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Cytotoxic Activity of Ethanolic Extract of *Aloe saudiarabica* and *Aloe shadensis* against Three Human Cancer Cell Line

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Author's contribution

The sole author designed, analyzed, interpreted and prepared the manuscript.

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ABSTRACT

Aloe saudiarabica and *Aloe shadensis* are a rare species of the genus *Aloe* found only in Saudi Arabia. The cytotoxic activity of both plants were evaluated in the current study using three different human cancer cell line, lung carcinoma (A-549), breast adenocarcinoma (MCF-7) and liver cancer (HepG2), assessed by WST-1 cell viability assays. The results indicate that the *Aloe saudiarabica* and *Aloe shadensis* showed weak cytotoxic effects against all three tested cancer cell lines, with an IC50 value of >300 µg/ml. In addition, HepG2 cells were more sensitive to *Aloe saudiarabica* treatment than MCF-7 and A549 cells, while MCF-7 cells were more sensitive to *Aloe shadensis* treatment than HepG2 and A549 cells. This study also identified the characteristic chemical constituents of the two plants using gas chromatography-mass spectrometry technique and the result indicated that 9-octadecenoic acid (Z)-, methyl ester (32.23%) was the main compound of *Aloe saudiarabica* while methyl 9-octadecenoate (17.28%) was the main compound of *Aloe shadensis*. In conclusion, the *in vitro* evaluation of *Aloe saudiarabica* and *Aloe shadensis* methanolic extraction showed low cytotoxicity on the viability of A-549, MCF-7 and HepG2 cell lines.

Keywords: Aloe shadensis; Aloe saudiarabica; cytotoxicity; Cancer; Cell line.

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1. INTRODUCTION

There is considerable evidence that cancer is a major threat to public health, as it is one of the leading causes of death in the world [1]. In fact, based on the findings of the International Agency for Research on Cancer (IARC), the most common cancer-related deaths occur due to liver, breast, lung and stomach cancers [2]. The understanding of cancer and its treatment has progressed rapidly in recent years. However, the improvement of many cancer drugs has not been enough to extend the life expectancy of cancer patients due to side effects and cancer drug resistance [3,4]. Therefore, natural products are receiving greater interest as treatment options for cancer [5]. In the development of drugs, natural products derived from plants have played a significant role. Today, more than 60% of all anticancer medications are derived from plants [6]. *Aloe* plants are widely recognized for their health, beauty, medicinal and skin care benefits and have been used as therapeutic agents for centuries [7,8]. *Aloe saudiarabica* is a rare species of the genus *Aloe* found only in Saudi Arabia. McCoy [9] first described the species under the name *saudiarabica*, which refers to its occurrence in Saudi Arabia. *Aloe shadensis* is characterized by its occurrence only in Jabal Shada in Albaha region, Saudi Arabia, and distinctive phenotypic characteristics. Several sources mention its characteristics, naming, and molecular identification [10-12].

The cytotoxic activity of *Aloe saudiarabica* and *Aloe shadensis* has not been reported in any published research. Therefore, this study assessed the cytotoxic activity of the methanolic extract of *Aloe saudiarabica* and *Aloe shadensis* against three human cell line, lung carcinoma (A-549), breast adenocarcinoma (MCF-7) and liver cancer (HepG2), assessed by WST-1 cell viability assays. Furthermore, the bioactive compounds of the *Aloe saudiarabica* and *Aloe shadensis* extracts were analyzed using gas chromatography-mass spectrometry (GC-MS).

2. MATERIALS AND METHODS

2.1 Plant Material and Extraction

The plant samples have collected in August 2020 from Albaha region in KSA. The plant was authentic and identified by Taxonomist Abdulwali Al-Khulaidi, Department of Biology, Albaha university. Plant leaves samples were dried under vacuum oven at 40 Co for 72 h, then were ground using mortar and pestle, after that were extracted with 70 % methanol (3 X 60 mL fresh solvent, 30 min ultrasonic each). Then, each mixture was filtered and the extract was collected and evaporated under vacuum at 50°C.

2.2 GC-MS Analysis

Chemical composition analysis of the methanol extract of *Aloe saudiarabica* and *Aloe shadensis* were performed using Trace GC-TSQ mass spectrometer and TG-5MS capillary columns. The column oven temperature was initially maintained at 50°C and subsequently increased by 5°C/ minutes to 250°C and held for 2 minutes. Then the temperature was increased to 300°C and held for 2 minutes. Temperatures of MS transfer line and injector were 270°C and 260°C, respectively; Helium was used as a carrier gas at a constant flow rate of 1 ml/min. The solvent delay was 4 min and diluted samples of 1 µL were injected automatically using Autosampler AS1300 coupled with GC in the split mode. El mass spectra were collected at 70 eV ionization voltages over the range of m/z 50-650 in full scan mode. The ion source temperature was set at 200 °C. The components were identified by comparison of their retention times and mass spectra with those of WLEY 09 and NIST 14 mass spectral database [13].

2.3 Cancer Cell Culture

Human cancer cell line (A-549, MCF-7 and HepG2) were obtained from Nawah Scientific Inc. (Egypt) and stored in the laboratory. All three cell lines were cultured in DMEM medium containing 100 mg/mL streptomycin, 100 U/mL penicillin and 10% FBS at 37°C in an incubator humidified with 5% CO_2.

2.4 WST-1 Assay

The WST-1 assay was performed using an Abcam® kit to measure cell viability. Briefly, 50µL of cell suspension were seeded in 96-well culture plates and incubated for 24 h in complete medium. Cells were treated with another aliquot of 50 µL media containing plant extract at 0-300 ug/ml concentrations. After 48 h of incubation, cells were treated with 10µL WST-1 reagent and the absorbance was measured after 1 h at 450 nm using a BMG LABTECH®- FLUOstar Omega microplate reader (Allmendgrün, Ortenberg, Germany).

2.5 Statistical Analysis

Using one-way ANOVA, the results were reported as mean ± standard deviation (Mean ± SD). P- values < 0.05 indicate significant differences in the data.

3. RESULTS AND DISCUSSION

The bioactive compounds present in medicinal plants can be synthesized in an unlimited amount and have a much lower risk of side effects than synthetic drugs. Over the years, bioactive compounds in plants have shown a wide variety of biological effects. Due to the biological activity and reliability of this compound, scientists have become more interested in its use in the formulation of new and novel drugs [14]. Table 1 presents the results of phytochemical analysis by GC-MS, which reveal that the methanolic extract of *Aloe saudiarabica* contains 19 chemical constituents. The major compound was 9-Octadecenoic acid (Z)-, methyl ester (32.23%) with the retention time value of 22.31(Fig. 1). The Gas chromatogram and respective mass spectrum for 9-Octadecenoic acid (Z)-, methyl ester were shown in Fig. 2 and Fig. 3. The other most abundant compounds were 3,11-tetradecadien-1-ol followed by 2.4.6-Tripropyl-1.3.5-trioxane with 16.22 and 10.10% peak areas, respectively. Table 2 presents the results of phytochemical analysis by GC-MS, which reveal that the methanolic extract of *Aloe shadensis* contains 28 chemical constituents. The major compound was Methyl 9-octadecenoate (17.28%) with the retention time value of 22.30 (Fig. 4). The Gas chromatogram and respective mass spectrum for Methyl 9-octadecenoate were shown in Fig. 5 and Fig. 6. The other most abundant compounds were 17-Octadecynoic acid followed by Boronic acid, ethyl-, dimethyl ester with 11.97 and 11.65% peak areas, respectively. In the case of plant bioactive compounds, a major concern is that some of these substances may be cytotoxic; therefore, safety is of paramount importance in the development of new drugs [15]. The cytotoxic effect of the methanol extract of *Aloe saudiarabica* and *Aloe shadensis* on lung cancer (A-549) and human breast adenocarcinoma (MCF-7), as well as on a hepatocellular carcinoma cell line (HepG2), was carried out using the WST-1 assay. The results indicate that the *Aloe saudiarabica* and *Aloe shadensis* showed weak cytotoxic effects against all three tested cancer cell lines, with an IC50 value of >300 µg/ml (Fig. 7 and 8). *In vitro*, both plant extracts produced limited reductions in cell viability and limited cytotoxicity [16,17]. Interestingly, it was observed that HepG2 cells were more sensitive to *Aloe saudiarabica* treatment than MCF-7 and A549 cell line. In addition, *Aloe shadensis* treatment significantly inhibited the growth of MCF-7 cells as compared to HepG2 and A549 cells. These results were in agreement with other study that reported weak cytotoxicity of other *Aloe* species. Moharram *et al*. [18] reported that *Aloe niebuhriana* latex extract exhibited a weak cytotoxicity effect against MCF-7, HepG2 and HCT-116 cancer cell line. Fox *et al.* [19] also reported that *Aloe vera, Aloe ferox* and *Aloe marlothii* exhibited weak cytotoxicity effect towards the HaCaT cells using MTT assay. In addition, This study also agrees with an earlier study by Du Plessis & Hamman [20], which reported that *Aloe vera*, *Aloe marlothii*, *Aloe speciosa* and *Aloe ferox* did not significantly affect the viability of three human cell lines *in vitro*, including human epithelial adenocarcinoma (HeLa), human hepatocellular carcinoma (HepG2) and human neuroblastoma (SH-SY5Y).

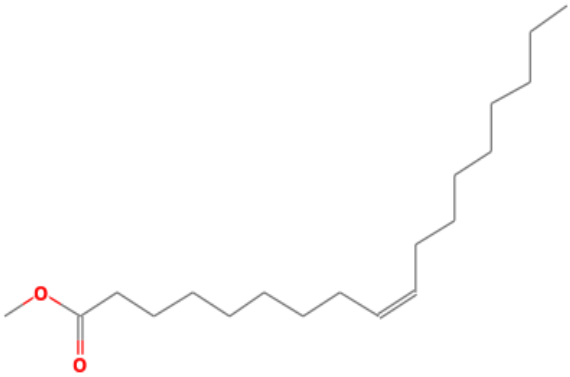

Fig. 1. 9-Octadecenoic acid (Z)-, methyl ester sturcture

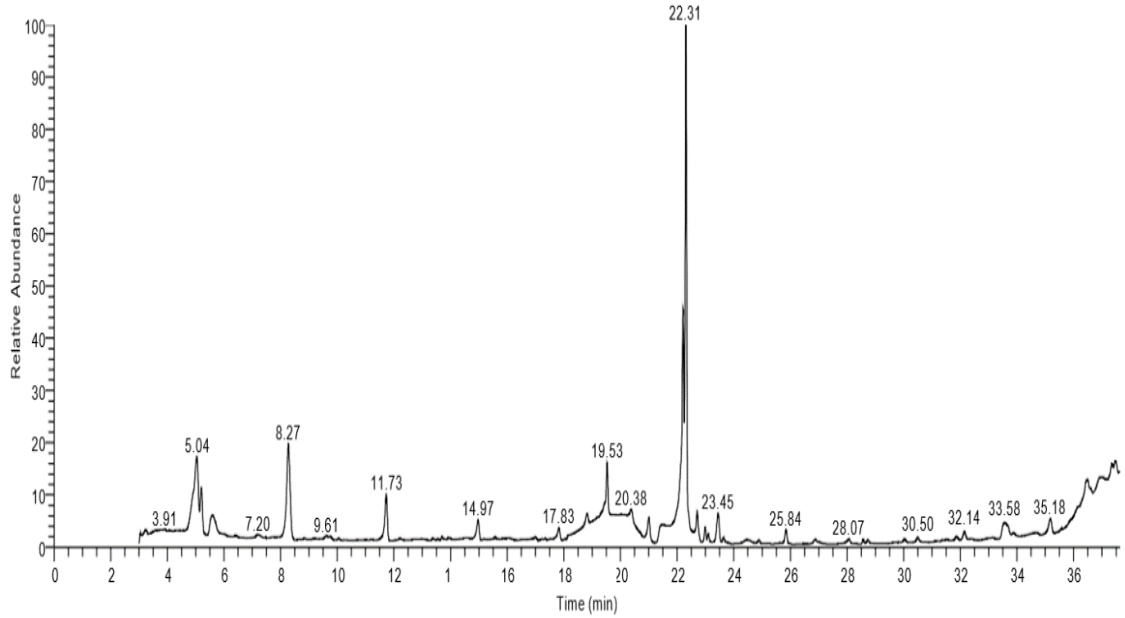

Fig. 2. Gas chromatography–mass spectrometry chromatogram of the methanolic extract of *Aloe Saudiarabica*

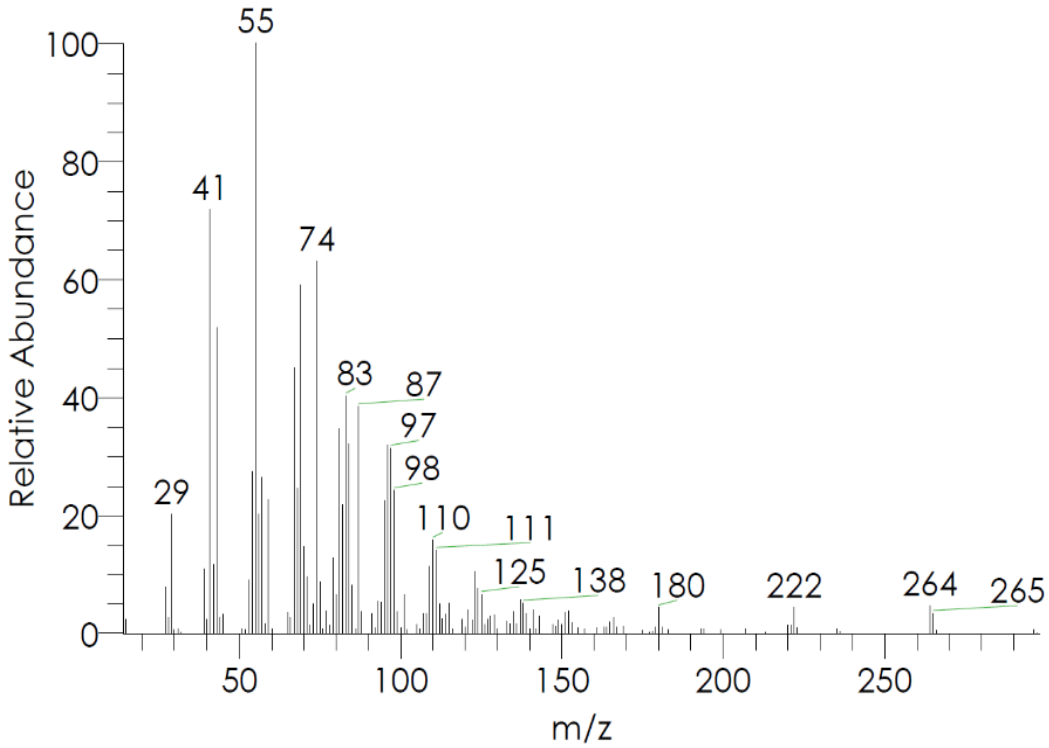

Fig. 3. Mass spectrum of 9-Octadecenoic acid (Z)-, methyl ester

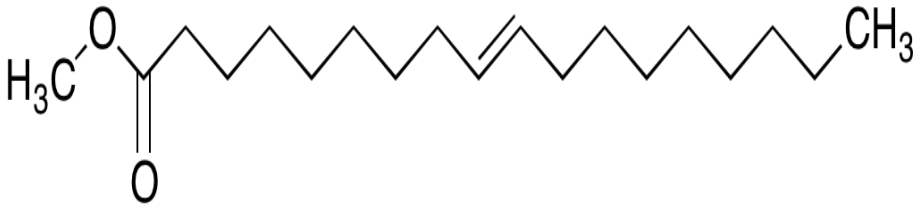

Fig. 4. Methyl 9-octadecenoate sturcture

Table 1. Phytochemical analysis of *Aloe Saudiarabica* present by GC-MS

	Compound Name	Molecular formula	RT	Area %
1	Boronic acid, ethyl-, dimethyl ester	C4H11BO2	5.05	5.72
2	Thiocyanic acid, methyl ester	C2H3NS	5.21	3.16
3	N-Benzyloxycarbonyl-D-aspartic acid	C12H13NO6	5.57	3.48
4	2.4.6-Tripropyl-1.3.5-trioxane	C12H24O3	8.28	10.10
5	2,2-Dimethyl-3,3-D2-Aziridine	C4H7D2N	11.73	4.30
6	1-Carbahexaborane	CH7B5	14.98	1.90
7	1,2-Epoxy-3-propyl acetate	C5H8O3	19.53	4.34
8	Amantadine	C10H17N	21.00	2.01
9	3,11-tetradecadien-1-ol	C14H26O	22.21	16.22
10	9-Octadecenoic acid (Z)-, methyl ester	C19H36O2	22.31	32.23
11	2-Methylmalonic acid	C4H6O4	22.71	2.46
12	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	C18H30O2	22.99	1.14
13	N,N-dimethyl-1,2,3-trithian-5-amine	C5H11NS3	23.45	3.00
14	3,4-Methylenedioxy-N-thylamphetamine	C12H17NO2	25.84	1.27
15	Benzeneethanamine, N, à,à- trimethyl -	C11H17N	33.57	3.11
16	Deoxyspergualin	C17H37N7O3	35.18	1.64
17	Nepetalactol	C10H16O2	36.44	0.96
18	Thionodecalactone	C10H18OS	37.37	1.41
19	11-Octadecenal	C18H34O	37.49	1.56

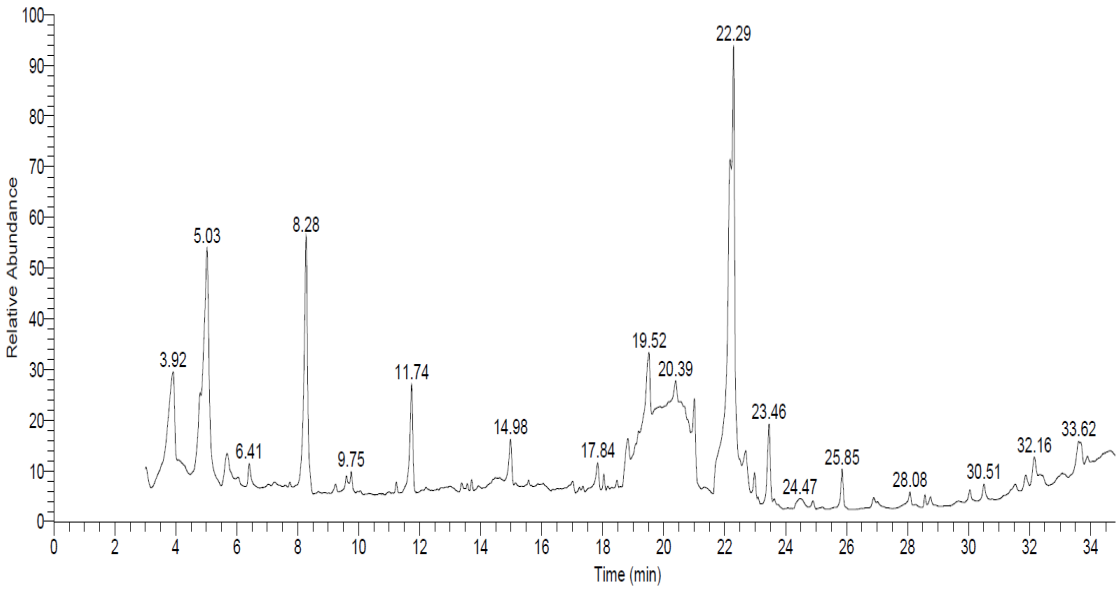

Fig. 5. Gas chromatography–mass spectrometry chromatogram of the methanolic extract of *Aloe shadensis*

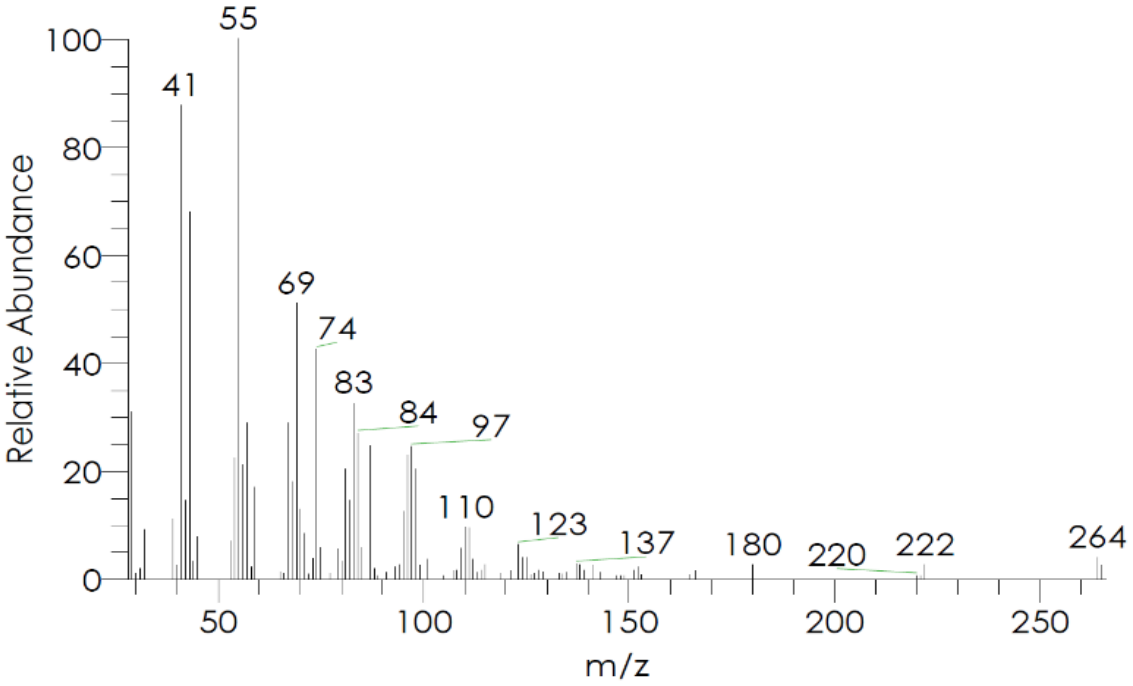

Fig. 6. Mass spectrum of Methyl 9-octadecenoate

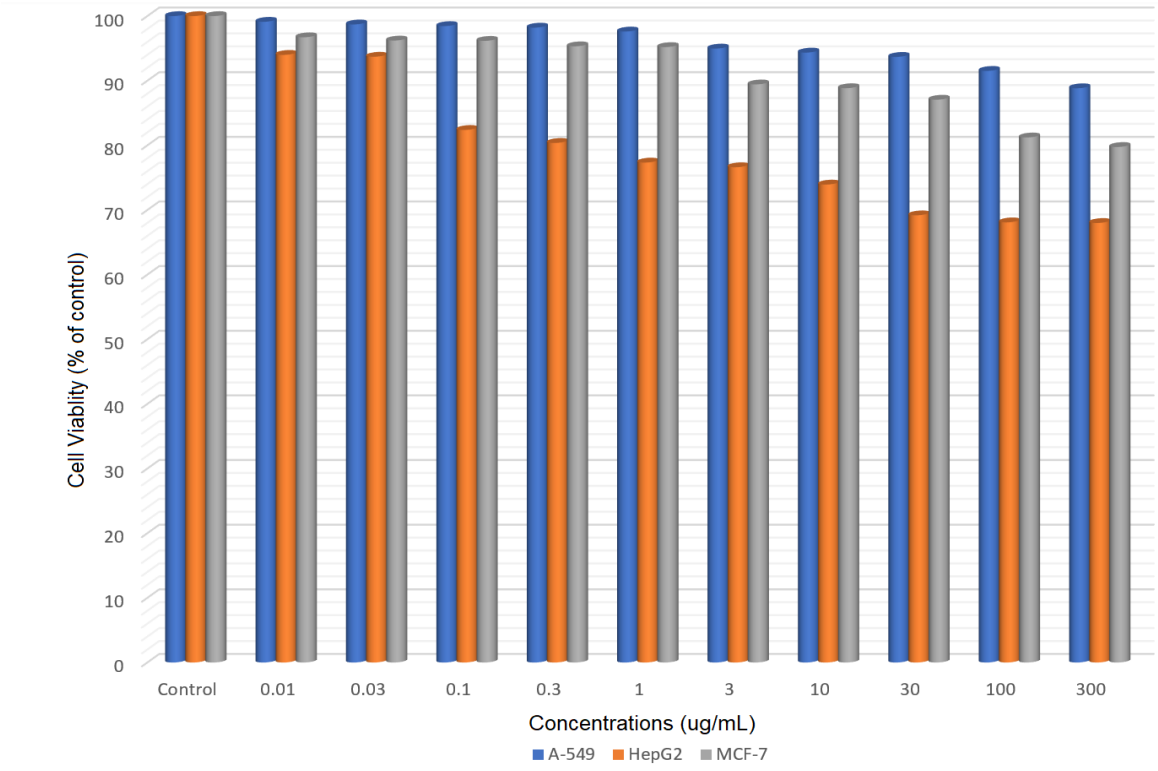

Fig. 7. Effect of Aloe saudiarabica on cell viability and growth of A549, HepG2 and MCF-7 cells

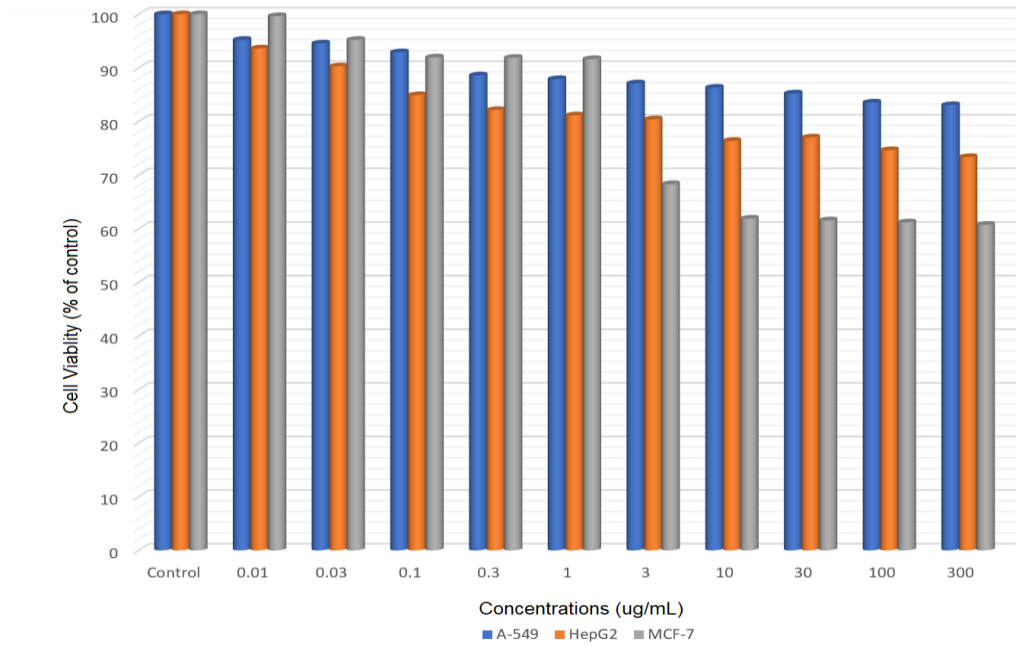

Fig. 8. Effect of Aloe shadensis on cell viability and growth of A549, HepG2 and MCF-7 cells

Table 2. Phytochemical analysis of *Aloe shadensis* present by GC MS

	Compound Name	Molecular formula	RT	Area %
1	Benzene, 1-chloro-2-methyl-	C7H7Cl	3.93	6.88
2	6-Deoxy-D-mannono-4-lactone	C6H10O5	4.78	0.85
3	Boronic acid, ethyl-, dimethyl ester	C4H11BO2	5.03	11.65
4	DL-Serine,N-[(phenylmethoxy)carbonyl]-	C11H13NO5	5.67	1.60
5	*methyl 13c Octadecatrienoate*	C19H32O2	6.41	1.04
6	Acetoacetamide	C4H7NO2	8.28	11.47
7	3,6-Dimethyl-3,6-dihydro-pyran-2-one oxime	C7H11NO2	9.60	0.81
8	10-Undecenoic acid, octyl ester	C19H36O2	9.75	0.78
9	Acetoacetamide	C4H7NO2	11.74	5.05
10	Alanine	C3H7NO2	14.98	2.24
11	*6-Deoxy-D-mannono-4-lactone*	C6H10O5	17.84	1.24
12	10-Methyl-E-11-tridecen-1-ol propionate	C17H32O2	18.04	0.57
13	d-Glycero-d-ido-heptose	C7H14O7	18.83	2.35
14	4-Cyclopentene-1,3-diol-D2, trans-	C5H6D2O2	19.52	3.56
15	1-Trideutero acetyl-2-methylaziridine	C5H6D3NO	20.40	1.08
16	Taurolidine	C7H16N4O4S2	21.01	3.31
17	*Trans-3-Deutero cyclopentene-3,5-diol*	C5H7DO2	21.70	1.24
18	17-Octadecynoic acid	C18H32O2	22.20	11.97
19	Methyl 9-octadecenoate	C19H36O2	22.30	17.28
20	2-Methylmalonic acid	C4H6O4	22.70	1.69
21	Methyl 12,13-tetradecadienoate	C15H26O2	22.98	1.11
22	N,N-Dimethyl-1,2,3-trithian-5-amine	C5H11NS3	23.46	4.06
23	hexaborane(10)	B6H10	25.86	1.68
24	Benzeneethanamine, N,N,α-trimethyl-	C11H17N	30.51	0.80
25	13-Tetradecynoic acid, methyl ester	C15H26O2	32.16	1.37
26	1,11-Undecanediol	C11H24O2	33.61	1.36
27	S-(2-Aminoethyl)-L-cysteine	C5H12N2O2S	33.69	0.89
28	9-Pentadecenoic acid	*C15H28O2*	35.19	2.08

4. CONCLUSION

In this study, we tested the cytotoxic activity of *Aloe saudiarabica* and *Aloe shadensis* against A-549, MCF-7 and HepG2 cell line using WST-1. The results show that the methanolic extract of both plant leaves showed weak cytotoxic effects on the three cancer cell lines tested. Further studies are required to evaluate the cytotoxicity of these two plants using other extraction solvents and against other cancer cell lines before any evaluation to their potential therapeutic applications.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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