2 (δ_C 117.4), H-5 (δ_C 115.6) and H-6 (δ_C 123.6), respectively.

Seven carbon signals were detected in the ^{13}C NMR spectra of 12, including a carbonyl at δ_C 168.7 corresponding to the carboxylic acid substituent at C-1, and two downfield shifted signals at δ_C 167.5 and δ_C 150.6 of the two hydroxyl substituents at C-3 and C-4. 3,4-dihydroxy benzoic acid (trivial name protocatechuic acid) was found to be compound 12. It was originally obtained from the *Aloe* genus (Dagne and Alemu 1991) and later found in the Ginkgoaceae, Hypericaceae, and Rosaceae families (Ellnain-Wojtaszek 1997; Jurgenliemk and Nahrstedt 2002; Lee and Yang 1994).

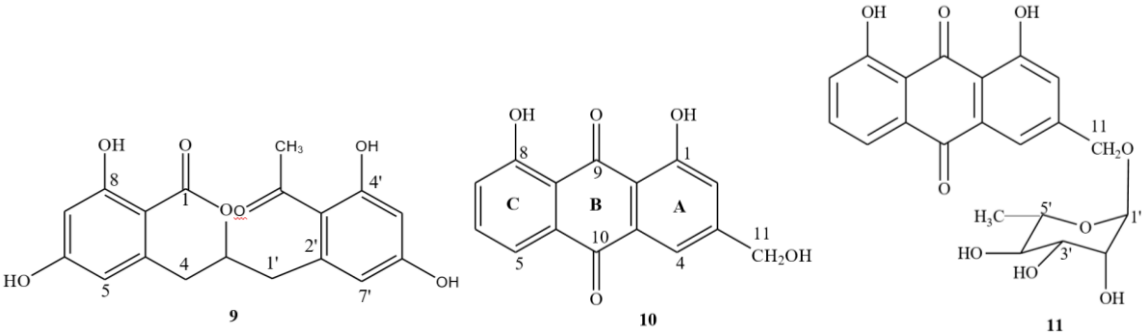


Table 8. ^{1}H (500 MHz), ^{13}C (125 MHz), and HMBC (500MHz) spectra data of compounds 10 and 11 (DMSO-d_6)

Carbon no.	Compound 10			Compound 11		
	^{1}H δ_H (*m*, *J* in Hz)	^{13}C	HMBC	^{1}H δ_H (*m*, *J* in Hz)	^{13}C	HMBC
1	-	161.6		-	161.4	
1a	-	114.6		-	115.3	
2	7.28 (1H, *d*, *J* = 1.6 Hz)	120.6	C-1, C-1a	7.32 (1H, *d*, *J* = 1.6 Hz)	121.8	C-1a, C-4
3	-	157.3		-	148.8	
4	7.68 (1H, *d*, *J* = 1.6 Hz)	117.1	C-1a, C-1, C-2	7.68 (1H, *d*, *J* = 1.6 Hz)	117.7	C-1a, C-2, C-4a, C-10
4a	-	133.3		-	133.4	
5	7.71 (1H, *dd*, *J* = 7.5, 1.1 Hz)	119.3	C-7, C-8a, C-8	7.41 (1H, *dd*, *J* = 8.4, 1.1 Hz)	119.3	C-8a, C-5, C-8
5a	-	133.1		-	133.3	
6	7.81 (1H, *t*, *J* = 8.0 Hz)	137.3	C-5a, C-8, C-7, C-5	7.82 (1H, *st*, *J* = 8.4 Hz)	137.4	C-5a, C-5, C-8
7	7.38 (1H, *dd*, *J* = 9.0, 1.1 Hz	124.4	C-8a, C-8, C-5	7.73 (*dd*, *J* = 5.0 Hz)	124.4	C-8a, C-7, C-8
8	-	161.3		-	161.3	
8a		115.9			115.9	
9	-	191.6		-	191.6	
10	-	181.4		-	181.4	
CH_2- 11	4.62 (*s*)	62.1		4.75 (1H, *d*, *J* = 14.0 Hz)	66.9	
				4.61 (1H, *d*, *J* = 13.9 Hz)		C-1', C-2, C-3, C-4
1'		-		4.71 (1H, *d*, *J* = 1.6 Hz)	99.9	C-11, C-2', C-3'
2'	-	-		3.73 (2H, *dd*, *J* = 3.4, 1.7 Hz)	70.4	C-3'
3'	-			3.62-3.28 (33H, *m*)	69.0	C-4'
4'	-	-		3.23 (2H, *s*)	71.8	CH_3, C-5'
5'	-	-		-	70.7	-
1-OH & 8-OH	11.93 (*s*)	-	-	-	-	-
CH_3	-	-	-	1.16 (*d*, *J* = 6.2 Hz)	17.9	C-5'

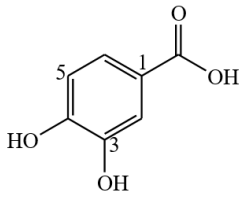
Chemotaxonomic importance of the isolated naphthoquinones

Because of its unique chemical components, Aloe has been categorized differently. The *A. secondiflora*, another member of the same plant family, also contains naphthoquinones (Induli et al. 2012). Reynolds (1996) used a chromatographic examination of leaf exudates to determine that *A. scabrifoli* and *A. turkanensis* are separate species. This study used two naphthoquinones, 3,5,8-trihydroxy-2-methylnaphthalene-1,4-dione (1) and 5,8-dihydroxy-3-methoxy-2-methylnaphthalene-1,4-dione (2), were extracted. Naphthoquinones have been found and reported in two other plant genera, and Aloe is the second. In the case of family 14 (Aloes with secund flowers), naphthoquinones may serve as a unique identifier for taxonomic purposes. It will be interesting to check if the closely related plant *A. scabrifolia* has any naphthoquinones.

In vitro anticancer activities

Effect of DMSO on cell viability

Cytotoxicity tests were performed on the extracts and the pure compounds in a DMSO solution. A series of DMSO concentrations were utilized to establish the point at which further testing would be called off due to cytotoxicity. Below 1 g/mL, DMSO has negligible effects on cell viability (Figure 2).



12

Table 9. ^{1}H (500 MHz) and ^{13}C (125 MHz) spectra data of compound 12 (acetone-d_6)

Carbon no.	δ_H (*m*, *J* in Hz)	δ_C
1	-	146.2
2	7.53 (*d*. *J* = 2.0 Hz)	117.4
3	-	167.5
4	-	150.6
5	6.89 (*d*, *J* = 4.9)	115.6
6	7.47 (*dd*, *J* = 6.5, 2.0)	123.6
COOH	-	168.7

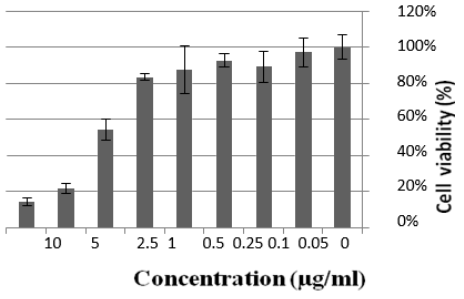


Figure 2. Effect of DMSO on TFK-1 cell viability

Anticancer test of crude extracts and compounds on TFK-1 cell line

The crude extracts and identified compounds were tested against a human extrahepatic bile duct carcinoma cell line (TFK-1). The cytotoxicity of an *A. turkanensis* rhizome extract in CH2Cl2/MeOH (1:1) against human extrahepatic bile duct cancer cells (TFK-1) is shown in Figure 3 at concentrations of 50-100 g/mL. In contrast, leaf extracts' cytotoxicity is similar at concentrations of 75 and 100 g/mL. Following the discovery of cytotoxic effects in crude extracts of *A. turkanensis* rhizomes and leaves (Figure 3), a panel of twelve compounds isolates from these extracts was tested on the human TFK-1 cell line. Compounds belonging to the anthraquinone, pre-anthraquinone, and naphthoquinone families exhibited activities.

Figure 4 shows that between 5 and 100 µg/mL ($p<0.001$), aloe-emodin (10) and its glycoside, α-L-11-*O*-rhamnopyranosyl aloe-emodin (11), reduced cell viability more than any other anthraquinone. For example, aloesaponarin II (4), which has a methyl group peri to carbonyl (at C-8), significantly decreased cell viability (10-100 µg/mL) (Figure 5). Figure 6 shows that the presence of a methyl group at C-8 (*peri* to the carbonyl) in alosaponarin II (4) is crucial for the observed cytotoxicity. In contrast, the isomeric structure chrysophanol (3), which has its methyl group at C-3, only showed significant cytotoxicity at concentrations of 2.5 and 25 µg/mL ($p<0.05$). Vis aloesaponarin I (5) and laccaic acid D methyl ester (6), two additional anthraquinones with a methyl group at C-8, likewise significantly decreased cell viability at concentrations of 10-100 g/mL and 50-100 g/mL, respectively (Figure 7). Figure 6 shows that at 5-100 g/mL concentrations, helminthosporin (8) considerably decreased cell viability, while chrysophanol (3) only did so at concentrations of 2.5 and 25 g/mL. (Figure 6). At concentrations between 5 and 100 g/mL, the pre-anthraquinone aloesaponol I (5) significantly decreases cell viability (Figure 5).

At 25-100 µg/mL doses, the naphthoquinone 5,8-dihydroxy-3-methoxy-2-methylnaphthalene-1,4-dione (2) showed substantial cytotoxicity, significantly decreasing cell viability by more than 90%. Moreover, this compound decreased cell viability significantly at doses between 5 and 15 µg/mL. Cell viability was significantly decreased at 100, 75, and 5 µg/mL concentrations for the second naphthoquinone evaluated, 3,5,8-trihydroxy-2-methylnaphthalene-1,4-dione (1) (Figure 8). However, compared to non-methylated naphthoquinone (1), methylation naphthoquinone (2) was more toxic to cells (1).

At concentrations of 25 and 50 µg/mL ($p < 0.05$), the benzoic acid derivative 3,4-dihydroxybenzoic acid (12) significantly decreased cell viability. At 5 µg/mL, feralolide (9)-a pyrone derivative was likewise significantly effective ($p < 0.05$) against the TFK-1 cell line (Figure 9). These compounds appear responsible for the reduction in TFK-1 cell viability caused by crude extracts.

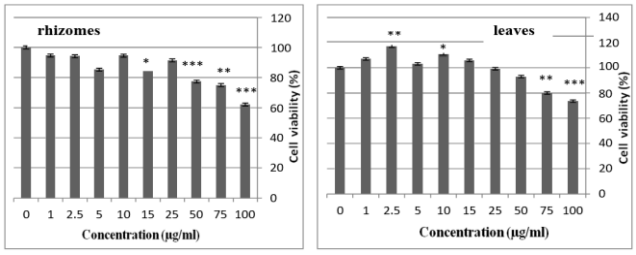


Figure 3. Effect of rhizomes and leaves extract on TFK-1 cell viability

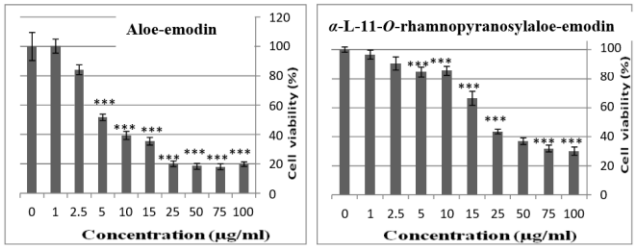


Figure 4. Effect of aloe-emodin and α-L-11-*O*-rhamnosyl aloe-emodin on TFK-1 cell viability

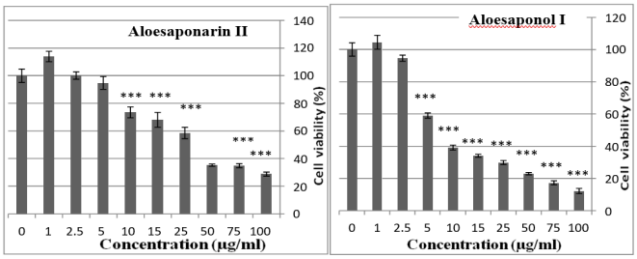


Figure 5. Effect of aloesaponarin II and aloesaponol I on TKF-1 cell viability

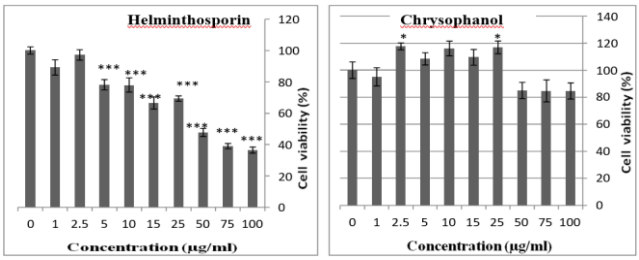


Figure 6. Effect of helminthosporin and chrysophanol on TFK-1 cell viability

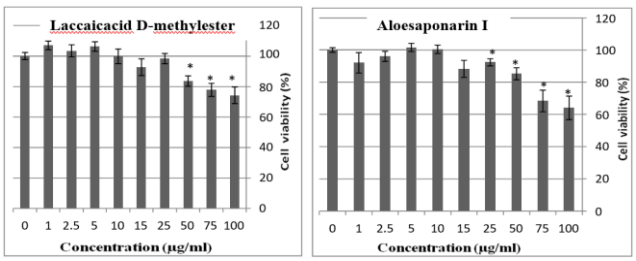


Figure 7. Effect of laccaic acid D- methyl ester and aloesaponarin I on TFK-1 cell viability

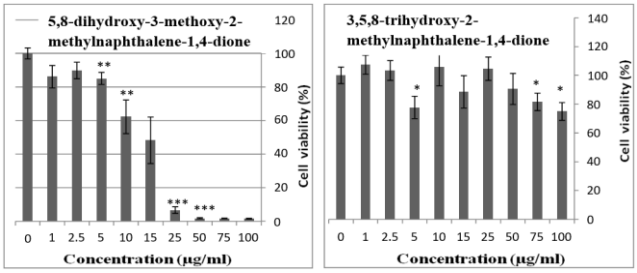


Figure 8. Effect of 5,8-dihydroxy-3-methoxy-2-methylnaphthalene-1-4-dione and 3,5,8-Tri hydroxyl-2-methylnaphthalene-1,4-dione on TFK-1 cell viability

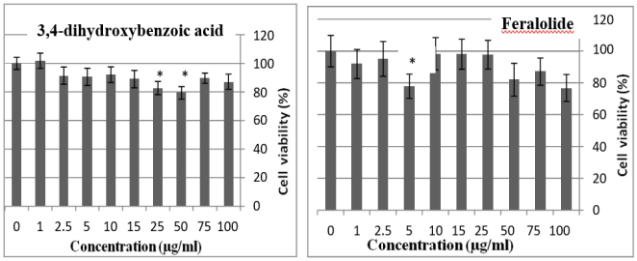


Figure 9. Effect of 3,4-dihydroxybenzoic acid and feralolide on TFK-1 cell viability

Anticancer test on selected compounds on HuH7 cell line

The human hepatoma carcinoma cell line (HuH7) was tested with the most cytotoxic compounds against the extrahepatic bile duct carcinoma cell line (TFK-1). The 25-100 µg/mL concentration range for the naphthoquinone derivative 5,8-dihydroxy-3-methoxy-2-methylnaphthalene-1,4-dione (2) resulted in the greatest decrease in cell viability (80%-97.5%). At 15 µg/mL, this compound was shown to drastically decrease cell viability (by 29.4%) (Figure 10).

At concentrations of 25-100 µg /mL, aloe-emodin (10) and its glycoside α-L-11-*O*-rhamnopyranosyl aloe-emodin (11) significantly reduced cell viability (Figure 10 and Figure 11). The higher concentrations (50-100 µg/mL) of the pre-anthraquinone aloesaponol I (7) and the anthraquinone aloesaponarin II (4) significantly decreased cell viability (Figure 12). As shown in Figure 9, the examined quantities of helminthosporin (8) had no appreciable effect on HuH7 cells.

Table 10 shows that aloe-emodin (10) is the most effective inhibitor of TFK-1 (IC_{50} = 6 µg/mL) and HuH7 (IC_{50} = 31 µg/mL) cell lines. The TFK-1 (IC_{50} values of 15 µg/mL) and HuH7 (IC_{50} values of 20 µg/mL) cell lines were likewise highly inhibited by the naphthoquinone derivative 5,8-dihydroxy-3-methoxy-2-methylnaphthalene-1,4-dione (2). With an IC_{50} value of 10 µg/mL, the pre-anthraquinone aloesaponol I (7) suppressed TFK-1 cell proliferation. Inhibition of TKF-1 cell viability was detected at IC_{50} values of 23 µg/mL and 34 µg/mL for Aloe-emodin glycoside, α-L-11-*O*-rhamnopyranosyl aloe-emodin (11) and aloesaponarin II (4), and at IC_{50} of 47 µg/mL and 55 µg/mL for HuH7 cell lines. The examined helminthosporin (8) had IC_{50} values of 46 µg/mL for inhibiting the proliferation of TFK-1 cells but did not affect the HuH7 cells at such concentrations. In contrast to their cytotoxic effects on the TFK-1 cell line, all of the chemicals tested exhibited minimal effects on the HuH7 cell line (Table 10).

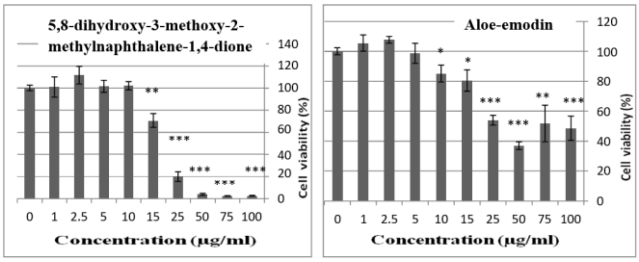


Figure 10. Effect of 5,8-dihydroxy-3-methoxy-2-methyl naphthalene-1,4-dione and aloe-emodin on HuH7 cell viability

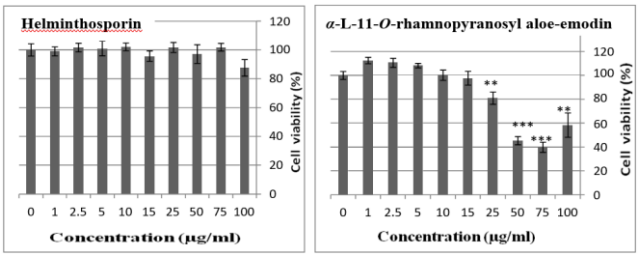


Figure 11. Effect of helminthosporin and α-L-11-*O*-rhampopyranosyl aloe-emodin on HuH7 cell viability

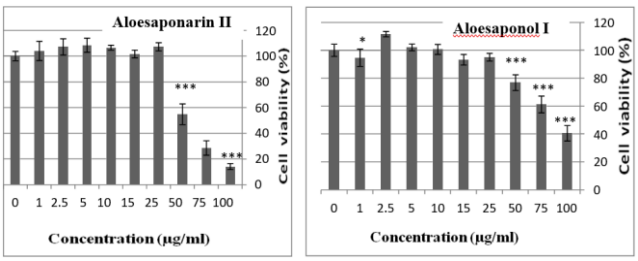


Figure 12. Effect of aloesaponarin II and aloesaponol I on HuH7 cell viability

Table 10. Cytotoxicity (IC_{50} value) of pure compounds and crude extracts from *Aloe turkanensis* against human cancer cell lines (TFK-1 and HuH7)

Compounds	IC_{50} (µg/mL)	
	TFK-1	HuH7
Crude extracts	>100	NT
3,5,8-Trihydroxy-2-methylnaphthalene-1,4-dione (1)	>100	NT
5,8-Dihydroxy-3-methoxy-2-methylnaphthalene-1,4-dione (2)	15.0	20.0
Chrysophanol (3)	>100	NT
Aloesaponarin II (4)	34.0	55.0
Aloesaponarin I (5)	>100	NT
Laccaic acid D-methyl ester (6)	>100	NT
Aloesaponol I (7)	10.0	88.0
Helminthosporin (8)	46.0	NA
Feralolide (9)	>100	NT
Aloe-emodin (10)	6.0	31.0
α-L-11-*O*-Rhamnopyranosylaloe-emodin (11)	23.0	47.0
3,4-Dihydroxybenzoic acid (12)	>100	NT

Note: NA = not active up to 100 µg/ml NT = not tested

Several studies have shown that aloe-emodin can slow the expansion of several cancer cells. For instance, aloe-emodin has demonstrated anticancer effects on the SCC-4 human tongue squamous carcinoma cells (Pecere et al. 2000) and lung squamous cell carcinoma (Lee 2001). (Chiu et al. 2009). However, aloe-reported emodin's ability to decrease mTORC2 activity and hence slow the progression of prostate cancer is relatively new (Liu et al. 2012).

Numerous synthetic and plant-based naphthoquinone structural isomers have been tested in vitro against various human cancer cell lines and in vivo against animal tumor models. Inhibition of human non-small cell lung cancer cell proliferation was seen using plumbagin (Hsu et al. 2006). Human myeloma RPMI 8226, human mammary cancer MCF-7, mouse fibroblasts LMTK, and main mouse fibroblast cell line (PMF) were all killed off by polyfluorinated 1,4- naphthoquinone derivatives (Zakharova et al. 2011). The antitumor effect of synthetic and natural naphthoquinones in several cancer cell lines was recently demonstrated by Bringmann et al. (2011). The anticancer effects of anthraquinones, pre-anthraquinone, and naphthoquinone derivatives on human extrahepatic bile duct (TFK-1) and liver cancer (HuH7) cell lines are described for the first time in this paper.

In conclusion, twelve isolates chemicals from *A. turkanensis* resulted from chromatographic separation. Among these were found to be 3,5,8-trihydroxy-2-methylnaphthalene-1,4-dione (1), 5,8-dihydroxy-2-methoxy-2-methylnaphthalene-1,4-dione (2), chrysophanol (3), aloesaponarin I (4), aloesaponarin II (5). laccaic acid D methyl ester (6) and aloesaponol I (7). The *A. turkanensis* was used to extract eight chemicals, including helminthosporin (8), feralolide (9), aloe-emodin (10), α-L-11-*O*-rhamnopyranosyl aloe-emodin (11)] and 3,4-dihydroxybenzoic acid (12). In addition, the rhizomes and leaves were analyzed for various compounds, including 3,5,8-trihydroxy-2-methylnaphthalene-1,4-dione (1), aloesaponarin I (4), aloesaponarin II (5). Herein is reported for the first time the occurrence of the naphthoquinone 3,5,8-trihydroxy-2-methylnaphthalene-1,4-dione (1) in the Asphodelaceae family. Cell viability was significantly decreased in an extrahepatic bile duct cancer cell line (TFK-1) exposed to the crude extracts. Six compounds [5,8-dihydroxy- 3-methoxy-2-methylnaphthalene-1,4-dione (2), aloesaponarin II (5), aloesaponol I (7), Helminthosporin (8), Aloe-emodin (10) and α-L-11-*O*-rhamnopyranosylaloe-emodin (11)] were found to be very strong inhibitors of the TKF-1 cell line. Higher inhibition was seen on the HuH7 cell line when exposed to the following five compounds: 5,8-dihydroxy-3-methoxy- 2-methylnaphthalene-1,4-dione (2), aloesaponarin II (5), aloesaponol I (7), Aloe-emodine (10) and α-L-11-*O*-rhamnopyranosylaloe-emodin (11). Extrahepatic (TFK-1) and liver (HuH7) cancer cell lines were used to evaluate the phytochemical and anticancer properties of the isolates chemicals, making this the first report of its kind.

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